Reduction in Toxicity of Coking Wastewater to Aquatic Organisms by Vertical Tubular Biological Reactor

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Abstract

We conducted a battery of toxicity tests using photo bacterium, algae, crustacean and fish to evaluate acute toxicity profile of coking wastewater, and to evaluate the performance of a novel wastewater treatment process, vertical tubular biological reactor (VTBR), in the removal of toxicity and certain chemical pollutants. A laboratory scale VTBR system was set up to treat industrial coking wastewater, and investigated both chemicals removal efficiency and acute bio-toxicity to aquatic organisms. The results showed that chemical oxygen demand (COD) and phenol reductions by VTBR were approximately 93% and 100%, respectively. VTBR also reduced the acute toxicity of coking wastewater significantly:

Toxicity Unit (TU) decreased from 21.2 to 0.4 for *Photobacterium phosphoreum*, from 9.5 to

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21 0.6 for *Isochrysis galbana*, from 31.9 to 1.3 for *Daphnia magna*, and from 30.0 to nearly 0
22 for *Danio rerio*. VTBR is an efficient treatment method for the removal of chemical
23 pollutants and acute bio-toxicity from coking wastewater.

24 **Keywords:** acute toxicity; bio-toxicity; *Daphnia*; phenol removal; toxicity removal; toxicity
25 unit (TU)

26

1. Introduction

China is one of the largest coke producer of coke in the world, e.g. nearly 48% of total coal production in the world was produced in China in the year of 2012 (BP, 2013). Thus the coking wastewater is a typical industrial wastewater in China. Coking wastewater is composed of high concentrations of organic and inorganic pollutants, e.g. phenols, nitrogen heterocyclic compounds, polycyclic aromatic hydrocarbons, cyanide, sulfide and ammonia nitrogen (Yang et al., 2007; Kim et al., 2008; Lu et al., 2009; Zhang et al., 2012). Most of these compounds are refractory, toxic and carcinogenic, which may pose potential ecological risk to the receiving water bodies (Lai et al., 2007; Zheng et al., 2007).

The bio-toxicity of coking wastewater was commonly tested with lux bacteria (Zhao et al., 2009; Ma and Wang, 2013; Zhao et al., 2014), alga (Zhao et al., 2014), invertebrate (Zitova et al., 2009; Zhao et al., 2014), fish and plants (Dong and Zhang, 2010; Han et al., 2011; Wei et al., 2012; Zhao et al., 2014), involving acute toxicity (Zitova et al., 2009; Zhu et al., 2013b; Zhao et al., 2014) or/and genotoxicity (Dong and Zhang, 2010; Han et al., 2011; Zhu et al., 2013a). However, there was only segmental information on the eco-toxicity profile of coking wastewater until now. A battery of toxicity test is demonstrated to be useful to clarify the toxicity profile for complex environmental samples (e.g. wastewater) using different aquatic test organisms (i.e. bacterium, algae, Daphnia and fish) (Fang et al., 2012; Zhao et al., 2014). Herein, a battery of toxicity test is needed to clarify the toxicity profile of coking wastewater.

Conventional activated sludge (CAS) technology has been widely used in industrial wastewater treatment. However, the refractory and toxic compounds in coking wastewater often inhibit the performance of microorganism in CAS. The effluents from CAS process often do not meet the regulation standards for discharge in China, therefore, modified CAS or some other advanced technology has been developed to remove chemical oxygen demand (COD), ammonia or phenolic compounds further (Zhao et al., 2009; Lu et al., 2010; Yuan et al., 2012; Jin et al., 2013; Wei et al., 2012; Zhao et al., 2014).

VTBR is a novel biological treatment technique (China patent: ZL00131550.1, ZL00131325.8 and ZL00131326.6). Based on deep-well process, VTBR integrates some treatment processes, i.e. biofilm reaction, compressed bio-contact oxidation, anaerobic denitrification, aerobic hydrolysis and sludge digestion, into single process with the aid of delicately-selected filler material and well-designed combined reactors. VTBR is characterized by high gas-liquid mass transfer efficiency and efficient oxygen utilization even under high COD load. VTBR has been applied in some kinds of industrial wastewater with good performance in COD and ammonia removal (Xu et al., 2010). However, there is no information on its toxicity removal performance until now.

In this study, the whole eco-toxicity profile of industrial coking wastewater was investigated before and after the VTBR process. A battery of acute toxicity test was conducted using lux bacteria (*P. phosphoreum*), alga (*I. galbana*), crustacean (*D. magna*) and fish (*D. rerio*). Meanwhile, conventional chemical indexes (e.g. COD, volatile phenol) were

also investigated. This study aims to clarify the whole acute toxicity profile of coking wastewater and to evaluate the acute toxicity removal efficiency of VTBR.

2. Materials and Methods

2.1. Wastewater sample

The industrial coking wastewater is the wastewater mixture from different processes in the steelwork, e.g. ammonia distillation, gas purification and/or recycles process. Our sample was collected from a typical steelwork in April, 2012 in Dalian, China. The water quality parameters of influent to VTBR were shown in **Table 1**. No additional nitrogen and phosphorus was added during the treatment process since the existing $\text{NH}_3\text{-N}$ and phosphorus in the wastewater were enough for the growth of micro-organisms.

2.2. Operation condition of VTBR

Laboratory scale VTBR (**Fig. 1**) was set up with three poly-methyl methacrylate columns (6 L) in series, filled with the elastic fibrous filler that enhance the volume of microorganisms. It can be seen that aeration was flowed to the VTBR from the first column only; therefore, the gas went through the vertical baffle in the same direction of the liquid inside the whole VTBR process. Microorganisms within active sludge grew well around the filler columns, where aerobic species dominated in the outside layer, anoxic species presented in the medium layer and anaerobic species dominated in the inner layer.

The operational conditions were as follows: inflow rate was 0.6 L h^{-1} , sludge reflux ratio

was 1:1, hydraulic retention time was 30 h, pH was 7.9 ± 0.2 , temperature was $21.5 \pm 0.5^{\circ}\text{C}$ and the first column was only aerated with air compressor. The activate sludge used to start VTBR was taken from a typical municipal wastewater treatment plant, Chunliuhe wastewater treatment plant, in Dalian, China.

2.3. Chemical analysis

Both influent and effluent water samples were subjected to the following chemical analyses: COD, measured with potassium dichromate titration; $\text{NH}_3\text{-N}$, analyzed according to the Nessler's reagent spectrophotometry; total nitrogen (TN), tested by the alkaline potassium persiflage digestion-UV spectrophotometric method; Phenolics, determined according to 4-AAP spectrophotometric method; dissolved oxygen (DO), analyzed using portable dissolved oxygen meter (Mettler toledo, USA); pH, determined by pH meter (Sartorius, Germany); biology oxygen demand (BOD), tested using BOD Trak meter (Hach, USA). Organic substances were detected by gas chromatography-mass spectrometer (GC-MS) (Agilent, USA). The pretreatment of the samples and the GC-MS analysis were performed in accordance with the methods described in Lai et al. (2008).

2.4. Acute bio-toxicity test

Wastewater samples (influent and effluent) were tested with a series of dilution in triplicate unless otherwise specified. The toxicities of the samples (EC_{50} or LC_{50}) were expressed by the dilution factor.

2.4.1. Bioluminescence inhibition test

Bioluminescence inhibition test was conducted according to the Luminescent bacteria test procedure (SEPA, 1995). The luminescence of *P. phosphoreum* was detected using Lumin Max-C (Maxwell, USA). The *P. phosphoreum* was provided by the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. The bioluminescence inhibition test was conducted after the *P. phosphoreum* exposed to the wastewater for 20 minutes. Then the luminescence was then measured after exposure immediately.

2.4.2. Alga growth inhibition test

Alga toxicity was conducted by measuring the growth inhibition of freshwater green alga *I. galbana* according to OECD 201 (2006). Here, *I. galbana*, friendly provided by Chinese National Marine & Environment Monitor Center (Dalian, China), was selected to be test microorganism based on our pre-tests results. Our pre-tests showed that *I. galbana* was the most sensitive algae species to the toxicity of coking wastewater among the four species of microalgae tested (*I. galbana*, *Scenedesmus obliquus*, *Chlorella pyrenoidosa* and *Chlorella spp.*).

2.4.3. *Daphnia magna* acute immobilization test

D. magna 48 h acute immobilization test was carried out according to the OECD 202 (2004) with a few modifications. Exposure experiment was conducted with five neonates (less than 24 h old) in 50 ml test solution. Here, five test concentration series were conducted with four replicates.

2.4.4. Zebra fish swimming behavior inhibition test

The lethal toxicity to zebra fish was firstly conducted within 96 h using static method according to ISO (1996) with some modifications. Six healthy zebra fish (length of 35 ± 5 mm, weight of 0.3 ± 0.1 g) were put in the same beaker (1 L test solution in 1.5 L container), containing four dilution series of coking wastewater influent (2%, 3%, 5% and 10%), and four VTBR effluent (20%, 40%, 80% and 100%). The concentrations of test solution were assigned on the basis of pre-test results, which conducted with triplicate per concentration. After 24 h, 48 h and 96 h, the lethal rate was recorded.

Another acute toxicity tests called “swimming behavior inhibition test” was also performed as described in Kang et al. (2009), using 3D-biosensor developed by Seiko Electric Ltd., Japan. One piece of healthy zebra fish was exposed to test solution for 60 min after pre-exposure with dechlorinated tap water for 30 min to let the fish adapt to the small size of test chamber. The test solutions were designed as the same concentration as the lethal toxicity test to fish. The swimming behavior of test fish was investigated for total 90 min, whose 3D data (x, y, z coordinates) were recorded three times per second. Surface behavior was defined as that the vertical position of fish stayed at 20 mm below the surface in the test chamber (10*10*15 cm). The frequency of swimming speed and duration time of surface behavior of test fish were used to reflect the toxicity of test solution. Each concentration of coking wastewater was repeated 8 times. Here, all of the parameters were retrieved from the raw records by 3D-biomonitor and analyzed at consecutive 10 min interval.

2.5. Data analysis

Toxicity data was expressed with EC_{50} (concentration for 50% of maximal effect) or LC_{50} (lethal concentration 50) values and its 95% confidence intervals, which were calculated with SPSS 20.0 (IBM, USA). One-way ANOVA was used to check the significant difference ($p < 0.05$) between the toxicity data of the influent and effluent within software SPSS 20.0. The data showed in this study were expressed as mean $\pm$ SD unless otherwise specified.

TU approach was also introduced to reflect the acute toxicity, which was expressed as $TU = 1/EC_{50}$. According to the toxicity classification system introduced by Persoone et al. (2003), the toxicity of the effluent was divided into five levels as follows: no acute toxicity: $TU < 0.4$; slight acute toxicity: $0.4 < TU < 1$; acute toxicity: $1 < TU < 10$; high acute toxicity: $10 < TU < 100$; very high acute toxicity: $TU > 100$.

3. Results and Discussion

3.1. Chemicals removal

3.1.1. COD and phenol removal

VTBR removed $93.0 \pm 3\%$ of COD, from $1340 \pm 263 \text{ mg L}^{-1}$ in influent to $100 \pm 51 \text{ mg L}^{-1}$ in effluent. The COD of effluent can meet the discharge standard of coking wastewater regulated in China (SEPA, 1996). The phenol in VTBR effluent decreased to $2.70 \pm 0.80 \text{ mg L}^{-1}$ from $605 \pm 128 \text{ mg L}^{-1}$, with the removal of about 100%, but it was still higher than the discharge standard of 0.5 mg L^{-1} that regulated by GB8978-1996 in China.

Studies on coking wastewater treatment using CAS showed that the COD removal efficiency was not as effective or stable as expected (Zhao et al., 2009). Jin et al. (2013)

found that 83% COD and 100% phenol were removed by A/A/O biological treatment. Yuan et al. (2012) concluded COD removal of coking wastewater improved from 69% to 89% after pretreating coking wastewater with the extraction replacement method for 5 min using a 1:1 ratio of extract agent to wastewater, which followed by biodegradation treatment. It is known that COD and phenol removal efficiency by conventional biological treatment process are heavily dependent on the influent loads. Consequently, the effluent should be further purified by advanced process or combined/modified biological process. Comparatively, VTBR, a novel biological treatment process, can remove more than 90% COD and 99% phenol, even under the relatively high COD and phenol influent load, which may be contributed to the high oxygen utilization and the high concentration of mixed liquid suspended solids (MLSS). Therefore, it can be concluded that the performance of VTBR is better than the conventional biological treatment process with or without pretreatment.

3.1.2. Organic compounds removal

The organic compounds in coking wastewater were analyzed with GC/MS. The organic compounds detected in the influent and in the effluent are shown in **Table 2**. The major organic compounds in influent were phenols. Meanwhile, some kinds of phenols in the influent could not be detected in the effluent, while other new compounds were found in the effluent. It illustrated that VTBR can remove most of the organic compounds in the influent, and some new organic compounds presented within VTBR process. This suggested that the toxicity profile may be different between influent and effluent.

3.2. Toxicity bioassay

The dilution factor of the influent/effluent was used to express the concentration of test solution. Here, the concentration of 100% means the test solution (influent or effluent) without dilution.

3.2.1. Microtox test

The luminous intensity inhibition rates for VTBR influent and effluent are shown in **Fig. 2A**. It can be seen that the inhibition rate of luminous bacteria was about 90% when exposed to 5% influent and it was only 9% when exposed to the whole effluent. TU of coking wastewater by VTBR decreased from 21.2 (influent) to 0.4 (effluent). According to the toxicity classification system raised by Persoone et al. (2003), the influent of coking wastewater was classified to be high acute toxicity, while the effluent of VTBR was slight acute toxicity. It can be seen that VTBR could reduce most portion of acute toxicity to luminous bacteria from the coking wastewater. Zhao et al. (2014) evaluated the toxicity of coking wastewater treated by four stages process (anoxic-oxic-hydrolytic-oxic couples with biological fluidized bed) with *E. Coli* HB 101 pUCD607 for 15-min inhibition, and found that the TU for influent was 16.2 and for effluent was 0. Compared with their four-stage-process, our VTBR can also remove the acute toxicity to luminous bacteria effectively.

3.2.2. Alga growth inhibition test

After 96 h exposure, the dose-response curves of influent and effluent on *I. galbana* are

shown in **Fig. 2B**. The calculated 96-h EC_{50} of coking wastewater influent was about 11%, nearly equal to the 96-h TU of 9.5; meanwhile, the 96-h TU of the effluent from VTBR was only 0.6. VTBR could reduce acute toxicity of coking wastewater to alga from acute toxicity level to slight acute toxicity level. Other study (Zhao et al., 2014) showed that their typical biological treatment process cannot remove the acute toxicity to algae with 72h inhibition test.

3.2.3. *D. magna* acute immobilization test

The dose-response curves of *D. magna* acute immobilization test for the VTBR influent and effluent are shown in **Fig. 2C** and **Fig. 2D**. It can be seen that the mean value of 48-h EC_{50} for the influent was about 3% (95% CI: 2.90% – 3.50%) ($p < 0.05$), while the 48-h EC_{50} for the effluent from VTBR were increased to 77% (95% CI: 73.6% – 80.7%) ($p < 0.05$). That is to say, TU (48h) of the influent was about 31.9 and TU (48h) of the effluent was about 1.3. It can be seen that acute toxicity of coking wastewater to *D. magna* was effectively reduced by VTBR. Although our VTBR were not as effective in acute toxicity removal as that of four-stage-process (anoxic-oxic-hydrolytic-oxic couples with biological fluidized bed) (Zhao et al., 2014), our VTBR was still comparative due to our simple treatment process.

3.2.4. Zebra fish swimming behavior inhibition test

According to the zebra fish 96 h acute toxicity pre-test exposed to the influent and VTBR effluent, the 96-h LC_{50} of influent was about 3% and no fish died after 96 h exposure to the VTBR effluent. That is to say, the TU of the influent was about 30 and for the effluent was 0.

Moreover, zebra fish swimming behavior inhibition test was conducted to evaluate the acute toxicity profile of coking wastewater influent and effluent, by examining the changes of swimming speed and vertical position. The changes of swimming speed of test fish exposed to control, influent and VTBR effluent are shown in **Fig.3 (A)**. When exposed to the control solution, the frequency of swimming speed did not change distinctly within 90 minutes, where the most frequent swimming speed was 21-40 mm s⁻¹. When exposed to the influent, the swimming speed decreased suddenly and the dominant swimming speed was 0-20 mm s⁻¹ within 90 minutes. Compared with that exposed to the control, it can be seen that the swimming speed hardly changed after exposed to VTBR effluent, which illustrated that the toxicity of influent to fish was mostly reduced by VTBR.

The duration time of surface behavior was also investigated and statistically analyzed (shown in **Fig.3 (B)**). From **Fig.3 (B)**, it can be seen the duration time of surface behavior of fish exposed to VTBR effluent was similar to that exposed to the control, while it differed significantly from that exposed to the influent. These results were consistent with the former study that found that the duration time of surfacing behavior increased with the increment of the toxic chemical concentration (Little and Finger, 1990). This suggested that VTBR could reduce most of the toxicity to the fish in the view of surface behavior.

Generally, both the swimming speed and the surface behavior changed significantly when the test fish exposed to influent, while there was no obvious changes after exposed to the control and VTBR effluent. This suggested that VTBR could reduce the toxicity of coking

wastewater to the swimming behavior of zebra fish effectively. The swimming behavior inhibition test was first used in the evaluation of toxicity profile for coking wastewater as we know.

3.2.5. Generalization of Toxicity

In this study, the acute toxicity profile of coking wastewater was demonstrated with four test organisms: bacteria (*Photobacterium phosphoreum*), algae (*Isochrysis galbana*), Crustacean (*Daphnia magna*) and fish (*Danio rerio*). The raw industrial coking wastewater presented high toxicity to all these four test species with TU ranging from 9.50 to 31.9. After treatment with our novel process VTBR, the toxicity of the effluent to four test species near zero except to the *Daphnia Magna*. From the values of TU of four test species to the coking wastewater given in **Fig.4**, it can be seen that *Daphnia magna* presented the relatively high sensitivity to the toxicity of coking wastewater influent and effluent. VTBR illustrated the effective performance on the toxicity removal of coking wastewater. Moreover, the swimming behavior inhibition test was also used to evaluate the toxicity profile for coking wastewater, which illustrated that it was more effective than the fish inhibition test, especially to the low toxicity wastewater.

4. Conclusions

In this study, the performance of VTBR in coking wastewater treatment was evaluated by a battery of bio-toxicity test, as well as chemical analysis. The findings are as follows: VTBR was shown to be effective not only in the reduction of conventional index (COD and phenol),

but also in the reduction of acute toxicity. *Daphnia* was found to be the most sensitive test organism within the selected test organisms to the toxicity of coking wastewater. VTBR can be an alternative for industrial coking wastewater treatment.

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Table 1 Chemical parameters of industrial coking wastewater.

Characterization of influent (n=5)		
Chemical parameters	Range	Mean
COD (mg L ⁻¹)	893 - 1790	1340 ± 263
NH ₄ ⁺ (mg L ⁻¹)	103 - 640	372 ± 194
Phenol (mg L ⁻¹)	415 - 792	605 ± 128
BOD (mg L ⁻¹)	760 - 1520	1140 ± 224
TN (mg L ⁻¹)	273 - 863	572 ± 251
pH (mg L ⁻¹)	7.7 - 8.1	7.9 ± 0.2
TP (mg L ⁻¹)	10 - 15	-
TOC (mg L ⁻¹)	1140 - 1340	1220 ± 85
Temperature (°C)	21 - 22	21.5 ± 0.7

Table 2 Organic compounds detected in influent and effluent of VTBR.

No.	Compounds	Influent	Effluent	No.	Compounds	Influent	Effluent
1	Phenol	+	-	15	N-Cyclohexyl-1,3-propanediamine	-	+
2	p-Cresol	+	-	16	Naphthalene	-	+
3	m-Cresol	+	-	17	1-Ethylidene-1H-indene	-	+
4	2,3-Dimethylphenol	+	-	18	1,3-Diazaspiro[4.5]decane-2,4-dione	-	+
5	2-Ethylphenol	+	-	19	1,1'-Sulfonyldibenzene	-	+
6	2,5-Dimethylphenol	+	-	20	4-Isopropyl-1-methyl-2-cyclohexen-1-ol	-	+
7	3,4-Dimethylphenol	+	-	21	2-(Azidomethyl)-1,3,3-trimethylcyclohexene	-	+
8	2,4-Dimethylphenol	+	-	22	2-Isopropyl-5-methylphenyl methylcarbamate	-	+
9	4-Ethyl-2-methylphenol	+	-	23	3,3-Dimethylpiperidine	-	+
10	3-Ethylphenol	+	-	24	1-Methylnaphthalene	-	+
11	2,4,6-Trimethylphenol	+	-	25	1-Methyl-2-piperidone	-	+
12	2-Isopropylphenyl methylcarbamate	+	-	26	3,5-Dimethyl-2-(2-methyl-2-propanyl)-4-imidazolidinone	-	+
13	4-(2-Aminopropyl)phenol	+	-	27	1-(1,3-Thiazol-2-yl)piperidinone	-	+
14	Benzene	+	-				

Note: “+” detected, “ - ” not detected.

Fig. 1 The schematic diagram of VTBR

Fig. 2 Dose-response of coking wastewater on lux bacteria, alga and daphnia

Fig. 3 Swimming behavior changes after exposure to test solutions

(A) Frequency of swimming speed of fish exposed to test solutions (n=8);

(B) Duration time of surfacing behavior of fish (n=8).

Fig. 4 Acute toxicity units of the influent and effluent for the tested organisms

Vertical Tubular Biological Reactor

1. glass rotameter(gas) 2. peristaltic pump 3. reaction column 4. sedimentation tank
5. exhaust 6. packing 7. valve 8. sampling point 9. gas diffuser

Fig. 1 The schematic diagram of VTBR

Fig. 2 Dose-response of coking wastewater on lux bacteria, alga and daphnia

(A): Luminous intensity inhibition rate of influent and VTBR effluent on

Photobacterium phosphoreum; (B): Dose-response curve on *Isochrysis galbana* at 96

h; (C) and (D) showed the Dose-response curve of *Daphnia magna* acute

immobilization test (48 h) to influent and effluent of VTBR respectively, the dotted line presents 95% confidence interval of 48 h effect concentrations.

$\ln C$ in (B) means the Napierian Logarithm of concentration.

Fig. 3 Swimming behavior changes after exposure to test solutions

(A) Frequency of swimming speed of fish exposed to test solutions (n=8);

(B) Duration time of surfacing behavior of fish (n=8).

Fig. 4 Acute toxicity units of the influent and effluent for the tested organisms