Study of the variation of ecotoxicity at different stages of domestic wastewater treatment using *Vibrio-qinghaiensis* sp.-Q67

Xiaoyan Y. Ma, Xiaochang C. Wang*, Yongjun J. Liu

Key Lab of Northwest Water Resources, Environment and Ecology, Ministry of Education, Xi'an University of Architecture and Technology, Xi'an 710055, PR China

ARTICLE INFO

ABSTRACT

A bioassay using the luminescent bacterium *Vibrio-qinghaiensis* sp.-Q67 associated with solid-phase extraction (SPE) was developed for evaluating the variation of ecotoxicity along with the reduction of organic substances in a domestic wastewater treatment plant employing an oxidation ditch process. With effective elimination of the interference from all inorganic substances by the SPE operation, the ecotoxicity of the water, as expressed by the toxicity impact index, TII_{50} (%) after a model-based regression and parameter transformation, was found to decrease from 50.51% in the influent to 25.84% after the grid chamber and then to 1.38% for the secondary effluent, but the final chlorination stage resulted in a slight TII_{50} increase. Of the two typical tertiary treatment processes, coagulation could not assist further reduction of the ecotoxicity while carbon adsorption could reduce the TII_{50} to as low as 0.96%. When enhanced primary treatment was conducted, the influent TII_{50} could be directly reduced to 7.36%. A good correlative relationship was found between the total COD concentration and the TII_{50} value at different stages of the wastewater treatment process.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Wastewater discharge is often the main reason for water pollution due to insufficient treatment of domestic and/or industrial wastewater. In order to protect the water environment, regulations are put forward in many countries regarding the maximum allowable limits of pollutants in the discharged effluent, such as the total concentration of organics (COD or BOD), nutrients (ammonia and phosphorus), and a number of specific inorganic and organic chemicals. These pollutants, if with higher concentrations in water, may affect the value of the water for its uses and services [1]. In addition to this, one issue drawing wide attention is the toxic effect of the pollutants on aquatic ecosystems [2,3]. In recent years, various studies were reported on using various ecotoxicity assays for evaluating the wastewater treatment processes with attention paid to the overall effect of effluent discharge on receiving water bodies [4–6].

Of the methods for ecotoxicity assessment of water, the bioassays using luminescent bacteria are widely applied for acute toxicity test. The most commonly used luminescent bacterium is *Vibrio fischeri* of marine water origin which needs a salty environment for conducting the ecotoxicity test [7,8]. The limit for applying *V. fischeri* to freshwater samples is that the requirement of NaCl

addition to a concentration as high as 2–3% may change the inherent property of the testing sample and increase the insolubility of some organic substances [9]. As an alternative approach, a freshwater bacterium *Vibrio qinghaiensis* sp.-Q67 (abbreviated to "Q67" in following paragraphs) is found to be with similar luminescent property and can be used for ecotoxicity test [10]. The suitability and reliability of ecotoxicity assays using Q67 have been shown by a number of studies regarding the toxicity caused by heavy metals [11], herbicides and insecticides [12,13], as well as direct analysis of environmental samples [14,15].

Although the procedures for bioassays using luminescent bacteria have been well established, it is found that the toxicity test results are often affected by the ways of sample pretreatment and bacteria manipulation [16]. The impurities in a water sample, such as color and turbidity, may largely affect the test results, and some inorganic substances, such as nutrients, may have very strong stimulatory effects on the luminescent measurements [16,17]. In a wastewater treatment plant (WWTP), the removal of organic pollutants is one of the main objectives of the treatment. Therefore, a question yet to be well answered is: with the removal of organic matter at different stages of the treatment process how does the water ecotoxicity vary? An effective assessment of the ecotoxicity caused by the organic substances will need a complete elimination of any interference from the coexisting inorganic substances in the water samples. For this purpose, the solid-phase extraction (SPE) may be a suitable pretreatment [18] before the bioassay using luminescent bacteria. However, valid methods have yet been provided

* Corresponding author.
E-mail addresses: xcwang@xauat.edu.cn, xcwang45@hotmail.com (X.C. Wang).

regarding the use of Q67 for ecotoxicity assay with appropriate pretreatment for enriching the toxic organic substances. It thus becomes the objective of the present study to develop a method combing the bioassay using Q67 with SPE for the assessment of the ecotoxicity caused by organic substances along the secondary wastewater treatment process in a WWTP. Attention is paid to the relationship between the organic content and ecotoxicity at different treatment stages and the role of unit process for the reduction of ecotoxicity.

2. Materials and methods

2.1. Sample collection

For investigating the variation of ecotoxicity at different stages of domestic wastewater treatment, water samples were collected from a WWTP (treatment capacity as 100,000 $m^3 d^{-1}$) in Xi'an, China. As shown in Fig. 1, an oxidation ditch process was applied in the WWTP where raw sewage, after entering the plant, was treated through a series of physical, chemical and biological units such as coarse and fine screens, grid chamber, oxidation ditch, secondary settler, and chlorination tank. An anaerobic selection tank with short hydraulic retention time (HRT, about 0.5 h) was constructed prior to the oxidation ditch for fully mixing the water with return sludge. 6 sampling locations (No. 1 to No. 6 as indicated in Fig. 1) were selected along the treatment train for collecting the water samples. The sample collection was conducted in selected sunny weekdays in the autumn of 2010 when the WWTP was operated under normal conditions. Toxicity tests and physicochemical analyses were performed within 24 h after sampling.

For a comparison of the conventional secondary treatment with enhanced primary treatment, the sample collected from location No. 1 was sent to the laboratory for an experiment of enhanced primary treatment by coagulation and sedimentation using a jartester. The treated water was denoted as No. 7 in Fig. 1. For evaluating the effects of typical tertiary treatment on ecotoxicity reduction of the secondary effluent, the sample collected from location No. 5 was sent to the laboratory for the experimental treatment by coagulation and carbon adsorption. The treated water was denoted as No. 8 and No. 9, respectively in Fig. 1.

2.2. Solid-phase extraction and preparation of testing samples

Each of the samples collected for ecotoxicity analysis was pretreated by solid-phase extraction (SPE).

The sample was firstly filtered by a 0.45 μm membrane filter for removing the suspended solids and then SPE was conducted for the extraction of organic matter from the sample. As the target substances for this study were the organic substances that might be ecotoxic, C18 SPE cartridges (Agilent SampliQ C18 3 ml, 500 mg) that could extract a broad range of organic substances were selected. A SPE kit (SUPELCO visiprep™ DL) was used. Before the SPE operation, methylene chloride, methanol, and milli-Q water, each with a volume of 7 ml were successively passed through the cartridge for activating the fillings and keeping them under a wetted condition. A given volume of the sample (150 ml for samples No. 1, No. 2 and No. 3 which were of comparatively high organic content, and 500 ml for samples No. 4, No. 5 and No. 6 which were of comparatively low organic content) was then passed through the cartridge at a flow rate of 5 ml min⁻¹ for extracting the organic substances. Afterwards, 3 ml milli-Q water was passed through for washing out the impurities.

After the above procedures, the cartridge was put in a centrifuging machine for removing the residual water. It was then eluted by 7 ml methylene chloride. A nitrogen evaporator (Sample Concen-

trator MD200) was used for evaporating the eluent at a constant temperature of 40 °C. The extracted organic substances were finally dissolved in 0.5% dimethyl sulphoxide (DMSO) solution and tested for ecotoxiciy with Q67.

2.3. Acute toxicity test with Q67

For the acute toxicity test, luminescent bacterium Q67 was purchased from Beijing Hammatsu Photon Techniques Inc. The Q67 was grown in the culture medium that could produce larger quantity of bacteria with fluorescence enzyme. The culture medium consisted of tryptone (0.5%), yeast extract (0.5%), glycerin (0.3%), $MgCl_2$ (0.32%), KBr (0.02%), $CaSO_4$ (0.01%), NaCl (0.4%) and KCl (0.4%), pH 8.5 ± 0.5, and was cultured at $22 \pm 1\,°C$ [19].

The bacteria strains from a stock culture medium which was maintained at 4 °C were inoculated to a liquid medium. The bacteria were grown in the liquid medium up to the logarithmic growth stage after 10–12 h under $22 \pm 1\,°C$ while shaking at 120 rpm. The acute toxicity test was then conducted using a Modulus™ Single Tube Multimode Reader. For each test, 4 testing tubes were prepared, 3 for parallel samples and 1 for blank control. 100 μl bacterial suspension was added into each tube, and the same volume of the sample or blank control liquid (0.5% DMSO) was added at 15 s interval. After 15 min exposure of the bacteria to the sample at $22 \pm 1\,°C$, the relative light unit (RLU) of Q67 was measured on the Modulus™, and the acute toxicity of the sample on Q67 was expressed as the inhibition value in percent (%).

In order to quantitatively compare the ecotoxicity of different water samples, the concentration (times) corresponding to the inhibition value of 50%, namely effective concentration (EC_{50}) was used. By definition, the higher the EC_{50} value, the lower the ecotoxic effect. For the convenience of comparison following the common sense, the reciprocal of EC_{50}, namely the toxicity impact index (TII_{50}) can be introduced as below [20]:

$$TII_{50}(\%) = \frac{1}{EC_{50}} \times 100 \tag{1}$$

2.4. COD and TOC analysis

In order to compare the ecotoxicity with the organic contents of the water samples, chemical oxygen demand (COD) was analyzed by closed reflux, colorimetric method [21], and total organic carbon (TOC) was measured using a total organic carbon analyzer (Shimadzu TOC-VC). For COD analysis, both the unfiltered and filtered (by 0.45 μm membrane filter) samples were used. The former was the total COD while the later was the dissolved COD.

3. Results and discussion

3.1. Inhibition–concentration relationships

The direct results from the acute toxicity tests with Q67 were the inhibition–concentration relationships shown in Fig. 2. For each water sample, the toxicity test was conducted at 7 different concentrations which were expressed as the times the water sample was concentrated, i.e. the reciprocal of the volume ratio of the DMSO solution with concentrated organic matter to the volume of the water sample. Regarding the samples collected from the 6 locations in the WWTP, there were apparently two distinguishable types of the relationships. The first type was the curves for the first 3 samples i.e. No. 1 (the influent), No. 2 (the grid chamber outlet) and No. 3 (the selection tank outlet) which was characterized by a faster increase of the inhibition value when the concentration (times) was low, gradual slow-down of the increase of the inhibition value when the concentration became higher, and a finally leveled
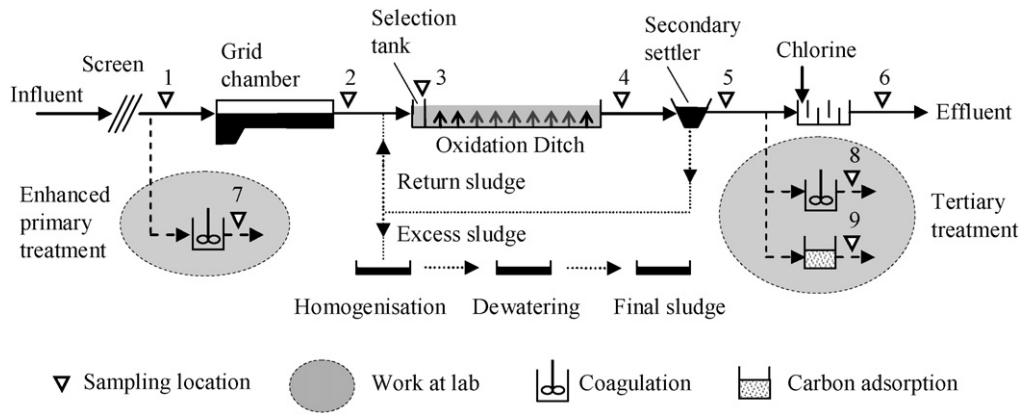
Fig. 1. Flow diagram of the oxidation ditch process in the WWTP and sampling locations.

section toward a maximum inhibition value. By data manipulating it was found that such kind of inhibition–concentration relationship well followed the following equation:

$$y = \frac{x}{a + bx} \tag{2}$$

where x is the concentration (times), y is the inhibition value (%), a and b are constants to be obtained by regression. As can be seen from Fig. 2, the 3 curves obtained well present the trend of the inhibition value varying with the concentration (regressive coefficients $R^2 = 0.94$–0.99).

Contrarily, the second type was the linear relationship for the rest samples i.e. No. 4 (the oxidation ditch outlet), No. 5 (after the secondary settler) and No. 6 (the final effluent after chlorination). By linear regression the 3 straight

lines in Fig. 2 were obtained (regressive coefficients $R^2 = 0.97$ –0.98).

From the fact that for the first 3 samples the maximum concentration was 30 times while for the rest samples it was 100 times, it could be roughly concluded that the first type might be the case for wastewater with a high content of toxicants and the second type might be for the case of low content of toxicants. The higher content of toxicants might have anesthesia effect to the bacteria, so that the inhibition–concentration relationship could not be linear [22,23].

3.2. Variation of ecotoxicity in the treatment process in the WWTP

By using the EC_{50} values obtained from Fig. 2 and calculating the corresponding TII_{50} values, Fig. 3 was obtained to show the
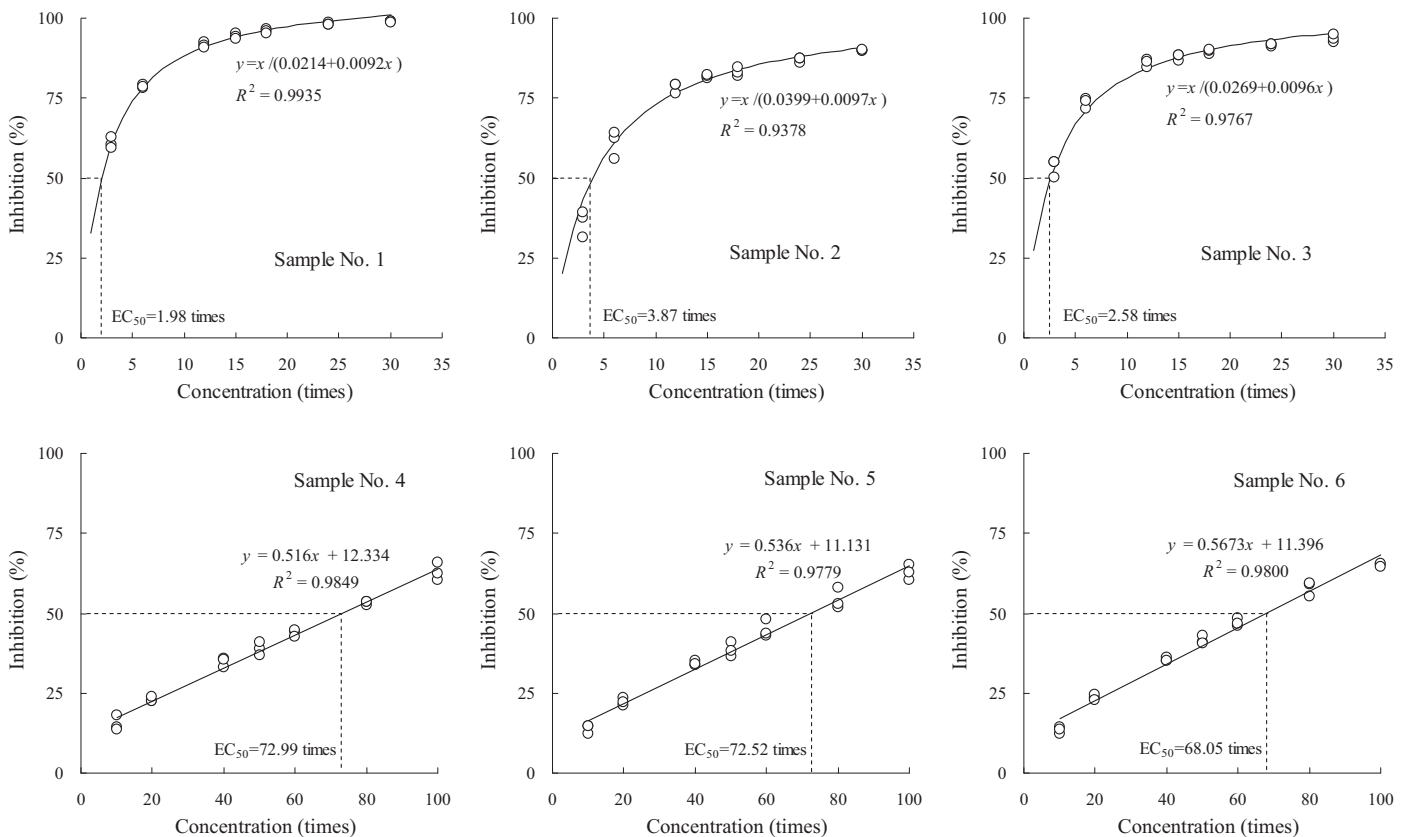

Fig. 2. Inhibition–concentration relationships of the samples collected from the 6 locations in the WWTP.
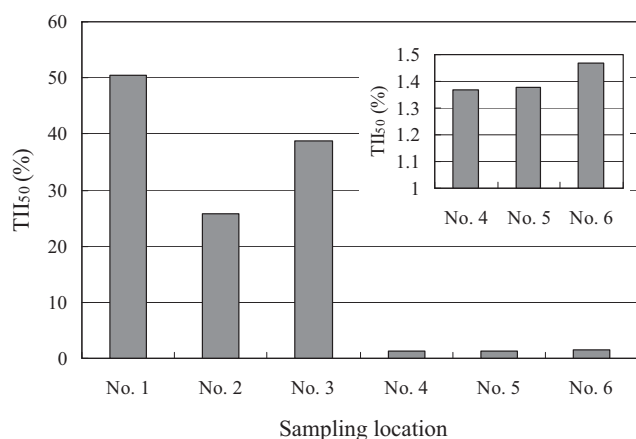
Fig. 3. Variation of ecotoxicity along the wastewater treatment process in the WWTP.

Fig. 5. The effect of coagulation on ecotoxicity reduction of the secondary effluent.

variation of ecotoxicity due to organic toxicants along the wastewater treatment process in the WWTP. For sample No. 1, i.e. the influent just after the screen, its TII_{50} was 50.51%. When the water flowed through the grid chamber (HRT as 6.3 min and with aeration), TII_{50} was reduced to 25.84% (sample No. 2). After the water entered the selection tank (HRT as about 0.5 h, anaerobic condition) and mixed with the return sludge (return ratio as 1.0), TII_{50} increased to 38.76% (sample No. 3). Very effective reduction of the ecotoxicity was seen after the biological treatment in the oxidation ditch (HRT as 18 h) as TII_{50} was reduced to 1.37% (sample No. 4). Significant change was no longer seen after the secondary settler ($TII_{50} = 1.38\%$ for sample No. 5). However, a slight increase was noticed (the inserted graph in Fig. 3) when the secondary effluent was disinfected by chlorination ($TII50 = 1.47\%$ for sample No. 6).

3.3. Effects of coagulation and carbon adsorption on the ecotoxicity

Fig. 4 shows the effect of enhanced primary treatment on ecotoxicity reduction when the influent was coagulated by jartest using polyaluminium chloride (PAC) as coagulant at a dosage of $100 \, mg \, l^{-1}$. The EC_{50} obtained was 13.59 times which corresponded to $TII_{50} = 7.36\%$ and indicated a substantial reduction of the ecotoxicity by enhanced primary treatment directly from $TII_{50} = 50.51\%$ for the influent. By comparing the two curves in Fig. 4, it can be seen that with the removal of toxic substances by coagulation from the
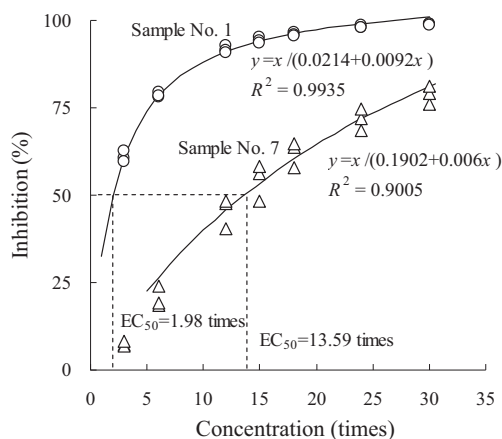
influent, the inhibition–concentration relationship showed a more linear trend.

Figs. 5 and 6 show the effects of two typical tertiary treatment processes, namely coagulation and carbon adsorption on the ecotoxicity reduction of the secondary effluent. For coagulation by jartest, the PAC was dosed at $50 \, mg \, l^{-1}$, and for carbon adsorption by batch experiment, powdery activated carbon was used.

As a result of coagulation, no improvement was found in the ecotoxicity of the finished water as EC_{50} was decreased from 72.52 times to 66.57 times and the corresponding TII_{50} was increased from 1.37% to 1.50% indicating rather a slight increase of ecotoxicity. In contrast to this, carbon adsorption showed a significant effect on reducing the ecotoxicity of the secondary effluent as EC_{50} was increased from 72.52 times to 105.18 times and the corresponding TII_{50} was decreased from 1.37% to 0.95%.

3.4. Correlations between ecotoxicity and organic matter

From Table 1 which compares the organic contents of the water samples with the ecotoxicity analysis results, it can be seen that by each stage of the treatment in the WWTP, the total COD could be effectively removed by certain extent. The total COD removal resulted in a substantial decrease of the ecotoxicity. When we compare the available data of the total COD with the corresponding TII_{50}, Fig. 7 can be obtained to show a very good correlative relationship on a log–log plot.

In the WWTP, the grid chamber was the first treatment unit where grits and coarse solids were settled resulting in about 27% SS
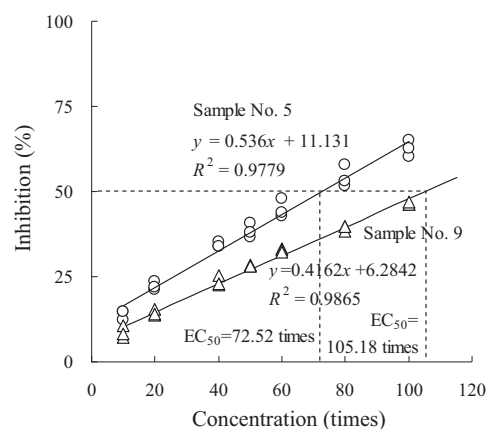

Fig. 4. The effect of enhanced primary treatment on ecotoxicity reduction of the influent.

Fig. 6. The effect of carbon adsorption on ecotoxicity reduction of the secondary effluent.

Table 1
Organic contents of water samples in comparison with the ecotoxicity analysis results.

Sample number	Sampling location	COD (mg l^{-1})		TOC (mg l^{-1})	EC$_{50}$ (times)	TII$_{50}$ (%)
		Total	Dissolved			
1	Influent	306.0	106.2	21.78	1.98	50.51
2	Grid chamber outlet	253.0	108.8	21.02	3.87	25.84
3	Selection tank outlet	–	43.4	10.97	2.58	38.76
4	Oxidation ditch outlet	–	35.5	6.11	72.99	1.37
5	Secondary settler effluent	36.7	35.2	6.93	72.52	1.38
6	Effluent after chlorination	–	37.3	6.80	68.05	1.47
7	After enhanced primary treatment	100.5	96.0	16.25	13.59	7.36
8	After tertiary treatment by coagulation	–	22.5	5.01	66.57	1.50
9	After tertiary treatment by carbon adsorption	-	23.5	5.69	105.18	0.95

removal (from 275 mg l^{-1} to 200 mg l^{-1}) and 17% total COD removal (from 306 mg l^{-1} to 253 mg l^{-1}). Since the dissolved organic contents (dissolved COD or TOC) did not show any significant change, the total COD reduction was believed to be the removal of part of the organic substances attached onto the suspended solids, thus bringing about a substantial reduction of the ecotoxicity.

In the selection tank, although more organic substances could be adsorbed by the high concentration activated sludge due to sludge return to result in a low concentration of the dissolved COD or TOC, a significant increase was seen in the TII$_{50}$ from 25.84% to 38.76%. As no aeration was provided, the selection tank was in fact under an anaerobic condition. This might be the main reason for the increase of the water ecotoxicity as pointed out by other studies [24].

The oxidation ditch, as the core biological treatment unit for the biodegradation of organic substances under aerobic/anoxic conditions and with a HRT as long as 18 h, could achieve the greatest reduction of the water ecotoxicity as the TII$_{50}$ decreased from 38.76% to 1.37% though the residual dissolved organic substances (35.46 mg l^{-1} as dissolved COD and 6.11 mg l^{-1} as TOC) were not at a very low level. This fact agreed with many studies that stressed the effect of ecotoxicity reduction through biological processes especially under an aerobic condition [25,26].

The secondary settler performed the final solid/liquid separation and achieved an almost complete removal of suspended matter as could be seen from the small difference between the total COD (36.7 mg l^{-1}) and dissolved COD (35.2 mg l^{-1}). The TII$_{50}$ value almost kept unchanged (1.37% vs. 1.38%), indicating that the secondary settling had no effect on ecotoxicity reduction. Comparing with this, the slight increase of TII$_{50}$ from 1.38% to 1.47% after chlorination might be an indication of the formation of the disinfection by-products that have ecotoxic effect [27].

By coagulation of the raw sewage, the total COD was removed by 67% (from 306 mg l^{-1} to 100.5 mg l^{-1}) but the dissolved COD and TOC were little removed. From the very close values of the total COD and dissolved COD, it can be concluded that the enhanced primary treatment is very effective to remove almost all organic substances attached onto suspended solids [28]. The effective total COD removal resulted in a remarkable reduction of the TII$_{50}$ from 50.51% to 7.36%, a much more effective ecotoxicity reduction than that by the grid chamber.

For tertiary treatment of the secondary effluent, both coagulation and carbon adsorption showed almost identical effects for the removal of the residual organic substances (dissolved COD and TOC), but much different effects on ecotoxicity reduction of the secondary effluent as indicated by the different TII$_{50}$ values of the finished waters (1.50% and 0.95%). Both coagulation and carbon adsorption are commonly used processes for tertiary treatment of the secondary effluent to remove residual contaminants [29–31]. The function of coagulation is recognized to be mainly the removal of the residual suspended and colloidal substances [29], while carbon adsorption can effectively remove the residual dissolved organic matter, including trace hazardous organic compounds that may be more ecotoxic [30]. The advantages of carbon adsorption over other treatment methods for controlling finished water quality have been pointed out by many studies [31,32]. The present study further provided evidence of the remarkable effect of carbon adsorption for the reduction of water ecotoxicity.

4. Conclusion

Through the bioassay using the luminescent bacterium *Vibrio-qinghaiensis sp.*-Q67 combined with SPE for extracting organic substances, the ecotoxicity of water at different stages of the WWTP was evaluated. With an effective elimination of any interference from inorganic matters, the method was found to be applicable to water samples with wide range of organic concentrations – from the WWTP influent to the finished water after tertiary treatment. Even a small variation in water ecotoxicity, such as that of the secondary effluent after chlorination, could be sensitively detected. A good correlative relationship was found between the total organic content, such as COD and the ecotoxicity in terms of TII$_{50}$. In a conventional secondary wastewater treatment process such as the oxidation ditch process investigated in this study, the biological treatment unit played the central role for ecotoxicity reduction. Other treatment unit such as the grid chamber could also bring about substantial reduction of ecotoxicity while the enhanced primary treatment showed more remarkable effect than
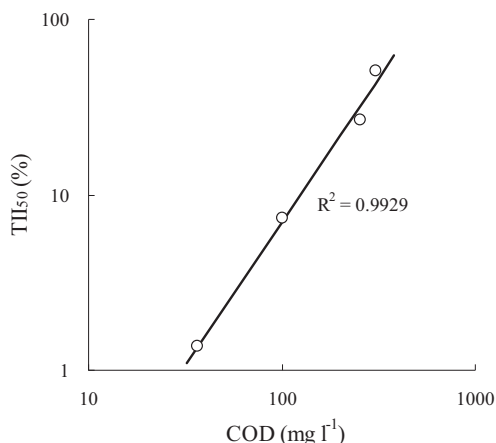

Fig. 7. The relationship between the total COD and TII$_{50}$.

the grid chamber for ecotoxicity reduction. The organic substances that were ecotoxic might include biodegradable, coagulable, and adsorptive parts, so that carbon adsorption was found to be the most effective method for further reduction of the ecotoxicity caused by the adsorptive organic mater residual in the secondary effluent.

Acknowledgements

References

[1] R.A. Haggarty, C.A. Ferguson, E.M. Scott, C. Iroegbu, R. Stidson, Extreme value theory applied to the definition of bathing water quality discounting limits, Water Res. 44 (2010) 719–728.

[2] J. Bayo, J.M. Angosto, M.D. Gómez-López, Ecotoxicological screening of reclaimed disinfected wastewater by Vibrio fischeri bioassay after a chlorination–dechlorination process, J. Hazard. Mater. 172 (2009) 166–171.

[3] S.E. Lopus, P.A.M. Bachand, A.C. Heyvaert, I. Werner, S.J. Teh, J.E. Reuter, Potential toxicity concerns from chemical coagulation treatment of stormwater in the Tahoe basin, California, USA, Ecotoxicol. Environ. Saf. 72 (2009) 1933–1941.

[4] M.D. Hernando, A.R. Fernández-Alba, R. Tauler, D. Barceló, Toxicity assays applied to wastewater treatment, Talanta 65 (2005) 358–366.

[5] E. Mendonça, A. Picado, S.M. Paixão, L. Silva, M.A. Cunha, S. Leitão, I. Moura, C. Cortez, F. Brito, Ecotoxicity tests in the environmental analysis of wastewater treatment plants: case study in Portugal, J. Hazard. Mater. 163 (2009) 665–670.

[6] A. Katsoyiannis, C. Samara, Ecotoxicological evaluation of the wastewater treatment process of the sewage treatment plant of Thessaloniki, Greece, J. Hazard. Mater. 141 (2007) 614–621.

[7] S. Parvez, C. Venkataraman, S. Mukherji, A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals, Environ. Int. 32 (2006) 265–268.

[8] X.W. Zhu, S.S. Liu, H.L. Ge, Y. Liu, Comparison between the short-term and the long-term toxicity of six triazine herbicides on photobacteria Q67, Water Res. 43 (2009) 1731–1739.

[9] M. Farré, D. Barceló, Toxicity testing of wastewater and sewage sludge by biosensors, bioassays and chemical analysis, Trends Anal. Chem. 22 (2003) 299–310.

[10] M. Ma, Z. Tong, Z. Wang, W. Zhu, Acute toxicity bioassay using the freshwater luminescent bacterium Vibrio-qinghaiensis sp.Nov.-Q67, Bull. Environ. Contam. Toxicol. 62 (3) (1999) 247–253.

[11] W.X. Liu, R.M. Coveney, J.L. Chen, Environmental quality assessment on a river system polluted by mining activities, Appl. Geochem. 18 (2003) 749–764.

[12] S.S. Liu, X.Q. Song, H.L. Liu, Y.H. Zhang, J. Zhang, Combined photobacterium toxicity of herbicide mixtures containing one insecticide, Chemosphere 75 (2009) 381–388.

[13] X. Zhou, W. Sang, S. Liu, Y. Zhang, H. Ge, Modeling and prediction for the acute toxicity of pesticide mixtures to the freshwater luminescent bacterium Vibrio qinghaiensis sp.-Q67, J. Environ. Sci. 22 (2010) 433–440.

[14] T.T. Liao, Y.L. Shi, J.W. Jia, L. Wang, Sensitivity of different cytotoxic responses of vero cells exposed to organic chemical pollutants and their reliability in the bio-toxicity test of trace chemical pollutants, Biomed. Environ. Sci. 23 (2010) 219–229.

[15] I. Mantis, D. Voutsa, C. Samara, Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods, Ecotoxicol. Environ. Saf. 62 (2005) 397–407.

[16] M.D. Hernando, O. Malato, M. Farré, A.R. Fernandez-Alba, D. Barceló, Application of ring study: water toxicity determinations by bioluminescence assay with Vibrio fischeri, Talanta 69 (2006) 370–376.

[17] S. Ore, J. Todorovic, H. Ecke, K. Grennberg, S. Lidelöw, A. Lagerkvist, Toxicity of leachate from bottom ash in a road construction, Waste Manage. 27 (2007) 1626–1637.

[18] M. Macova, B.I. Escher, J. Reungoat, S. Carswell, K.L. Chue, J. Keller, J.F. Mueller, Monitoring the biological activity of micropollutants during advanced wastewater treatment with ozonation and activated carbon filtration, Water Res. 44 (2010) 477–492.

[19] J.M. Zheng, S.X. Zhao, W.J. Zhu, D.C. Yang, F.X. Jing, Environmental factors on the growth and glowing of Vibrio-qinghaiensis sp.-Q67, J. East China Normal Univ. (Nat. Sci.) 18 (1999) 98–102 (in Chinese).

[20] M.I. Farré, M.J. García, L. Tirapu, A. Ginebreda, D. Barceló, Wastewater toxicity screening of non-ionic surfactants by Toxalert® and Microtox® bioluminescence inhibition assays, Anal. Chim. Acta 427 (2001) 181–189.

[21] State Environmental Protection Administration, Detection of water and wastewater analysis, Fourth Ed., China Environmental Science Press, Beijing, 2002 (in Chinese).

[22] J. Zhang, S.S. Liu, H.L. Liu, Effect of ionic liquid on the toxicity of pesticide to Vibrio-qinghaiensis sp.-Q67, J. Hazard. Mater. 170 (2009) 920–927.

[23] S. Ren, P.D. Frymier, Kinetics of the toxicity of metals to luminescent bacteria, Adv. Environ. Res. 7 (2003) 537–547.

[24] J.Y. Zhao, Y.M. Li, Q. Zhou, Toxicity reduction of synthetic muncipal wastewater treated by anerobic, anoxic or aerobic reactors, Environ. Sci. 27 (2006) 2314–2317 (in Chinese).

[25] A. Gerhardt, L. Janssens de Bisthoven, Z. Mo, C. Wang, M. Yang, Z. Wang, Short-term responses of Oryzias latipes (Pisces: Adrianichthyidae) and Macrobrachium nipponense (Crustacea: Palaemonidae) to municipal and pharmaceutical waste water in Beijing, China: survival, behaviour, biochemical biomarkers, Chemosphere 47 (2002) 35–47.

[26] D.T. Sponza, P. Demirden, Relationships between chemical oxygen demand (COD) components and toxicity in a sequential anaerobic baffled reactor/aerobic completely stirred reactor system treating Kemicetine, J. Hazard. Mater. 176 (2010) 64–75.

[27] L.S. Wang, D.B. Wei, J. Wei, H.Y. Hu, Screening and estimating of toxicity formation with photobacterium bioassay during chlorine disinfection of wastewater, J. Hazard. Mater. 141 (2007) 289–294.

[28] X.C. Wang, P.K. Jin, H.M. Zhao, L.B. Meng, Classification of contaminants and treatability evaluation of domestic wastewater, Front. Environ. Sci. Eng. China 1 (2007) 57–62.

[29] M. Petala, V. Tsiridis, P. Samaras, A. Zouboulis, G.P. Sakellaropoulos, Wastewater reclamation by advanced treatment of secondary effluents, Desalination 195 (2006) 109–118.

[30] S. Bhattacharjee, S. Datta, C. Bhattacharjee, Improvement of wastewater quality parameters by sedimentation followed by tertiary treatments, Desalination 212 (2007) 92–102.

[31] I. Vázquez, J. Rodríguez-Iglesias, E. Marañón, L. Castrillón, M. Álvarez, Removal of residual phenols from coke wastewater by adsorption, J. Hazard. Mater. 147 (2007) 395–400.

[32] M. Petala, L. Kokokiris, P. Samaras, A. Papadopoulos, A. Zouboulis, Toxicological and ecotoxic impact of secondary and tertiary treated sewage effluents, Water Res. 42 (2009) 5062–5074.