



Variations in toxicity of semi-coking wastewater treatment processes and their toxicity prediction



Xiaoyan Ma^{a,b,c,d,e,*}, Xiaochang Wang^{a,b,c,d,e,*}, Yongjun Liu^{a,b,c,d,e},
Jian Gao^{a,b,c,d,e}, Yongkun Wang^{a,b,c,d,e}

^a Xi'an University of Architecture and Technology, Xi'an 710055, China

^b Key Lab of Environmental Engineering, Shaanxi Province, China

^c Engineering Technology Research Center for Wastewater Treatment and Reuse, Shaanxi Province, China

^d Key Lab of Northwest Water Resource, Environment and Ecology, MOE, China

^e International Science & Technology Cooperation Center for Urban Alternative Water Resources Development, China

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ABSTRACT

Chemical analyses and bioassays using *Vibrio fischeri* and *Daphnia magna* were conducted to evaluate comprehensively the variation of biotoxicity caused by contaminants in wastewater from a semi-coking wastewater treatment plant (WWTP). Pretreatment units (including an oil-water separator, a phenols extraction tower, an ammonia stripping tower, and a regulation tank) followed by treatment units (including anaerobic-oxic treatment units, coagulation-sedimentation treatment units, and an active carbon adsorption column) were employed in the semi-coking WWTP. Five benzenes, 11 phenols, and five polycyclic aromatic hydrocarbons (PAHs) were investigated as the dominant contaminants in semi-coking wastewater. Because of residual extractant, the phenols extraction process increased acute toxicity to *V. fischeri* and immobilization and lethal toxicity to *D. magna*. The acute toxicity of pretreated wastewater to *V. fischeri* was still higher than that of raw semi-coking wastewater, even though 90.0% of benzenes, 94.8% of phenols, and 81.0% of PAHs were removed. After wastewater pretreatment, phenols and PAHs were mainly removed by anaerobic-oxic and coagulation-sedimentation treatment processes respectively, and a subsequent active carbon adsorption process further reduced the concentrations of all target chemicals to below detection limits. An effective biotoxicity reduction was found during the coagulation-sedimentation and active carbon adsorption treatment processes. The concentration addition model can be applied for toxicity prediction of wastewater from the semi-coking WWTP. The deviation between the measured and predicted toxicity results may result from the effects of compounds not detectable by instrumental analyses, the synergistic effect of detected contaminants, or possible transformation products.

1. Introduction

Coal production in China rose from 1107.6 million tonnes oil equivalent in 2004 to 1844.6 million tonnes oil equivalent in 2014 (46.9% of global coal production), accompanied by an increase of wastewater produced from coal carbonization (BP, 2015). The wastewater from coal carbonization generally contains a high concentration of toxic organic chemicals, e.g., phenols, polycyclic aromatic hydrocarbons (PAHs), and benzenes, and may pose a potential ecological risk to the aquatic environment (Li et al., 2003; Marañón et al., 2008; Liu et al., 2016a; Zhang et al., 2013). Han et al. (2011) measured the toxic effects of coking wastewater on *Zea mays* L., suggesting that it affected the growth, fresh biomass, and cell division, and showed genotoxicity

via an obvious increase of micronucleus frequency in root tips. The genotoxicity of coking wastewater was further verified using *Vicia faba* and *Hordeum vulgare* root tips (Dong and Zhang, 2010). Biotoxicity of coking wastewater was also investigated by using various aquatic organisms from different trophic levels, such as luminescent bacteria, algae, crustaceans, and fish embryos (Zhao et al., 2009a, 2014; Zhu et al., 2013).

The wastewater from coal carbonization was divided into two categories: the semi-coking wastewater produced from the middle- (700–800 °C) and low- (500–600 °C) temperature carbonization processes and the coking wastewater produced from the high-temperature carbonization process (950–1050 °C). The semi-coking and coking wastewater exhibit similar properties, but the concentrations of con-

* Corresponding authors at: Xi'an University of Architecture and Technology, Xi'an 710055, China.
E-mail address: maxiaoyan@xauat.edu.cn.

taminants, such as PAHs, phenols, etc., in semi-coking wastewater are typically higher than the levels in coking wastewater (Wang et al., 2014; Liu et al., 2016b). Semi-coke production is an emerging industry. The treatment, discharge, and environmental impact of semi-coking wastewater have become a difficult issue because of its complexity, heterogeneity, and toxicity. Therefore, it is necessary to comprehensively study the removal of contaminants and the variation of biotoxicity in the semi-coking wastewater treatment processes using chemical analyses and bioassays to ensure ecological safety.

The biotoxicity of a mixture is dependent on the species, concentration, toxicity, and interaction of contaminants in water (Villa et al., 2012; Tian et al., 2012; Escher et al., 2013). Accurate prediction of the toxicity of actual wastewater based on the concentrations and toxicity of detected compounds is complicated due to the potential synergism and antagonism. Nevertheless, it has been concluded that as the number of components in the mixture increase, the synergistic or antagonistic effects will weaken to the additive effects if the added component cannot yield a much stronger joint effect with an existing component (Tian et al., 2012). This facilitates the toxicity prediction for actual water samples, because the joint toxicity of a mixture with additive effects could be predicted by concentration addition (CA), independent action (IA), two-step prediction, etc. Many researchers have shown that the CA model is typically more rational because it can provide reliable estimates of the toxicity of a broad range of mixtures composed of various chemicals, especially for the mixtures with unspecific mechanisms of action (Altenburger et al., 2003; Arrhenius et al., 2004; Zhou et al., 2010). However, the predictability of CA for semi-coking wastewater with a high concentration of contaminants has not been demonstrated.

The objective of the present study was to evaluate the treatment performance of a semi-coking wastewater treatment plant (WWTP) employing anaerobic-oxic (A/O) biological treatment combined with coagulation and adsorption treatment using chemical analyses, to investigate the variation of biotoxicity during treatment processes using the bioluminescence inhibition assay, and the immobilization and lethality assay with *D. magna*, and to analyze the predictability of CA for semi-coking wastewater. The work will contribute to understanding the removal of contaminants and the potential biotoxicity during semi-coking wastewater treatment processes, and will demonstrate the validity of its toxicity prediction.

2. Materials and methods

2.1. Semi-coking WWTP and sampling

Wastewater from a semi-coking WWTP located in Yulin, Shaanxi Province, China, with an average treatment capacity of 3000–5000 m³/d was studied. The WWTP applied a series of pretreatment and treatment processes, as shown in Fig. 1. The pretreatment units included an oil-water separator, a phenols extraction tower, an ammonia stripping tower, and a regulation tank. In the phenols extraction tower, the extractant (mixture of tributyl phosphate and kerosene) and wastewater were mixed in the proportion 1:7, and the extraction time was 20 min. The effluent from the phenols extraction tower was pumped into the ammonia stripping tower after adjustment of the pH to 11, and the air was blown into the wastewater in the ammonia stripping tower to recover the ammonia. The treatment units included A/O treatment, a coagulation-sedimentation unit, and an active carbon adsorption column. After pretreatment, the wastewater was directed to the A/O treatment unit with a hydraulic retention time (HRT) of 20 h. The effluent of the biological treatment processes was passed through the coagulation-sedimentation unit, containing polymeric ferric sulfate as coagulant and semi-coke as coagulant aid, followed by an active carbon adsorption column for further treatment. The effluent of the semi-coking WWTP was reused for coal carbonization in the semi-coke plant. Sampling locations along the wastewater

treatment processes are shown in Fig. 1. The collected samples were immediately transferred from the site to the laboratory at 4 °C and then stored in a refrigerator.

2.2. Sample extraction and chemical analyses

Individual standard solutions of five benzenes, benzene, toluene, ethylbenzene, cumene, and styrene, and five PAHs, naphthalene, acenaphthylene, fluorene, phenanthrene, and fluoranthene, were purchased from o2si smart solutions (USA). The phenols standard solution containing 11 compounds, i.e., phenol, 2-chlorophenol, o-cresol, 2-nitrophenol, 2,4-dimethylphenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 4-nitrophenol, 2,3,4,6-tetrachlorophenol, 2-methyl-4,6-dinitrophenol and pentachlorophenol, were obtained from Sigma–Aldrich (USA).

Before extraction, the water samples were filtered through 0.8 glass-fiber filters to remove suspended particulates. Sodium chloride was added to the extracting sample to improve recovery rate, to avoid emulsification and to shorten the separation time of the aquatic and organic phases. First, 10% NaOH was added to adjust the pH \geq 12. Basic and neutral organic compounds were extracted with dichloromethane using an ultrasonic vibrating device, and then the aqueous and organic phases were separated using a centrifuge. Secondly, sulfuric acid was added to the aquatic phase to adjust the pH \leq 2. Acid organic compounds were extracted similarly to the basic and neutral organic compound extraction. The two parts of the organic phase were mixed, and anhydrous sodium sulfate was added to remove the water. The volume of extract was concentrated to 1.8 mL using a rotary evaporator.

The determination of target compounds was carried out by Agilent GC-2014 gas chromatography (USA) equipped with a DB-5MS capillary column (30 m \times 0.25 mm \times 0.25 μ m) and flame ionization detector. The detailed instrument conditions for the different categories of chemicals are as follows. The injection port temperature was maintained at 250 °C. Nitrogen was the carrier gas at a flow rate of 1.0 mL/min. For the benzenes, the column temperature was programmed from 60 °C for 2 min and then increased by 8 °C/min to 150 °C for 1 min, and the split ratio was 20:1. For the phenols, the column temperature was programmed from 80 °C for 2 min and then increased by 8 °C/min to 300 °C for 5 min, and the split ratio was 10:1. For the PAHs, the column temperature was programmed from 60 °C for 2 min and increased by 10 °C/min to 250 °C, and then increased by 5 °C/min to 310 °C for 5 min. The split ratio was 10:1.

After filtering the water samples collected from the different treatment units, conventional indices including total phosphorus (TP), total nitrogen (TN), NO₃⁻-N, NO₂⁻-N, NH₄⁺-N, chemical oxygen demand (COD), and total organic carbon (TOC) were measured according to the Water and Wastewater Analytical Methods to evaluate the performance of the semi-coking WWTP (SEPA, 2006).

2.3. Bioassays

2.3.1. Bioluminescence inhibition assay with *Vibrio fischeri*

The acute toxicity test using luminescent bacteria is the most widely used method for monitoring environmental pollution because of its high throughput and sensitivity, cost effectiveness, and simple operation. The intensity reduction of the luminescence emitted by the marine bacteria *V. fischeri* is used to quantify the toxicity of pollutants. *V. fischeri* was purchased from the China Center of Industrial Culture Collection and was used for toxicity test (15 min exposure), according to a modified ISO 11348 (2008) procedure. Briefly, 100 μ L of bacterial suspension prepared from logarithmically growing bacteria was exposed to 100 μ L of the testing sample in a cell of the microplate for 15 min. Based on the result of the preliminary test, the testing sample was diluted into a series of different concentrations (as percentages). Three replicates were performed for each concentration. The relative

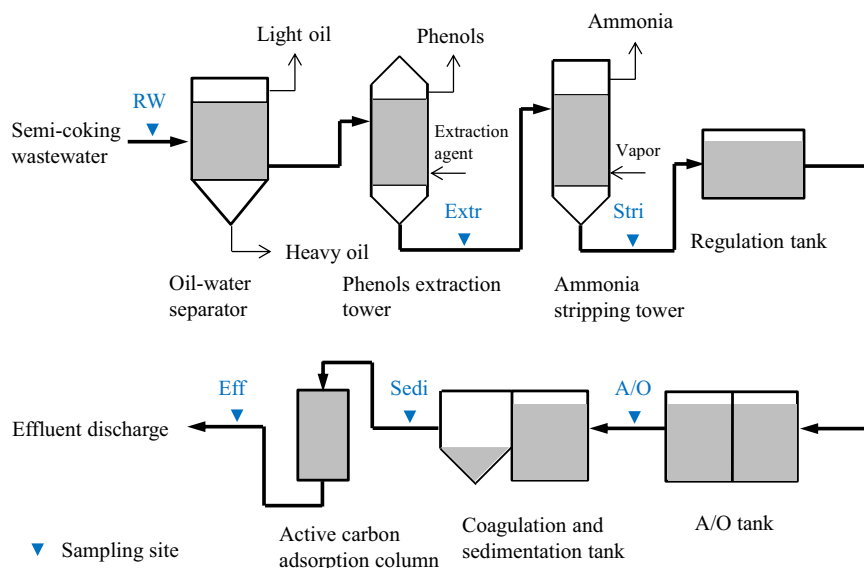


Fig. 1. Flow scheme of pretreatment and treatment at the semi-coking WWTP and the sampling sites. RW: raw wastewater; Extr: effluent of phenols extraction tower; Stri: effluent of ammonia stripping tower; A/O: effluent of A/O treatment units; Sedi: effluent of coagulation and sedimentation tank; Eff: effluent of active carbon adsorption column.

light units were measured on a Centro LIAPc LB962 Microplate Luminometer (Berthold Technologies Company, Germany). The tests for every water sample were conducted in triplicate in different microplates. The effective concentration, $EC_{50}(\%)$, was calculated using the concentration-inhibition curve. The toxicity unit (TU) was used to express the acute toxicity of wastewater toward *V. fischeri* according to Eq. (1).

$$TU = \frac{100}{E(L)C_{50}(\%)} \quad (1)$$

2.3.2. Immobilization and lethality assay with *Daphnia magna*

Twenty-four-hour immobilization and 48-h lethality tests with *D. magna* neonates were implemented to investigate the acute toxicity of wastewater from semi-coking wastewater treatment processes following the standard ISO, 6341 (1998). The *D. magna* strain was obtained from the Institute of Environmental Health and Related Product Safety, China CDC. Because the sensitivity of *D. magna* to toxicants is age-dependent, neonates less than 12 h old were used in the tests. A preliminary test was conducted to determine the range of dilution for every water sample. In the test, six *D. magna* neonates were exposed to 20 mL testing samples in a container and cultured in 8 h dark/16 h light cycles at $20 \pm 2^\circ\text{C}$. Every sample was tested in triplicate. The number of immobilized *D. magna* in each container was recorded after 24 h of exposure. At the end of the test period of 48 h, the lethal *D. magna* in each container was counted. The EC_{50} and lethal concentration (LC_{50}) as percent were obtained from the concentration-effect (immobilization or lethality) curve. TU was used to assess the immobilization and lethality of water samples toward *D. magna* according to Eq. (1).

2.4. Predicting the toxicity of mixtures to aquatic organisms

The toxicities of mixtures toward *V. fischeri* and *D. magna* were predicted with CA as shown in Equation (2).

$$EC_{50\text{-pre}} = \left(\sum_{i=1}^n p_i \cdot EC_{50,i} \right)^{-1} \quad (2)$$

where n is the number of mixture components, p_i and $EC_{50,i}$ is the fraction and the EC_{50} value of component i in the mixture, respectively.

The $E(L)C_{50}$ values of individual chemicals toward *V. fischeri* and *D. magna* were obtained from the quantitative structure-activity relationships (QSARs) or databases, and then applied for CA model prediction.

2.4.1. EC_{50} value predictions for chemicals toward *V. fischeri*

Because there is no available database to predict the toxicity of chemicals toward luminescent bacteria, the prediction of EC_{50} values of pollutants to luminescent bacteria could resort to QSARs. The typical baseline QSAR models using the octanol-water partition coefficient K_{ow} as hydrophobicity descriptor were established for neutral organic molecules, which is suitable for benzenes and PAHs. EC_{50} values (15 min exposure) of benzenes and PAHs were predicted using baseline toxicity QSAR models as shown in Eqs. (3) and (4) respectively (Vighi et al., 2009; Lee et al., 2013). The K_{ow} values of chemicals were from the Kowwin v. 1.68 database.

$$\log \frac{1}{EC_{50}(\text{mmol/L})} = 0.856 \cdot \log K_{ow} - 2.42 \quad (3)$$

$$\log \frac{1}{EC_{50}(\text{mmol/L})} = 1.07 \cdot \log K_{ow} - 1.87 \quad (4)$$

However, in the case where the investigated chemicals, such as phenols, are not neutral organic molecules, K_{ow} is an unsuitable descriptor for uptake into biological membranes. Therefore, the liposome-water distribution coefficient K_{lipw} at a defined pH of 7, $D_{lipw}(\text{pH } 7)$, has replaced the K_{ow} in the QSAR models for phenols, because partitioning into membranes depends not only on the hydrophobicity of a compound but also on its charge and specific interactions with the membrane. $D_{lipw}(\text{pH } 7)$ was estimated using Eq. (5) (Escher et al., 2011). The fraction of neutral chemical species, f_{neutral} , was calculated based on the acidity constant pK_a using the online prediction program SPARC, and the logarithm of K_{lipw} was calculated using Eq. (6) (Vaes et al., 1997; Hilal et al., 2005).

$$D_{lipw}(\text{pH } 7) = f_{\text{neutral}} \cdot K_{lipw} + (1 - f_{\text{neutral}}) \cdot \frac{K_{lipw}}{10} \quad (5)$$

$$\log K_{lipw} = 0.905 \cdot \log K_{ow} + 0.515 \quad (6)$$

The QSAR model derived for polar chemicals (Eq. (7)) was employed to predict the EC_{50} values of phenols with 30 min exposure ($EC_{50\text{-}30\text{ min}}$) (Tang et al., 2013). Then the EC_{50} values with 15 min exposure ($EC_{50\text{-}15\text{ min}}$) were estimated from $EC_{50\text{-}30\text{ min}}$ values

Table 1
Conventional indices of wastewater from semi-coking WWTP.

Index	RW	Extr	Stri	A/O	Sedi	Eff
TP (mg/L)	29.0 ± 5.00	28.4 ± 4.50	28.2 ± 3.00	3.91 ± 1.00	0.91 ± 1.00	0.63 ± 0.30
TN (mg/L)	33,400 ± 600	9860 ± 400	4220 ± 300	3770 ± 200	130 ± 20.0	25.5 ± 2.00
NO ₃ ⁻ -N (mg/L)	1580 ± 280	2380 ± 500	1330 ± 500	992 ± 200	80.0 ± 10.0	6.90 ± 3.00
NO ₂ ⁻ -N (mg/L)	2840 ± 365	1570 ± 300	926 ± 300	742 ± 130	20.0 ± 5.00	9.20 ± 2.00
NH ₄ ⁺ -N (mg/L)	10,800 ± 800	5510 ± 200	1620 ± 200	681 ± 100	18.6 ± 1.00	9.60 ± 4.00
COD (mg/L)	88,000 ± 7000	56,300 ± 5000	50,300 ± 5000	359 ± 50	130 ± 10	70.3 ± 11.0
TOC (mg/L)	3670 ± 580	4330 ± 600	3520 ± 600	229 ± 36.0	105 ± 10.0	42.5 ± 10.0

Note: RW: raw wastewater; Extr: effluent of phenols extraction tower; Stri: effluent of ammonia stripping tower; A/O: effluent of A/O treatment units; Sedi: effluent of coagulation and sedimentation tank; Eff: effluent of active carbon adsorption column.

using Eq. (8), to keep conditions between predictions and experiments the same (Ribo and Kaiser, 1983).

$$\log 1/EC_{50-30\text{min}} (\text{mmol/L}) = 0.6978 \cdot \log D_{\text{lipw}} (\text{pH7}) + 3.9578 \quad (7)$$

$$\log 1/EC_{50-15\text{min}} (\text{mmol/L}) = 0.976 \cdot \log 1/EC_{50-30\text{min}} (\text{mmol/L}) + 0.011 \quad (8)$$

2.4.2. LC₅₀ value predictions for chemicals toward *D. magna*

The LC₅₀ values (48 h exposure) of chemicals toward *D. magna* were obtained from the ECOSARv1.1 database. Considering ecological safety, the highest conservation effect level is selected when predictions are identified from multiple classes.

3. Results and discussion

3.1. The performance of a semi-coking WWTP based on conventional indices

The semi-coking wastewater was firstly subjected to multi-step pretreatment for the recovery of compounds, such as phenols and ammonia. This was followed by A/O biological treatment, coagulation-sedimentation, and active carbon adsorption units for further purification. Table 1 shows the variation of conventional indices of wastewater in treatment processes. For the systematic treatment, the removal of detected indices including TP, TN, NO₃⁻-N, NO₂⁻-N, NH₄⁺-N, COD, and TOC was greater than 90.0%. Previous studies showed that the coking wastewater treatment processes could reduce more than 80.0% COD, more than 95.0% NH₄⁺-N, and around 90.0% TN, which was in accordance with the present study (Qi et al., 2007; Marañón et al., 2008; Li et al., 2011; Liu et al., 2016a, 2016b).

Comparing the raw wastewater (sample RW) with the pretreated wastewater (sample Stri), TP, NO₃⁻-N, and TOC were almost unchanged, while TN, NO₂⁻-N, NH₄⁺-N, and COD were notably reduced, which may be attributed to the recovery of phenols and ammonia from the wastewater. During the A/O biological treatment processes (sample A/O), the organic matter quantified by COD and TOC was efficiently removed from the wastewater (99.3% and 93.5%, respectively). However, nitrogen compounds, expressed as indices of TN, NO₃⁻-N, NO₂⁻-N, and NH₄⁺-N, were not effectively removed in the process. Although 58.1% NH₄⁺-N removal efficiency could be achieved, 25.1% NO₃⁻-N and 19.9% NO₂⁻-N removal resulted in a low removal efficiency of TN (10.6%). This indicated that the denitrification process needed to be enhanced for the removal of nitrogen compounds in the biological treatment process. Zhao et al. (2009b) utilized an anaerobic-anoxic-oxic membrane bioreactor to treat coke wastewater and stated that the anaerobic reactor primarily acted to convert refractory compounds into biodegradable organic substances, the anoxic reactor denitrified nitrate and removed more than 50.0% COD, and the oxic reactor nitrified ammonium-nitrogen to nitrate and degraded 33.0% COD. Therefore, the absence of an anoxic reactor led to unsatisfactory

denitrification in the present system. During subsequent treatment that coupled coagulation-sedimentation with activated carbon adsorption, the determined indices listed in Table 1 were gradually decreased and achieved more than 80% removal. The removal of most NO₃⁻-N, NO₂⁻-N, and NH₄⁺-N by the coagulation-sedimentation treatment suggested that this step supplemented the poor denitrification in the A/O biological treatment processes.

3.2. Variation of target contaminants

Twenty-one target chemicals including five benzenes, 11 phenols, and five PAHs, were analyzed in the water samples. The total concentrations of the three categories of contaminants and the concentrations of individual chemicals are presented in Fig. 2 and Table 2. The highest total concentration of contaminants and the highest number of chemicals were detected in the WWTP influent (sample RW). The concentrations decreased through the wastewater treatment processes (Fig. 2 and Table 2). The contaminants in the collected wastewater were dominated by phenols and PAHs, while benzenes only accounted for a small fraction (Fig. 2).

The mass percentages of the three categories of contaminants varied with the semi-coking wastewater treatment processes. Of the three categories of contaminants, hydrophilic phenols accounted for the majority of detected contaminants in the WWTP influent (63.7%). It was reported previously that biodegradable chemicals such as phenols represent the major part of the total organic compounds in raw coke wastewater, but hydrophobic refractory chemicals such as PAHs account for only a minor part (Yu et al., 2015). However, after the pretreatment and A/O biological treatment processes, the proportion of phenols decreased to 17.3%, and the proportion of PAHs

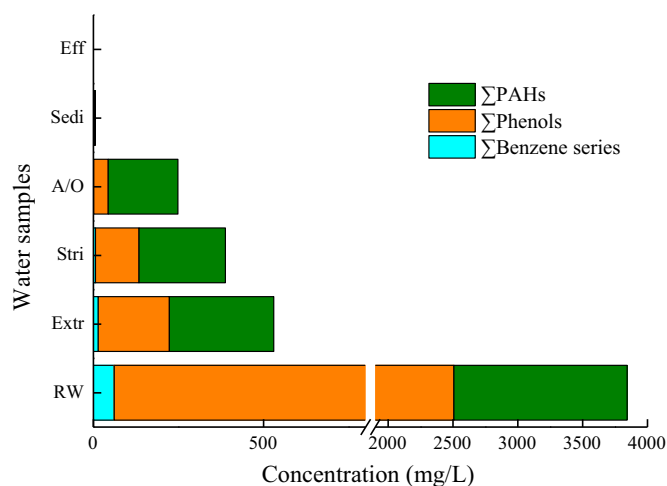


Fig. 2. Distribution of PAHs, phenols and benzenes in wastewater from a semi-coking WWTP. RW: raw wastewater; Extr: effluent of phenols extraction tower; Stri: effluent of ammonia stripping tower; A/O: effluent of A/O treatment units; Sedi: effluent of coagulation and sedimentation tank; Eff: effluent of active carbon adsorption column.

Table 2
Concentration of contaminants in wastewater (mg/L).

Chemicals	K _{ow}	RW	Extr	Stri	A/O	Sedi	Eff
<i>Benzenes</i>							
Benzene	1.99	11.5	ND	ND	ND	ND	ND
Toluene	2.54	34.5	4.65	3.24	0.81	ND	ND
Ethylbenzene	3.03	10.9	4.12	1.44	ND	ND	ND
Cumene	3.45	1.50	ND	ND	ND	ND	ND
Styrene	2.89	2.61	5.20	1.42	ND	ND	ND
<i>Phenols</i>							
Phenol	1.51	1330	83.2	36.4	7.20	ND	ND
2-Chlorophenol	2.16	19.8	0.22	0.25	ND	ND	ND
o-Cresol	2.06	279	24.8	25.6	3.96	ND	ND
2-Nitrophenol	1.91	43.9	3.60	3.58	ND	ND	ND
2,4-Dimethylphenol	2.61	282	40.0	8.64	5.40	1.36	ND
2,4,5-Trichlorophenol	3.45	316	11.5	11.2	13.0	0.79	ND
2,4,6-Trichlorophenol	3.45	60.8	12.6	10.8	12.1	1.71	ND
4-Nitrophenol	1.91	26.6	1.80	1.44	1.40	0.39	ND
2,3,4,6-Tetrachlorophenol	4.09	48.6	1.44	1.08	ND	ND	ND
2-Methyl-4,6-dinitrophenol	2.27	20.0	30.1	29.3	ND	ND	ND
Pentachlorophenol	4.74	16.0	ND	ND	ND	ND	ND
<i>PAHs</i>							
Naphthalene	3.17	1330	275	223	179	0.41	ND
Acenaphthylene	3.94	5.93	5.63	5.40	4.32	ND	ND
Fluorene	4.02	0.43	25.7	24.5	21.2	0.79	ND
Phenanthrene	4.35	0.29	ND	ND	0.23	0.17	ND
Fluoranthene	4.93	0.93	0.72	0.64	0.46	0.10	ND

Note: K_{ow} is the octanol-water partition coefficient of the chemical. ND: not detected. RW: raw wastewater; Extr: effluent of phenols extraction tower; Stri: effluent of ammonia stripping tower; A/O: effluent of A/O treatment units; Sedi: effluent of coagulation and sedimentation tank; Eff: effluent of active carbon adsorption column.

increased from 34.8% to 82.4%. This may be attributed to the recovery of 91.5% of the phenols in the pretreatment processes and the removal of 66.5% of the phenols in the biological treatment process. Subsequently, the proportion of phenols in the effluent of the coagulation-sedimentation process rose to 74.3%, due to the significant removal of hydrophobic benzenes and PAHs (> 99.0%) in this process.

Of the 21 chemicals, the dominating contaminants present in the semi-coking wastewater with a concentration level exceeding 100 mg/L, were phenol, o-cresol, 2,4-dimethylphenol, 2,4,5-trichlorophenol and naphthalene (Table 2). Zhang et al. (2013) who studied the occurrence and fate of phenolic compounds in coking WWTP pointed out that 3-cresol and phenol were the main compounds in raw coking wastewater, and that chlorophenols and nitrophenols, at the µg/L level, had lower concentrations. Song et al. (2013) investigated the concentration of phenols, anilines, and PAHs in wastewater from six coking WWTPs and showed that of the target phenolic compounds, phenol and 3-methylphenol were present at mg/L level and 15 PAHs were present at the µg/L level. Zhao et al. (2014) determined that the total concentration of 16 PAHs was only 139 µg/L in raw coking wastewater and the concentration of phenanthrene was the highest. It was obvious that the concentrations of contaminants in the semi-coking wastewater determined here were higher than those reported previously for levels in coking wastewater. Additionally, the dominant compounds in semi-coking and coking wastewater were completely distinct.

Concerning the removal of contaminants in the semi-coking WWTP, 90.0% benzenes, 94.8% phenols, and 81.0% PAHs were initially removed from the wastewater during the pretreatment processes. Then 86.7% benzenes and 66.5% phenols were effectively removed by the A/O biological treatment processes, which may explain the significant decrease of COD and TOC. It was previously reported that anaerobic and aerobic tanks played an important role in the elimination of chlorophenols and phenols, which is consistent with the present study (Zhang et al., 2013). Only 19.3% PAHs were eliminated from the wastewater during the A/O treatment processes in the present study. However, Zhang et al. (2012) indicated that the biological treatment stage played a key role in 47%–92% of PAH removal. This

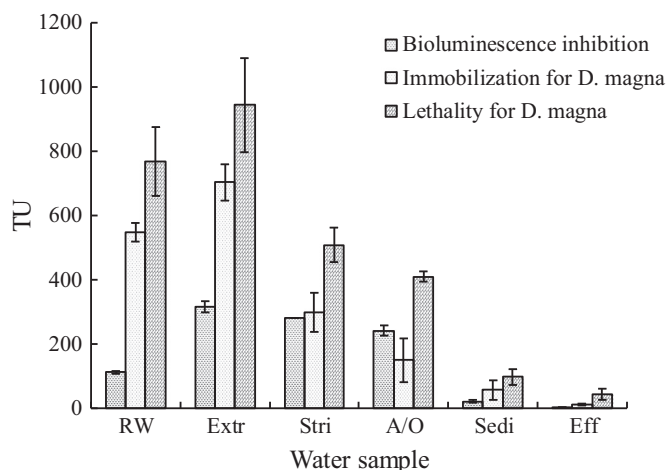


Fig. 3. Results of the bioluminescence inhibition test and the *D. magna* test, including the immobilization assay with 24 h exposure and the lethality assay with 48 h exposure. RW: raw wastewater; Extr: effluent of phenols extraction tower; Stri: effluent of ammonia stripping tower; A/O: effluent of A/O treatment units; Sedi: effluent of coagulation and sedimentation tank; Eff: effluent of active carbon adsorption column.

remarkable treatment performance may be due to the longer HRT of the anaerobic tank (26 h) in their study, where sufficient adsorption between PAHs and sludge and degradation by bacteria was induced (Chun-Hua et al., 2010). In the present study, residual contaminants were removed with a high efficiency by coagulation and adsorption processes, and could not be detected in the effluent of the WWTP. In particular, the concentrations of PAHs that were not removed by biological treatment processes were notably reduced during the coagulation-sedimentation processes. The efficient removal of contaminants was closely related to the removal property of the coagulation-sedimentation and activated carbon adsorption treatments. Hydrophobic and high molecular weight compounds were prone to elimination through aggregating colloid contaminants by charge neutralization and bridge action during the coagulation process (Lai et al., 2007; Han et al., 2015). Activated carbon adsorption, which was based on nonspecific dispersive interplays and complex reactions between contaminants and functional groups or surface charges of adsorbents, had the advantage of eliminating hydrophilic organic matter, particularly phenols (Han et al., 2015; Zhang et al., 2013).

3.3. Biototoxicity assessment

Fig. 3 displays the biotoxicity variation of wastewater from the semi-coking WWTP toward *V. fischeri* and *D. magna*. The TU values were calculated to quantify the toxicity for the two species and ranged from 1.24 to 943 for wastewater samples at different stages of treatment. Interestingly, TU values did not steadily decrease along the semi-coking wastewater treatment processes. Furthermore, the effluent of the WWTP still showed toxicity to the two species to some extent, although the concentrations of all the target compounds in the effluent were below detection limits.

3.3.1. Acute toxicity to luminescent bacteria

The TU of raw semi-coking wastewater to *V. fischeri* increased from 112 to 315 when subjected to oil-water separation and phenols extraction processes, then decreased to 280 after the ammonia stripping process. The observed toxicity increase during the pretreatment processes may be due to the contributions of residual extractant to the toxicity. The TU of wastewater decreased to 1.24 after the wastewater flowed through the A/O biological treatment, the coagulation-sedimentation treatment, and the activated carbon adsorption treatment in sequence. The toxicity removals of the three treatment units were 13.9%, 91.8%, and 93.7%, respectively. This result indicated that an

effective reduction of toxicity was seen in the coagulation and adsorption processes, accompanied by the elimination of target chemicals in wastewater. However, significant toxicity reduction was not found after the pretreatment wastewater underwent biological treatment (280 for sample Stri vs. 241 for sample A/O). In the A/O treatment processes, 86.7% of the benzenes, 66.5% of the phenols and 19.3% of the PAHs were removed. The poor reduction of PAHs at this stage suggested that residual PAHs contributed significantly to the toxicity of wastewater after A/O biological treatment. The short HRT resulting in the low removal efficiency of PAHs might explain the weak decrease of toxicity in the biological treatment processes. Zhou et al. (2015) set up a novel biological treatment process (vertical tubular biological reactor), which effectively reduced the toxicity of coking wastewater to *Photobacterium phosphoreum*. Zhao et al. (2014) showed that the toxicity of coking wastewater after pretreatment (degreasing tank and regulation tank) to *V. fischeri* decreased by 74.1% during anaerobic/aerobic1 biological treatment processes and further decreased by 62.3% during hydrolytic/aerobic2 treatment processes. Thus, the toxicity removal efficiency varied for different biological treatment processes.

3.3.2. Toxicity to *D. magna*

Immobilization assays with 24 h exposure and lethality assays with 48 h exposure using *D. magna* were conducted to evaluate the toxicity variation of semi-coking wastewater from the semi-coking WWTP. As Fig. 3 shows, similar with the observed acute toxicity to luminescent bacteria, the TU of raw semi-coking wastewater to *D. magna* increased from 548 to 703 for the immobilization assay and from 768 to 943 for the lethality assay when the wastewater flowed through the phenols extraction tower. It then decreased to 297 for the immobilization assay and 508 for the lethality assay after the ammonia stripping process. The TU of the final effluent decreased to 11.4 for the immobilization assay and 43.0 for the lethality assay after the pretreated wastewater passed through a series of treatment processes. After biological treatment, the immobilization toxicity and lethality to *D. magna* were decreased by 50.4% and 19.6%, respectively, and then the coagulation and adsorption processes effectively reduced them further by 92.3% and 89.5%, respectively, following the efficient removal of contaminants. The coagulation and adsorption processes showed the highest efficiency for decreasing the toxicity of wastewater to *D. magna*.

Comparing the bioluminescence inhibition assay with the *D. magna* toxicity assay, it can be seen that, for the same water sample, the toxicity to *D. magna* was higher than the toxicity to *V. fischeri*, suggesting that *D. magna* was an appropriate organism for toxicity assessment of semi-coking wastewater. Zhou et al. (2015) evaluated the biotoxicity of raw coking wastewater and its treated effluent using the luminescent bacteria (*P. phosphoreum*), algae (*Isochrysis galbana*), crustacea (*D. magna*), and fish (*Danio rerio*), and also found that *D. magna* was the most sensitive species. In addition, the *D. magna* toxicity assay did not exhibit a significant linear correlation with the luminescent bacteria toxicity assay ($R^2=38.8\%$ with immobilization assay and $R^2=54.3\%$ with lethality assay). However, for the two *D. magna* toxicity assays, the immobilization assay showed a good linear relationship with the lethality assay ($R^2=95.6\%$).

3.4. Comparison of measured and predicted biotoxicity

The measured $E(L)C_{50}$ (EC_{50} -mea or LC_{50} -mea) can be expressed as the total concentration of detected chemicals by calculating the product of the $E(L)C_{50}$ value (from the toxicity test) and the total concentration of the target compounds. For bioluminescence inhibition toxicity, the EC_{50} -mea value decreased dramatically from 34.4 mg/L for the RW sample to 1.68 mg/L for the Extri sample (95.1% removal), after oil-water separation and phenols extraction. It then decreased slowly from 1.38 mg/L for the Stri sample to 1.03 mg/L after biological treatment (25.6% removal), and decreased to 0.29 mg/L after the coagulation-sedimentation processes. Similarly, for the *D. magna* lethal toxicity,

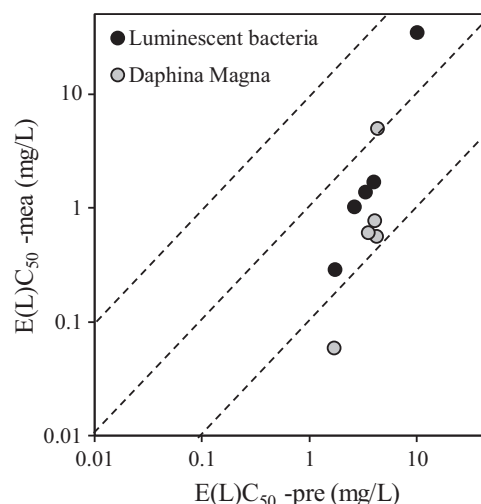


Fig. 4. Comparison of the measured and predicted biotoxicity results of wastewater from the semi-coking WWTP. EC_{50} and LC_{50} were used to express the toxicity of wastewater toward *V. fischeri* after 15 min exposure and the lethality toward *D. magna* after 48 h exposure, respectively.

the LC_{50} -mea decreased remarkably from 5.01 mg/L to 0.56 mg/L after oil-water separation and phenols extraction (88.7% removal). It then decreased from 0.77 mg/L to 0.61 mg/L after biological treatment (20.6% removal) and decreased to 0.06 mg/L after the coagulation-sedimentation processes. This reveals that the semi-coking wastewater treatment processes in the system first removes the pollutants characterized by high concentrations and relatively low toxicity in oil-water separation and phenols extraction processes. The residual contaminants in the subsequent treatment units showed similar integrated biotoxicity and were difficult to remove by biological treatment. Hence, it is vital to consider the residual pollutants in biological treatment processes and to optimize the subsequent treatment processes for the removal of these residual contaminants.

The predicted $E(L)C_{50}$ ($E(L)C_{50}$ -pre) values estimated by CA were in approximate agreement with their $E(L)C_{50}$ -mea values (Fig. 4), generally within one order of magnitude, except for the Sedi sample for *D. magna* lethal toxicity. Of the detected samples, only the $E(L)C_{50}$ -mea of the RW sample for bioluminescence inhibition toxicity and *D. magna* lethal toxicity assays were higher than the $E(L)C_{50}$ -pre, while the others were overestimated by the CA model (Fig. 4). The deviation between the measured and predicted toxicity results may be because some unknown compounds in semi-coking wastewater were not detected by instrumental analyses and were responsible for the measured toxicity. Additionally, the synergistic effect of detected contaminants and transformation products may have occurred that could decrease the predictability of the CA model. The most popular application of the CA model is to derive the patterns of joint effects of mixtures whose components and concentrations were fixed (Ji et al., 2013; Liu et al., 2013; Mater et al., 2014). Consequently, few studies have applied the CA model to the toxicity prediction of actual environmental samples, most of which are domestic wastewater, reclaimed water, and drinking water (Escher et al., 2013; Tang et al., 2014). Limited studies have addressed the toxicity prediction of industrial wastewater, especially semi-coking wastewater. Carbajo et al. (2015) reported that the CA model could accurately predict the toxicity to *V. fischeri*, *Pseudomonas putida*, *Tetrahymena thermophila*, and activated sludge for 86% of the binary mixtures that are preservatives combined with the industrial wastewater from a cosmetics manufacturing facility. It is basically consistent with the present study. This study provides a reasonable reference for toxicity prediction of semi-coking wastewater using the CA model, based on detected contaminants.

4. Conclusion

The semi-coking WWTP employing A/O biological treatment combined with coagulation and adsorption treatment, showed satisfactory performance in decreasing the conventional indices by achieving more than 90.0% removal, and the concentrations of 21 target contaminants, including five benzenes, 11 phenols, and five PAHs. Nevertheless, the final effluent still showed bioluminescence inhibition toxicity, and immobilization and lethal toxicity to *D. magna*. The variation of biotoxicity in the semi-coking wastewater treatment processes implies that the coagulation and adsorption processes resulted in more efficient biotoxicity removal than A/O biological treatment, due to the high removal of PAHs during the coagulation and adsorption processes. Alternatively, the promising treatment units, such as the membrane bioreactor, which was regarded as an efficient industrial wastewater treatment process, were recommended for reducing the ecological risk of semi-coking wastewater and protecting the aquatic environment. Additionally, the CA model, calculated from the concentration and toxicity of the dominating individual contaminants in water, was demonstrated to be valid for the toxicity prediction of wastewater from a semi-coking WWTP.

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