SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 33, No. 9, pp. 1967–1975, 2014

Printed in the USA

MULTISPECIES ACUTE TOXICITY EVALUATION OF WASTEWATERS FROM DIFFERENT TREATMENT STAGES IN A COKING WASTEWATER-TREATMENT PLANT

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(Submitted 27 February 2014; Returned for Revision 13 April 2014; Accepted 15 May 2014)

Abstract: Coking wastewater contributes approximately 5% of the total discharge volume of industrial wastewaters every year in China. The toxicity of coking wastewater to aquatic organisms is still unknown. The authors evaluated the toxicity of wastewater from different treatment stages in a coking wastewater treatment plant, South China, using 5 test species belonging to different trophic levels: luminous bacteria, green alga, a crustacean, duckweed, and zebrafish embryos. The raw influent displayed the highest toxicity to the test species, with toxic units ranging from 16.2 to 1176. The toxicity in the wastewater was then gradually removed by sequential primary treatment, biological fluidized-bed treatment, and secondary clarifier treatment. The toxic unit of the final effluent was reduced to 2.26 for the green alga (*Pseudokirchneriella subcapitata*) and to 0 for the other 4 organisms. Quantitative analysis of metals and polycyclic aromatic hydrocarbons (PAHs) and qualitative scanning by gas chromatography–mass spectrometry showed the presence of a variety of pollutants in the coking wastewaters. Multivariate statistical analysis revealed that the toxicity in the coking wastewater was correlated to the chemical oxygen demand, total nitrogen, ammonia nitrogen, volatile phenols, sulfide, metals (Cr, As, Sb, Hg, Pb, and Ni), and ΣPAHs. Based on the results, it is required to set a safety emission limit value for the discharge of coking wastewater to protect aquatic organisms in the receiving water bodies. *Environ Toxicol Chem* 2014;33:1967–1975. © 2014 SETAC

Keywords: Coking wastewater Aquatic toxicity Toxic unit Green alga Toxicant

INTRODUCTION

Coking wastewater is generated from coking plant production processes including coal carbonization, coal gas purification, and chemical product refining. Chemical oxygen demand values in coking wastewater are often found up to several thousands of milligrams per liter [1,2]. Coking wastewater contains a cocktail of toxic and hazardous substances including metals, phenols, cyanides, polycyclic aromatic compounds, and nitrogen-, oxygen-, and sulfur-containing heterocyclic compounds [1,2]. These contaminants could have negative impacts on aquatic organisms in the receiving environment [3].

It is reported that crude coking wastewater can inhibit the growth of maize (*Zea mays* L.) and affect embryo development in maize seeds [4,5]. The inhibitory effect of the coking wastewater on the development of maize embryos at different treatment stages (anaerobic, anaerobic/aerobic, anaerobic/aerobic/photodegradation, anaerobic/aerobic/ozone oxidation treatment) decreased gradually with the proceeding of treatment stages, which indicated that toxic chemicals in the coking wastewater can be effectively removed or partly removed by the biological treatment [5]. The European Commission and the US Environmental Protection Agency (USEPA) recommend the use of multiple test species belonging to different trophic levels in the evaluation of wastewater toxicity and identification of potential toxicants [6,7]. Fang et al. [8] used luminescent bacteria, duckweed, green alga, crustacean, and zebrafish to evaluate the toxicity of various effluents from textile and dyeing plants, pulp and paper mills, fine chemical factories, and municipal wastewater-treatment plants. Recently, Zhu et al. [9] evaluated the toxicity of coking wastewaters from different treatment units using Japanese medaka (*Oryzias latipes*) embryos. However, studies on the toxicity and toxicity changes of wastewaters from different treatment stages of coking wastewater-treatment plants based on multiple test species are very limited.

The production of hard coke in China was estimated to be 23.6 million tons in the year 2007, which accounted for approximately 60% of the total global production [10]. The discharge amounts of coking wastewater in China in terms of chemical oxygen demand, NH_3-N (ammonia nitrogen), and oils are 125 kt, 19 kt, and 2 kt, respectively, which account for 2.5%, 4.6%, and 8.5% of the total national industrial discharges [10]. Recently, the Chinese government has adopted a control measure for industrial wastewaters based on the total pollutant discharge in each industrial sector. Advanced biological and physicochemical treatment technologies have increasingly been applied in the treatment of coking wastewater in China to meet the requirements of the national discharge standards. Zhang et al. [1] reported that most of the chemical oxygen demand in coking wastewater was removed by the biological fluidized-bed treatment in the Songshan coking wastewater-treatment plant in Guangdong Province, south China. But the toxicity removal efficiency by this treatment process remains unknown. Hence, it is essential to understand the toxicity of wastewaters from different treatment units in a coking wastewater-treatment plant and to identify the toxicants in the coking wastewaters of different treatment stages.

The objective of the present study was to evaluate the acute toxicity of wastewaters from different treatment stages in a coking wastewater-treatment plant in South China using multiple test species belonging to different trophic levels. The organisms used were luminous bacteria (*Escherichia coli*

All Supplemental Data may be found in the online version of this article.
* Address correspondence to cechwei@scut.edu.cn.
Published online 9 July 2014 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2638

HB101 pUCD607), duckweed (*Lemna minor*), green alga (*Pseudokirchneriella subcapitata*), a crustacean (*Daphnia magna*), and zebrafish (*Danio rerio*) embryos. Toxicity removal efficiency and effluent emission limit were then assessed for the coking wastewaters based on the results of the toxicity tests. The wastewaters were also quantatively analyzed for metals and polycyclic aromatic hydrocarbons (PAHs) and qualitatively scanned for potential organic pollutants by gas chromatography–mass spectrometry under electron impact mode (GC-EI-MS). Multivariate statistical analysis was also used to analyze the linkage between the toxicity reduction and removal of toxicants in wastewaters.

MATERIALS AND METHODS

Coking wastewater-treatment plant and sampling scheme

The wastewater-treatment plant for the Songshan coking plant in Shaoguan city, Guangdong Province, South China, was selected in the present study because it has typical wastewater-treatment facilities for coking wastewater in China. The wastewater-treatment plant receives the effluents from ammonia stilling and cleaning plants, with a treatment capacity of 2000 m^3/d. The treatment technologies used in the wastewater-treatment plant include primary treatment, biological treatment, and coagulation treatment. In the primary treatment, a flotation-degreasing tank coupled with an equalization basin is used to separate particles and tar from the raw influent. The primary effluent is then subjected to the biological treatment, which is composed of an anoxic–oxic–hydrolytic–oxic system coupled with a biological fluidized bed. In the coagulation treatment, the biological effluent is mixed with polyacrylamide and polyferric sulfate solution for 4 min. Then, the mixed effluent enters into the secondary clarifier with its hydraulic retention time of 4 h. After these treatments, the final effluent is discharged into the receiving environment.

A schematic diagram for the coking wastewater-treatment plant with the sampling locations is displayed in Figure 1. A sampling campaign was carried out in June 2012. Using flow proportional samplers (cooled at 4 °C) with a sampling interval of 2 h, 24-h composite water samples of raw influent, primary effluent, biological effluent (anaerobic effluent, the first aerobic effluent, hydrolytic effluent, and the second aerobic effluent), and final effluent were collected. The collected wastewater samples were transported in coolers to the laboratory and stored in a cold room at 4 °C for toxicity tests and chemical analysis.

Toxicity testing

D. magna acute lethality test. The 48-h acute lethality test for *D. magna* was conducted by following the guidance of standard methods from Environment Canada [11]. The test procedure was described in our previous study [8]. At first, a preliminary toxicity test was conducted to set suitable dilution factors for the wastewaters from different treatment stages. Wastewater was 10-fold diluted with moderately hard water in series from the original concentration to 10^4 dilution before conducting toxicity testing. Based on the preliminary toxicity testing results, raw influent was diluted 100 times with moderately hard water, and primary effluent and anaerobic effluent were diluted 10 times with moderately hard water; the others were not diluted for the following toxicity testing. Then, each sample was 2-fold diluted using moderately hard water in 5 series. The moderately hard water is prepared by dissolving the salts of $NaHCO_3$ (96 mg), $CaSO_4 \times 2H_2O$ (60 mg), $MgSO_4$ (60 mg), and KCl (4 mg) in deionized water on a per-liter basis. Copper sulfate solution with an initial concentration of 100 mg/L Cu^{2+} was used as a positive control. Four replicates of 5 neonates (24-h-old) per vessel were used for each concentration and the control. Exposure experiments were conducted in 50-mL glass beakers containing 20 mL of test solution. Mortality, defined as lack of movement after gentle prodding, was recorded at 24-h and 48-h intervals.

L. minor growth inhibition test. The duckweed (*L. minor*) growth inhibition test was carried out in accordance with the methods recommended by the Organisation for Economic Co-operation and Development (OECD) [12]. Similar preliminary toxicity testing was conducted as described for the *D. magna* acute lethality test. Based on the results of preliminary toxicity, raw influent was diluted 100 times with the Swedish standard (SIS) *L. minor* growth medium, and primary effluent, anaerobic effluent, first aerobic effluent, and hydrolytic effluent were diluted 10 times with SIS medium; the others were not diluted. Then, each sample was 2-fold diluted using SIS medium in 5 series. A 10-mL aliquot of the diluted test samples for each concentration was transferred to 6 20-mL beakers. Among them, 4 replicates for the toxicity test and 2 replicates for pH determination after the test were terminated. A 3-frond duckweed was transferred into each beaker, including pH

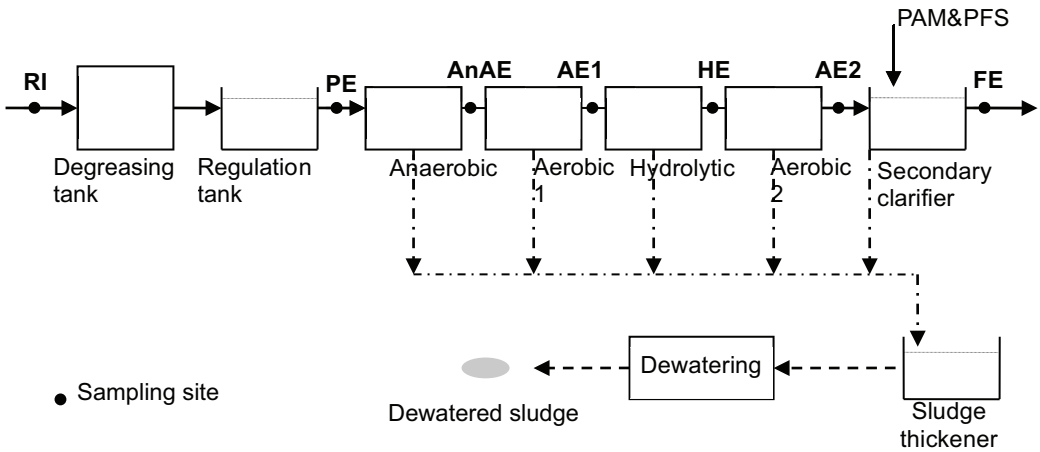

Figure 1. Flowchart of coking wastewater-treatment plant and sampling scheme. RI = raw influent; PE = primary effluent; AnAE = anaerobic effluent; AE1 = first aerobic effluent; HE = hydrolytic effluent; AE2 = second aerobic effluent; FE = final effluent; PAM&PFS = mean polyacrylamide and polyferric sulfate solution, respectively.

beakers. The beakers were randomly placed into an incubation cabinet at 24 ± 2 °C with static incubation. Also, the incubation was maintained on a continuous fluorescent light cycle (cool white light at 60–80 mmol photons/s/m^2). The number of fronds of each replicate beaker was recorded on day 2, day 5, and day 7.

D. rerio embryo acute toxicity test. The 96-h acute toxicity test using *D. rerio* embryos was modified from the methods reported by Nagel [13] and the OECD [14]. Based on the preliminary toxicity tests, raw influent, primary effluent, and anaerobic effluent were 10-times diluted with standard dilution water before the following toxicity testing. Each sample was further 2-fold diluted with the standard dilution water to obtain the concentration series of 100%, 50%, 25%, 12.5%, and 6.25%. The standard dilution waters were prepared according to International Organization for Standardization (ISO) 7346/3 (294.0 mg/L $CaCl_2 \times 2H_2O$, 123.3 mg/L $MgSO_4 \times 7H_2O$, 63.0 mg/L $NaHCO_3$, and 5.5 mg/L KCl, which was diluted 5 times before being used), and pH was adjusted to 7.8. Dilution waters were stored at 26 ± 1 °C for later use. Embryo acute lethality testing was carried out on a 24-well flat-bottom plate. Two milliliters of each diluted wastewater was transferred to each of 20 wells for replicates of each dilution in the plate, and the remaining 4 wells were used for blank controls. One newly fertilized embryo (<2 h) was placed into each well. The plates were then incubated at 26 ± 1 °C and at a light density of 700 lux with a 14:10-h light:dark cycle. At 96 h postfertilization, lethal, sublethal, and teratogenic end points were recorded using a dissecting microscope. The chemical 3,4-dichloroaniline with a concentration of 3.7 mg/L was used as a positive control. For a test to be classed as valid, the lethality rate for the positive control should be more than 80% and the lethality rate for the blank control should be less than 10%.

Toxicity to zebrafish embryos was expressed as the fish teratogenicity index. Standards used for scoring are listed in Supplemental Data, Table S1. The fish teratogenicity index is calculated based on the equation

$$\mathrm{FTI} = \frac{E_{\mathrm{other}} \times 1 + E_{\mathrm{sublethal}} \times 2 + E_{\mathrm{lethal}} \times 3}{N}$$

where FTI is the fish teratogenicity index, N is the number of embryos for each replicate, E_{other} is the worst other effect, $E_{sublethal}$ is the worst sublethal effect, and E_{lethal} is the worst lethal effect.

Green alga growth inhibition test. The green alga (*P. subcapitata*) 72-h growth inhibition test was conducted according to the OECD method [15]. Similar preliminary toxicity tests were conducted as for the *Daphnia magna* acute lethality test to set suitable dilution factors for each wastewater. In brief, raw influent and primary effluent were 1000 times diluted; anaerobic effluent, the first aerobic effluent, and hydrolytic effluent were 100 times diluted; and the second aerobic effluent was 10 times diluted with the culture medium before toxicity testing. Then, the test solutions for each sample were prepared by 2-fold dilution using the culture medium in 5 series. From each prepared test solution, 50 mL was transferred into 3 flasks of each concentration. Concentrated green algal cell suspension was added into each flask to obtain the initial cell concentration of 1×10^4 cells/mL. The flasks were placed randomly in an incubator and incubated at 24 ± 1 °C under continuous illumination (4000 lux, cool white fluorescence) for 72 h. The final cell yield after the 72-h exposure was determined by measuring the optical density of the cells at a wavelength of 430 nm using a multifunctional microplate reader (FLUO star Omega; BMG LABTECH), and then the biomass was calculated using a linear relationship. Percentage inhibition of algal growth was calculated and compared with the control.

Toxicity to the green alga was measured based on cell yield. Percentage inhibition of algal growth was calculated using the equation

$$I = \frac{Rc - R}{Rc} \times 100$$

where I is the percentage inhibition of algal growth for each test concentration replicate, R_C is the mean cell yield for the control, and R is the cell yield for each test concentration replicate.

Luminous bacteria toxicity test. The luminous bacteria toxicity test was performed in accordance with the methods described by Preston et al. [16]. The test organism used was *E. coli* HB101 pUCD607, which had been genetically modified to contain the plasmid pUCD607 with the lux CDABE genes from *Vibrio fischeri* encoded under the control of the tetracycline resistance promoter.

Strains for the test were prepared by growing cells in Luria-Bertani broth containing 30 mg/L kanamycin at 25 °C and shaking for about 18 h until late log phase. After the late log phase was reached, the optical density at 550 nm (= 1) and light output (1.4×10^6 relative light units) of the culture medium were measured. The culture was stored at 4 °C for later use within 2 d to 3 d. When required, 30 mL of the culture was centrifuged at the speed of 2000 *g* at 4 °C for 40 min, and the supernatant was discarded. Prior to the test, the strains were resuscitated for 10 min in 10 mL of 0.1 M KCl at 25 °C.

The effluent was 2-fold diluted by 0.1 M KCl in 12 series. Then, 200 μL of each test solution was transferred into a white 96-well microplate. The bioassay was carried out in triplicate for each concentration, blank control, and Zn reference test. A blank control and the Zn standard curve were also included in each microplate. Then, 50 μL of resuscitated strains was transferred to a microplate filled with test solutions. The bioluminescence, after being exposed for 5 min and 15 min, was measured using a BMG microplate reader, and the toxicity response was expressed as a reduction percentage of relative light units, which was calculated as

$$R = \frac{Lc - L}{Lc} \times 100$$

where R is a reduction percentage of the relative light units using *E. coli* HB101 pUCD607 for each test concentration, L_C is the mean relative light units for the control, and L is the relative light units for each test concentration.

Chemical analysis

Basic physicochemical parameters of the coking wastewaters, including electrical conductivity and pH, were measured on site immediately after collection (Table 1). Water-quality parameters including chemical oxygen demand, NH_3-N, total phosphorus, total nitrogen, volatile phenols, cyanide, sulfide, and oil were determined after the samples were transferred to the laboratory (Table 1). Concentrations of selected elements (Al, Cr, Fe, Mn, Ni, Cu, Zn, As, Cd, Pb, Sn, Sb, and Hg) were measured with the following procedure: 20 mL of each wastewater sample was digested with 30 μL of 1:1 (v/v) nitrate:water solution for 48 h and filtered through a 0.22-μm water-phase membrane filter into a polypropylene plastic tube. Then, the target elements in each sample were determined by inductively coupled plasma–mass spectrometry (ICP-MS).

Table 1. Physicochemical characteristics of the coking wastewater at different treatment stages

Wastewater	pH	Conductivity (mS/cm)	COD (mg/L)	NH_3-N (mg/L)	TP (mg/L)	TN (mg/L)	Volatile phenols (mg/L)	Cyanide (mg/L)	Sulfide (mg/L)	Oil (mg/L)
RI	10.1	6.19	2141	180	0.09	313	282	52.3	28.5	149
PE	9.31	7.19	1530	142	0.17	232	212	22.4	5.05	—[a]
AnAE	9.38	6.55	1398	186	6.67	258	195	19.4	20.0	—[a]
AE1	7.92	5.47	351	101	1.85	166	0.06	—[a]	—[a]	—[a]
HE	8.01	5.46	236	88.9	4.15	180	—[a]	—[a]	—[a]	—[a]
AE2	7.17	5.05	197	11.1	2.62	200	—[a]	3.73	—[a]	—[a]
FE	6.98	4.18	69.3	6.84	0.09	168	0.02	0.23	0.04	1.07

[a]Not available.
COD = chemical oxygen demand; NH_3-N = ammoniac nitrogen; TP = total phosphorus; TN = total nitrogen; RI = raw influent; PE = primary effluent; AnAE = anaerobic effluent; AE1 = first aerobic effluent; HE = hydrolytic effluent; AE2 = second aerobic effluent; FE = final effluent.

Organic contaminants in wastewater samples were enriched by a solid-phase extraction method. Waters Oasis HLB cartridges (6cc, 500 mg sorbents) were conditioned using 10 mL methanol, followed by 10 mL Milli-Q water. Then, 1 L of each wastewater sample was passed through the HLB cartridge at a speed of 3 mL/min to 5 mL/min. After the samples were loaded, 10 mL of Milli-Q water was used to rinse each cartridge. The HLB cartridges were then eluted by 10 mL dichloromethane and 10 mL methanol in sequence. The dichloromethane and methanol eluates were combined and dried under a gentle nitrogen stream, and the final extract was reconstituted in 1 mL dichloromethane for later analysis. Qualitative scanning of potential toxicants was carried out using GC-EI-MS (an Agilent 5975B GC coupled with an Agilent 5975B MS with an electron ionization source). Potential organic contaminants were identified by NIST MS Search software (version 2.0; National Institute of Standards and Technology). Quantitative analysis of PAHs was also conducted according to the method described by Zhang et al. [1].

Data analysis

The median lethal concentrations (LC50) of effluents for *D. magna* and *D. rerio* embryos were calculated by probit analysis with their 95% confidence intervals using the software SPSS 16.0. Median effect concentrations (EC50) of industrial wastewaters for luminous bacteria, duckweed, and green alga were calculated using the EC50 calculator program developed by the Commonwealth Scientific and Industrial Research Organisation [17]. Toxic units were calculated by dividing the 100% by the LC50 or by the EC50. If there was no significant difference for the lethal rate or inhibitory rate between the sample with 100% concentration and the blank control, the toxic unit of the sample could be regarded as 0.

Multivariate statistical analysis was applied to assess the relationship between toxicity reduction and pollutants in coking wastewater. The data sets were sorted into classes of species data and environmental factors, where the toxic units of 5 organisms were species data, and the water-quality parameters (pH, conductivity, chemical oxygen demand, NH_3-N, total phosphorus, and total nitrogen), volatile phenols, cyanide, sulfide, metals, and total PAHs (ΣPAHs) were environmental factors. Prior to multivariate analysis, the data sets were $\log(x + 1)$–transformed. A detrended correspondence analysis was carried out to detect the length of the ordination gradient along the first axis. If the value is smaller than 3, a redundancy analysis model should be selected to best fit the data set. Monte Carlo permutation tests with 499 permutations were used to give the variability and statistical significance of each variable. The ordination plots were generated based on the toxic units and environmental parameters.

Quality assurance and quality control

Quality-control procedures were applied in the bioassays and chemical analyses. As for the bioassays, with each set of samples analyzed, a blank control and reference substances were included to test the stability of test species and the experimental conditions. As for the chemical analyses, a solvent blank, a standard, and a procedure blank were run in sequence with each set of samples analyzed to check for background contamination, peak identification, and quantification.

RESULTS

Toxicity of coking wastewaters

The acute toxicity of wastewaters from different treatment stages of the Songshan coking wastewater-treatment plant for 5 species—crustacean (*D. magna*), duckweed (*L. minor*), zebrafish (*D. rerio*) embryos, green alga (*P. subcapitata*), and luminous bacteria (*E. coli* HB101 pUCD607)—are displayed in Table 2. The toxic units for the 5 species ranged from 16.2 to 1176 in raw influent, whereas the toxic units decreased to single-digit numbers or showed no acute toxicity in the final effluent. It can also be seen that the acute toxicity of coking wastewaters varied with different test species. Green alga (*P. subcapitata*) was the most sensitive organism for the toxicity of raw influent, primary effluent, and biological effluents (i.e., anaerobic effluent, first aerobic effluent, hydrolytic effluent, and the second aerobic effluent).

Toxicity to D. magna. As shown in Table 2, the highest toxic unit (up to 550) was found in raw influent based on the *Daphnia magna* 48-h acute immobilization test. The toxicity of raw influent was removed stepwise when the coking wastewater went through the primary treatment, biological treatment, and secondary clarification treatment in sequence. The toxic unit of wastewater decreased dramatically after the primary treatment and anaerobic biological treatment (Figure 1), which contributed 60.7% and 36.7% of the reduction from the initial raw influent toxicity. The final effluent had no significant toxicity to *D. magna* with the 24-h or 48-h acute immobilization test. Thus, the acute toxicity of the raw coking wastewater to *D. magna* was completely removed with the present coking wastewater-treatment processes.

Toxicity to L. minor. As shown in Table 2, the toxicity of raw influent was reduced gradually with the sequential wastewater-treatment processes. The toxic units for raw influent were 41.4 and 187 with the growth rate inhibition and biomass inhibition

Table 2. Toxic units of the coking wastewater at different treatment stages tested by the 5 species

	Crustacean[a]		Duckweed[b]		Zebrafish embryo[c]	Green alga[d]	Luminous bacteria[e]	
Wastewater	24-h lethality	48-h lethality	Growth rate	Biomass	96-h FTI	Biomass	5-min inhibition	15-min inhibition
RI	185	550	41.4	187	62.9	1176	56.2	16.2
PE	45.1	216	9.79	15.4	68.5	1087	65.9	19.8
AnAE	10.0	14.4	10.4	16.9	24.4	833	17.7	10.3
AE1	1.82	1.47	7.91	17.9	3.93	357	6.07	5.12
HE	1.04	1.07	3.54	7.36	3.22	297	3.92	3.41
AE2	0	0	2.61	5.33	1.36	43.8	2.40	1.93
FE	0	0	0	0	0	2.26	0	0

[a]Acute lethality test (24 h and 48 h) for *Daphnia magna* with lethality as effect end point.
[b]Growth inhibition test (72 h) for *Lemna minor* with growth rate and biomass as effect end points.
[c]Acute toxicity test (96 h) for *Danio rerio* embryos with fish teratogenicity index (FTI) as effect end point.
[d]Growth inhibition test (72 h) for *Pseudokirchneriella subcapitata* with biomass as effect end point.
[e]Luminous inhibition test (5 min and 15 min) for *Escherichia coli* HB101 pUCD607 with luminous inhibition as effect point.
RI = raw influent; PE = primary effluent; AnAE = anaerobic effluent; AE1 = first aerobic effluent; HE = hydrolytic effluent; AE2 = second aerobic effluent; FE = final effluent.

effects as the duckweed test end points, respectively, whereas the toxic unit for final effluent decreased to 0. Thus, the acute toxicity for *L. minor* was completely removed from the coking wastewater. The primary treatment process of the tar separation from raw influent by air flotation brought a significant reduction (76.4% and 91.7% for growth rate inhibition and biomass inhibition effects, respectively) of the toxicity. Then, the biological treatment of the anoxic–oxic–hydrolytic–oxic system removed the majority of the toxicity from primary effluent. However, the final effluent was found to enhance the growth of *L. minor*, which was probably a result of the higher nutrient contents in final effluent than the control after the removal of toxic contaminants.

Toxicity to D. rerio embryos. The toxic units of coking wastewaters for *Danio rerio* embryos reached 62.9 in raw influent and 68.5 in primary effluent, which were much higher than those for the other effluents (Table 2). The toxicity in primary effluent was removed by the biological treatment processes and the final clarifying–coagulation process. Biological treatment processes (anoxic–oxic–hydrolytic–oxic system) obviously removed the large proportion (90.3%) of toxicity from primary effluent, especially by the anaerobic treatment process and the first aerobic treatment process. The toxic unit in final effluent was less than 1 because the LC50 value was higher than 100%, suggesting complete removal of acute toxicity for zebrafish embryos with the present treatment processes.

Toxicity to green alga. Table 2 also displays the toxicity to green alga for the wastewaters from different treatment stages of the coking wastewater-treatment plant. The toxic unit for raw influent reached 1176, whereas the toxic unit for final effluent was reduced to 2.26. Thus, more than 98% of the acute toxicity in raw influent was removed by the treatment processes used in the coking wastewater-treatment plant. Similar to the other test organisms, a stepwise removal of toxicity was found in the effluents from different treatment stages. The first and second aerobic treatment processes contributed 40.5% and 20.5% of the total toxic unit removal, respectively. This result suggested that green alga was the most sensitive test organism to the toxicity of coking wastewater.

Toxicity to luminous bacteria. Toxic substances in wastewater can inhibit the luminous effect of the recombinant luminous bacteria *E. coli* HB101 pUCD607; thus, the strain is used to evaluate the toxicity of wastewater [8]. In the present study, the toxic unit was calculated based on the inhibition effects of luminous times of 5 min and 15 min. The toxic units of the influent and effluents from different treatment stages in the coking wastewater-treatment plant are listed in Table 2. The maximum toxic unit was 65.9 in primary effluent, which was slightly higher than the toxicity of raw influent. It can be seen that the biological treatment processes of anaerobic, aerobic, and hydrolytic processes played an important role in toxicity removal. The toxic unit of final effluent was 0 since the EC50 value was higher than 100%, suggesting complete removal of toxicity of coking wastewater using the present treatment processes.

Characterization of chemical contaminants in coking wastewaters

Basic properties of the coking wastewaters are listed in Table 1. In the raw influent, the chemical oxygen demand and NH_3-N were 2141 mg/L and 92.5 mg/L, respectively, and other parameters such as volatile phenols, cyanide, sulfide, and oil were at levels of dozens to hundreds of milligram per liter. The above wastewater-quality parameters were found to decrease gradually after the primary treatment (degreasing and regulating), biological treatment, and secondary clarifying treatment processes. The chemical oxygen demand and NH_3-N levels in the final effluent decreased to 69.3 mg/L and 6.84 mg/L, respectively. In general, based on the measured wastewater parameters, the final effluents meet the required national standards for indirect discharge of coking wastewaters [18].

The dissolved concentrations of 13 elements (Al, Cr, Fe, Mn, Ni, Cu, Zn, As, Cd, Pb, Sn, Sb, and Hg) in 7 wastewater samples from different treatment stages are given in Table 3. The concentrations of Fe, Al, Cr, Ni, and As were found to be greater than 10 μg/L in the raw influent and effluents from different treatment stages. Most of the metals were partially removed by the wastewater-treatment processes. However, the concentrations of Zn in hydrolytic effluent, second aerobic effluent, and final effluent were 15 to 30 times higher than those in raw influent, primary effluent, and anaerobic effluent. No metal in the final effluent was higher than the Chinese water-quality standards for class I (Table 3). This indicated that the metals in the final effluent may not cause toxic effects to organisms when discharged into the aquatic environment.

Qualitative GC-MS scan facilitated the identification of some organic contaminants in the wastewater samples from different treatment stages. The total ion chromatograms of the 7 samples analyzed are shown in Figure 2. The peaks with relatively high

Table 3. Concentrations of dissolved metals in different treatment stages of the coking-wastewater treatment plant

	Metals (μg/L)												
Wastewater	Al	Cr	Fe	Mn	Ni	Cu	Zn	As	Cd	Pb	Sn	Sb	Hg
RI	62.6	60.2	1107	2.50	24.3	<LOD	1.96	12.4	0.06	2.93	<LOD	1.28	0.31
PE	178	35.1	2585	2.03	5.35	<LOD	1.59	3.98	0.01	5.11	0.30	0.18	0.07
AnAE	28.2	36.4	2621	0.34	17.3	<LOD	0.41	4.94	<LOD	0.57	<LOD	0.14	0.11
AE1	28.5	21.1	1787	3.70	5.43	<LOD	6.02	2.78	0.01	0.81	0.19	0.15	0.02
HE	53.0	19.0	810	2.45	6.84	<LOD	40.5	3.19	0.01	0.78	0.21	0.16	0.03
AE2	64.8	14.77	327	4.75	4.82	<LOD	56.2	3.45	0.03	0.75	<LOD	0.17	0.04
FE	24.1	8.69	19.4	2.10	8.86	<LOD	31.7	0.24	0.04	0.49	<LOD	0.05	0.04
WQS[a]	≤200	≤10	≤300	≤100	≤20	≤10	≤50	≤50	≤1	≤10	—	≤5	≤0.05
LOD	0.01	0.01	0.01	0.01	0.01	0.57	0.01	0.01	0.002	0.01	0.14	0.01	0.01

[a]China's water-quality standard for class I (GB3838-2002).
RI = raw influent; PE = primary effluent; AnAE = anaerobic effluent; AE1 = first aerobic effluent; HE = hydrolytic effluent; AE2 = second aerobic effluent; FE = final effluent; WQS = water-quality standard; LOD = method limit of detection.

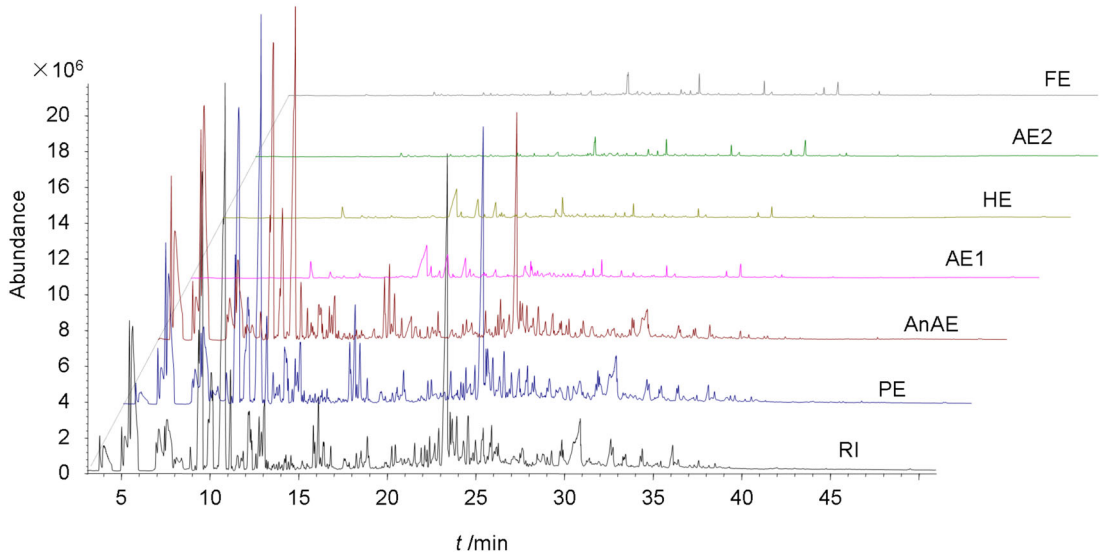

Figure 2. Scanned total ion chromatographs of water samples from different Songshan coking wastewater-treatment stages by gas chromatography–mass spectrometry. RI = raw influent; PE = primary effluent; AnAE = anaerobic effluent; AE1 = first aerobic effluent; HE = hydrolytic effluent; AE2 = second aerobic effluent; FE = final effluent. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

abundances were identified as the potential contaminants. The classes of identified organic compounds are summarized in Table 4. Detailed identification of organic compounds in the wastewater samples are provided in Supplemental Data, Table S2. Various organic compounds, such as phenol, quinolone, pyridine, indole, furan, benzylamine, anthracene, acridine, and pyrene, were detected in the raw influent and effluents.

Raw influent, primary effluent, and anaerobic effluent displayed similar patterns of their total ion chromatographs (Figure 2), suggesting that the organic compounds in these wastewaters were similar and that the removal of toxicity was achieved only by reduction in the concentrations of these compounds. The total ion chromatographs for first aerobic effluent and hydrolytic effluent displayed similar patterns, but the peaks of first aerobic effluent and hydrolytic effluent were

Table 4. Identified organic pollutants of wastewater eluates from different treatment stages in Songshan coking wastewater-treatment plants by gas chromatography–mass spectrometry scanning

Wastewater	Chemical class
RI, PE, and AnAE	Acenaphthenone, acetamide, acridine, acridone, alkane, anthracene, anthracenecarbonitrile, azafluorene, benzimidazole, benzofuran, benzylamine, carbazole, carboline, dibenzofuranol, indole, isoindole, isoquinoline, naphthalenamine, naphthalenol, norharmane, phenanthridine, phenanthrol, phenol, pyrene, quinoline, quinolinol
AE1 and HE	Acridine, alkane, benzimidazole, cyanodiphenyl, indazole, indole, isocyanate, isoquinoline, naphthalenol, naphthonitrile, pyridine, quinoline
AE2 and FE	Alkane, aminoindole, anisole, benzonitrile, indol, isoquinoline, naphthol, phenanthridine, pyridine, quinolinamine, quinoline, sulfone

RI = raw influent; PE = primary effluent; AnAE = anaerobic effluent; AE1 = first aerobic effluent; HE = hydrolytic effluent; AE2 = second aerobic effluent; FE = final effluent.

quite different from the previous raw influent, primary effluent, and anaerobic effluent wastewater samples. The peak abundances of first aerobic effluent and hydrolytic effluent also decreased dramatically compared with those of raw influent, primary effluent, and anaerobic effluent (Figure 2). This suggested that some of the compounds had been removed or transformed into other classes of compounds. Finally, the patterns of total ion chromatographs for second aerobic effluent and final effluent were also similar and the peak abundances of second aerobic effluent and final effluent were further decreased (Figure 2). In general, the decreased peak abundances from raw influent to final effluent implied an effective decomposition of pollutants with the processing of wastewater.

The concentrations of PAHs in the wastewaters from different treatment stages are listed in Table 5. The concentrations for most PAH monomers decreased with the stepwise treatment. The concentrations for PAH monomers in the raw influent ranged from 0.16 μg/L to 47.2 μg/L, and the total concentration of PAH monomers (ΣPAHs) in raw influent was 139.4 μg/L. The concentrations of naphthalene, acenaphthylene, phenanthrene, anthracene, and fluoranthene in raw influent were all higher than 10 μg/L. The concentrations for PAH monomers in final effluent ranged from not detected to 1.7 μg/L, which were much lower than those in raw influent. For most of the PAH monomers, the concentrations were lower than 1 μg/L or not detected in final effluent. This suggests effective removal of PAHs in the coking wastewater-treatment plant.

DISCUSSION

Bioassays with crustacean (*D. magna*), duckweed (*L. minor*), green alga (*P. subcapitata*), zebrafish (*D. rerio*) embryos, and luminous bacteria (*E. coli* HB101 pUCD607) showed different toxicities in raw influent and effluents from different treatment stages (Table 1). The raw influent obviously displayed higher toxicity to all 5 test species, with toxic units up to several hundreds. A previous study also showed toxicity of coking wastewater to maize (*Zea mays* L.) seed [4]. Wei et al. [5] further demonstrated that coking wastewaters from different treatment stages (anaerobic, anaerobic/aerobic, anaerobic/aerobic/photo degradation, anaerobic/aerobic/ozone oxidation treatment) could affect the amylase and protease activity in maize embryos. Coking wastewaters treated with Fenton/electro-Fenton, membrane bioreactor, and coagulation also showed toxicity to embryos and larvae of Japanese medaka [9]. The present study demonstrated that the present biological fluidized-bed treatment technologies are effective for the removal of most toxicity from raw coking wastewater. The toxic units in the final effluent to the 5 organisms were much lower than those in raw influent. In fact, the treatment technologies have been proved to be effective in the removal of PAHs in the Songshan coking wastewater-treatment plant [1].

It is acknowledged that the toxicity of wastewaters is related to the toxicant concentrations in wastewater. Some previous studies have investigated the metals and organic chemicals present in coking wastewaters [2,10,19,20]. Among the 13 tested metals, the present study found that most of the elements in raw influent and effluents from different treatment stages were below the Chinese water-quality standards, except for Cr and Fe, for which concentrations were higher than the Chinese water-quality standard (Table 2). The acute toxicity of Cr has been reported in the literature, with the EC50 values for *Daphnia magna*, *V. fischeri*, and green alga being 430 μg/L, 12.4 μg/L, and 208 μg/L, respectively [21–23]. The maximum concentration for Cr of 60.2 μg/L was higher than the EC50 of *V. fischeri*; hence, Cr could be a potential toxicant in raw influent to organisms. In addition to metals, various organic contaminants were present in coking wastewater, as shown in Table 4 and Supplemental Data, Table S2. Phenol, benzofuran, quinoline, isoquinoline, indole, isoindole, naphthalenol, naphthalenamine, pyridine, quinolinol, anthracene, acridine, azafluorene, norharmane, pyrene, and cyanide were found at high abundances in total ion chromatographs of raw influent, primary effluent, and anaerobic effluent (Figure 2). Hence, these organic chemicals might contribute to the toxicity in raw influent, primary effluent, and anaerobic effluent. As shown in Figure 2, the peak abundances in the chromatograms for first aerobic effluent and hydrolytic effluent decreased to lower levels, and some peaks present in the chromatograms of raw influent, primary effluent, and anaerobic effluent disappeared. Finally, the peak abundances in the chromatograms for second aerobic effluent and final effluent decreased to much lower levels compared with the effluents from previous treatment stages. This suggests effective removal of these organic compounds from the raw coking wastewater by the treatment processes.

A multivariate analysis was also conducted to assess the potential relationships between the toxicity and coking wastewater factors, including coking wastewater-quality parameters, metal element concentrations, and total concentration of

Table 5. Concentrations of polycyclic aromatic hydrocarbons (PAHs) in different treatment stages of the coking wastewater treatment plant

	PAHs (μg/L)															
Wastewater	Nap	Acy	Ace	Flo	Phe	Ant	Fla	Pyr	BaA	Chr	BbF	BkF	Bap	IcdP	DahA	BghiP
RI	16.8	14.4	3.37	9.52	47.2	16.3	11.5	7.59	3.23	2.32	0.94	0.37	1.91	1.15	2.67	0.16
PE	5.51	8.45	1.88	5.75	20.7	16.4	6.20	3.91	1.11	0.57	0.17	0.16	0.45	1.13	4.12	0.06
AnAE	5.42	6.46	1.48	4.08	10.1	6.02	2.32	1.45	0.55	0.32	0.19	0.16	0.50	1.69	0.22	0.05
AE1	1.84	1.85	0.08	0.4	0.18	0.22	0.03	<LOD	0.11	0.14	<LOD	0.18	0.25	ND	ND	ND
HE	1.97	1.78	0.38	0.45	0.22	0.4	0.49	<LOD	<LOD	0.22	ND	0.17	0.20	ND	ND	ND
AE2	1.92	1.17	0.19	0.37	0.16	0.18	0.29	<LOD	<LOD	0.19	ND	0.31	0.31	ND	ND	ND
FE	1.7	1.07	0.75	0.31	0.13	0.21	0.31	<LOD	<LOD	0.12	ND	0.08	ND	ND	ND	ND
WQS[a]													0.0028			
LOD	0.02	0.09	0.024	0.07	0.01	0.01	0.08	0.08	0.06	0.015	0.07	0.06	0.06	0.03	0.02	0.02

[a]China's water quality standard (WQS) for class I (GB3838-2002).

RI = raw influent; PE = primary effluent; AnAE = anaerobic effluent; AE1 = first aerobic effluent; HE = hydrolytic effluent; AE2 = second aerobic effluent; FE = final effluent; LOD = method limit of detection; Nap = naphthalene; Acy = acenaphthylene; Ace = acenaphthene; Flo = fluorene; Phe = phenanthrene; Ant = anthracene; Fla = fluoranthene; Pyr = pyrene; BaA = benz[*a*]anthracene; Chr = chrysene; BbF = benzo[*b*]fluoranthene; BkF = benzo[*k*]fluoranthene; Bap = benzo[*a*]pyrene; IcdP = indeno(1,2,3,-cd)pyrene; DahA = dibenzo[*a*,*h*]anthracene; BghiP = benzo[*ghi*]perylene; ND = not detected.

PAH monomers (Tables 1, 3, and 5). Detrended correspondence analysis showed that the lengths of the first ordination gradient were 1.353 for the toxic units for different test species, so a redundancy analysis was chosen. The correlations between the toxic units and environmental factors with the first 2 axes of redundancy analysis are shown in Figure 3. The first axis of correlation and variation in toxic units and wastewater factors revealed by redundancy analysis was 93.6%, and the second axis of correlation between them revealed by redundancy analysis was 6.2%. The toxic units of the 5 test species were clustered together, suggesting that the toxicity profile and toxicity reduction of coking wastewater-treatment processes are similar (Figure 3). Moreover, pH, chemical oxygen demand, total nitrogen, NH_3-N, volatile phenols, sulfide, some metals (Cr, As, Sb, Hg, Pb, and Ni), and ΣPAHs were strongly correlated to the toxicity of coking wastewaters, while the factors of total phosphorus, conductivity, and some metals (Zn, Mn, Fe, and Al) were less correlated to the toxicity of wastewaters. The test species of green alga, luminous bacteria, and zebrafish were found to be sensitive to pH, chemical oxygen demand, volatile phenols, sulfide, total nitrogen, Cr, and Pb, whereas duckweed and the crustacean were more sensitive to metals such as Sb, Hg, and As. Because of the complexity of chemicals in coking wastewaters, however, it is difficult to work out the specific toxicants in the coking wastewaters. But the present redundancy analysis definitely indicated that a series of pollutants were correlated to the toxicity of coking wastewater.

The safety emission limit value for wastewater discharge was also evaluated according to USEPA regulation [7]. Although many factors need to be considered when deriving wastewater emission limit values, the toxicity of wastewater to organisms in the receiving environment is probably the most important factor. In the present study, green alga (*P. subcapitata*) was found to be the most sensitive organism among the 5 test species when exposed to the final effluent. The toxic unit for green alga in final effluent was larger than 1.0 (Table 2), suggesting potential risks when the whole effluent is discharged into the environment in the "worst-case scenario." Hence, dilution of the final effluent is needed before discharging into the receiving aquatic environment. Currently, however, there are no national industrial whole effluent discharge criteria or coking wastewater discharge criteria based on toxicity benchmarks in China. The USEPA's recommendations for whole effluent toxicity are as follows: a criteria maximum concentration to protect against acute (short-term) effects and a criteria continuous concentration to protect against chronic (long-term) effects. For acute toxicity protection, the criteria maximum concentration should be set at 0.3 toxic units to the most sensitive organisms of 3 test species [7]. Based on the criteria maximum concentration and alga 2.26 toxic units in final effluent, the dilution factor should be 8 (which is equal to 2.26/0.3) before final effluent is discharged into the natural environment. The present study suggests further measures should be taken in regard to the emission of coking wastewater into the receiving environment.

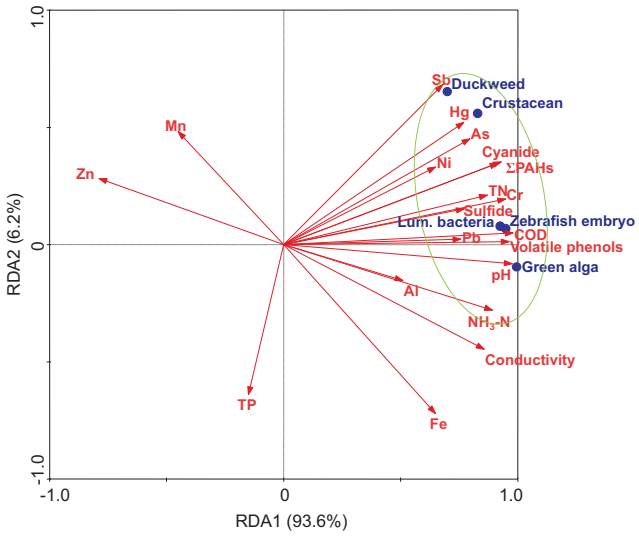

Figure 3. Redundancy analysis (RDA) ordination plots based on the toxic units for 5 organisms and coking wastewater parameters. Solid circles represent the toxic units of 5 organisms. Coking waste parameters are expressed as red arrows. The lengths of the arrows reveal the strength of the relationship, and the intersection angle between the arrows can express the correlation. The percentage of variation explained by each axis is shown, and the relationship is significant ($p = 0.0020$). COD = chemical oxygen demand; NH_3-N = ammoniac nitrogen; TP = total phosphorus; TN = total nitrogen; ΣPAHs = total polycyclic aromatic hydrocarbons. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

CONCLUSION

Coking wastewater was effectively assessed with the combined tools of battery toxicity tests and chemical analyses. The toxicity in coking wastewater could be gradually removed by stepwise treatment processes including primary treatment, biological fluidized-bed treatment, and secondary clarifying treatment processes used in the coking wastewater-treatment plant. The final effluent showed no toxicity to crustacean, duckweed, zebrafish embryos, and luminous bacteria, except for the low toxicity to green alga. Chemical analysis of coking wastewaters found a variety of potential pollutants. Multivariate statistical analysis found that a series of pollutants such as chemical oxygen demand, ammoniac nitrogen, volatile phenols, metals, and total PAHs contributed to the toxicity of the coking wastewater; but further toxicity evaluation is required to identify specific toxicants. For the purpuse of protecting sensitive aquatic species, emission limit values need to be set before final effluent is discharged into the receiving aquatic environment.

SUPPLEMENTAL DATA

Tables S1–S2. (987 KB DOC).

Acknowledgment—The present research was supported by the State Key Program of the National Natural Science Foundation of China (21037001) and the program of NSFC (41101462). This is a Contribution No. 1898 from GIG CAS.

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