**Evaluation of the detoxication efficiencies for acrylonitrile
wastewater treated by a combined anaerobic oxic-aerobic biological
fluidized tank (A/O-ABFT) process: acute toxicity and zebrafish
embryo toxicity**

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Abstract

Acrylonitrile (ACN) wastewater generated during ACN production has been reported to be toxic to many aquatic organisms. However, few studies have evaluated toxicity removal of ACN wastewater during and after the treatment process. In this study, the detoxication ability of an ACN wastewater treatment plant (WWTP) was evaluated using *Daphnia magna*, *Danio rerio* and zebrafish embryo. This ACN WWTP has a combined anaerobic oxic-aerobic biological fluidized tank (A/O-ABFT) process upgraded from the traditional anaerobic oxic (A/O) process. Moreover, the potential toxicants of the ACN wastewaters were identified by gas chromatography-mass spectrometry (GC-MS). The raw ACN wastewater showed

high acute and embryo toxicity. 3-cyanopyridine, succinonitrile and a series of nitriles were detected as the toxic contributors of ACN wastewater. The A/O process was effective for the acute and embryo toxicity removal, as well as the organic toxicants. However, the A/O effluent still showed acute and embryo toxicity which was attributed by the undegraded and the newly generated toxicants during the A/O process. The residual acute and embryo toxicity as well as the organic toxicants in the A/O effluent were further reduced after going through the downstream ABFT process system. The final effluent displayed no significant acute and embryo toxicity, and less organic toxicants were detected in the final effluent. The upgrade of this ACN WWTP results in the improved removal efficiencies for acute and embryo toxicity, as well as the organic toxicants.

Keywords: Acrylonitrile wastewater; upgrade; *Daphnia magna*; *Danio rerio*; GC-MS; toxicant.

1. Introduction

Acrylonitrile (ACN) is an important intermediate used to produce a variety of organic products, such as plastics, acrylic fibers, synthetic rubber and resins (Wyatt and Knowles, 1995b; Lu et al., 2000; Fechter et al., 2003). The yield of ACN has increased with the increasing demand for those products. To date, the production of ACN exceeds 5 million tons worldwide and approximately 1.4 million tons are produced in China each year (Zheng et al., 2014; Zheng et al., 2015).

The Sohio process is widely used in industry to produce ACN (Cespi et al., 2014). Generally, one cubic meter of ACN wastewater is generated in producing one ton of

45 ACN in Sohio process (Léonard et al., 1999; Blom et al., 2008). The ACN wastewater
46 is characterized to be complicated, refractory, and very toxic (Han et al., 2014). It has
47 been reported that ACN wastewater contains many organic components, such as ACN,
48 acryl amide, acrylic acid, acrolein, cyanopyridine, fumaronitrile, succinonitrile,
49 maleimide and many more (Wyatt and Knowles, 1995a; Kumar et al., 2008). Many of
50 these components are toxic and hazardous to aquatic organisms as well as to human
51 beings (Fechter et al., 2003; Pu et al., 2006). But the toxicity removal efficiency of
52 ACN wastewater remains unknown. Hence, it is essential to understand the toxicity of
53 wastewaters from different treatment units in an ACN wastewater treatment plant and
54 to identify the toxicants in the ACN wastewaters from different treatment stages.

55 Biodegradation treatment process is one of the most applied process for ACN
56 wastewater treatment (Shin et al., 2009), and the effluent quality is mainly evaluated
57 by physicochemical parameters, such as chemical oxygen demand (COD), ammonia
58 nitrogen ($\text{NH}_4^+\text{-N}$), and total organic carbon (TOC). However, even though the effluent
59 has met the discharge standard, some refractory pollutants in the ACN wastewater still
60 cannot be efficiently removed and the residual pollutants may cause adverse effects
61 on aquatic organisms even at a low concentration (Bolong et al., 2009). Biological
62 toxicity tests are useful tools for assessing the toxic effect of the wastewater samples
63 to evaluate the safety of the wastewater samples (Ma et al., 2005). For example, the
64 whole effluent toxicity testing (WET) proposed by the United States Environment
65 Protection Agency (USEPA) recommend the use of multiple test species, such as
66 bacteria, invertebrates and fishes, in the evaluation of wastewater toxicity (Zhu et al.,

2013; Zhao et al., 2014).

In this work, the detoxification efficiencies of the biodegradation treatment process in an ACN wastewater treatment plant in northeast China were evaluated. The acute toxicity was tested using *Daphnia magna* and *Danio rerio*, and the fish embryo toxicity was tested using zebrafish egg. Furthermore, we used gas chromatography-mass spectrometer (GC-MS) to investigate the organic pollutants in the ACN wastewaters from different treatment stages in order to indentify the toxicants.

2. Materials and methods

2.1. ACN wastewater treatment plant and sampling scheme

The ACN wastewater treatment plant in this study is located in northeast China. It receives the ACN production wastewater and municipal sewage generated in the company range with 15-20 T/h and 60-70 T/h, respectively. **Fig. 1** briefs a flowchart of the treatment process in the ACN wastewater treatment plant. Anaerobic oxic (A/O) process was firstly applied in this plant. However, with the increasing production of ACN and the stricter requirements for wastewater discharge standard, the effluent couldn't meet the integrated wastewater discharge standard (GB8978-2002). Thus, it was upgraded from A/O process to anaerobic oxic-aerobic biological fluidized tank (A/O-ABFT) process by combining an ABFT process system, which was composed of anoxic tank, ABFT, nitrifying tank and the third sedimentation tank.

The wastewater samples were collected according to the composite sampling methods in July 2014 (USEPA, 2002). We collected the wastewater samples at eight

different points marked by stars as shown in **Fig. 1**, including the wastewaters from adjusting tank as raw influent (RI), anaerobic tank effluent (A), oxalic tank effluent (B), A/O process effluent (AOE) from secondary sedimentation tank, anoxic tank effluent (C), ABFT effluent (D), nitrifying tank effluent (E) and final effluent (FE). All the wastewater samples were kept in dark at 4 °C for the toxicity tests.

Fig. 1. Flowchart of ACN wastewater treatment plant and sampling scheme. RI = Raw influent, A = Anaerobic tank effluent, B = Oxidation tank effluent, AOE = A/O process effluent, C = Anoxic tank effluent, D = ABFT effluent, E = Nitrifying tank effluent, FE= Final effluent

2.2. Physicochemical analysis of water quality

Physicochemical analysis was performed for water quality parameters such as dissolved oxygen (DO), pH, temperature, $\text{NH}_4^+\text{-N}$, COD, TOC and total phosphorus (TP) (Muna et al., 1995). Among these parameters, DO, pH and temperature were measured on site immediately after collection, and the remaining were measured in the laboratory using the supernatants after standing at 4°C.

2.3. Toxicity tests

2.3.1. Acute toxicity tests

The acute toxicity of ACN wastewaters was tested using *Daphnia magna* and

Danio rerio.

D. magna acute immobilisation test. 48 h *D. magna* acute immobilisation test was conducted according to the OECD Guideline 202 (OECD, 2004). *D. magna* were cultured in the medium under a constant temperature of 19 ± 1 °C and a 16 h: 8 h light: dark cycle in incubator. Fresh green algae (*Scenedesmus obliquus*) was served as food once a day (Besseling et al., 2014). The exposure experiment was conducted in the same conditions as stock culture. For each wastewater sample at least five concentrations diluted by a constant factor were tested, with four replicates of each test concentration. After exposure, the 48 h median lethal concentration (LC₅₀) values and toxic units (TUs) were used to evaluate the acute toxicity (Bianchini and Wood, 2008).

D. rerio acute toxicity test. 96 h *D. rerio* acute toxicity test was carried out following the OECD Guideline 203 (OECD, 1992). Zebrafish used in this study were obtained from Model Animal Research Center of Nanjing University (Nanjing, China) and raised in tap water treated by aeration and active carbon filter. The zebrafish stock cultures were kept at 25 ± 1 °C under a 14 h: 10 h light: dark cycle. They were fed with fresh *Artemia franciscana* daily. The bioassay was performed in the same conditions as stock cultures. Adult zebrafish were exposed to five concentrations of wastewater samples in triplicate for 96 h without feeding (Zhang et al., 2013). The 96 h LC₅₀ values and TUs were used to characterize the acute toxicity.

2.3.2. Fish embryo toxicity test

Short-term Toxicity Test on Fish Embryo and Sac-Fry Stages was conducted

according to OECD Guideline 212 with zebrafish embryo (OECD, 1998). Newly fertilized embryos in a same stage of cleavage were collected. The embryos were incubated at 26 ± 1 °C under a 14 h: 10 h light: dark cycle in incubator. At least five concentrations were tested per wastewater sample. The raw influent was diluted into a concentration series of 2.8%, 4%, 5.6%, 8% and 11.2%, the anaerobic tank effluent, oxic tank effluent, A/O process effluent and anoxic tank effluent were diluted into a concentration series of 7%, 10%, 14%, 20% and 28%, the ABFT effluent, nitrifying tank effluent and final effluent were diluted into a concentration series of 31.5%, 40%, 50%, 63% and 80%. The embryos were exposed under semi-static condition in four replicate beakers with test solution renewal every 48 h for 9 days. During the exposure, the following endpoints were investigated under a stereoscopic microscope daily: mortality, hatching and abnormalities of the embryos or larvae. The median effective concentration (EC_{50}) values and TUs for hatching rate (HR) (in the 5th day of exposure), total survival rate (TSR), survival rate after hatching (SRAH) and survival index (SI) (in the 8th day of exposure) were used as measurements to evaluate the embryo toxicity of the wastewater samples. The survival index was calculated based on the equation

$$\text{Survival Index} = \frac{\text{Hatching Rate} \times \text{Survival Rate after Hatching}}{1}$$

Besides that, the effects of the wastewater exposure on the phenotypes of lived larvae (at the end of exposure) were also assessed.

2.4. *Chemicals analysis*

To better understand the bioassay results, we investigated the potential organic

pollutants in the ACN wastewater samples from different treatment stages by gas chromatography-mass spectrometer (GC-MS) (Lai et al., 2012). At first, wastewater samples were pretreated by liquid-liquid extraction as follows : 100 mL of water sample were extracted twice with 10 mL CH₂Cl₂ (HPLC grade) at pH 2.0, 7.0 or 12.0, respectively, all the extracts were combined, dehydrated with anhydrous sodium sulfate, dried under a gentle N₂ stream, and then redissolved the residues in 1 mL CH₂Cl₂ for GC-MS detection. At least two replicates were performed for each treatment, and ultrapure water pretreated at identical procedures was set as blank. samples (μ L) were injected into a GC-MS system equipped with a HP-5MS capillary column (3 m . 25 mm . 25 μ m). e lium (purity 99.999%) was used as carrier gas (1.0 mL min⁻¹). The injector temperature was kept at 220 °C and splitless mode was used for injection. The mass detector was operated in the scan mode with mass range m/z 30-500. The temperatures for transfer line and ion source were set at 250 °C and 230 °C, respectively.

2.5. Statistical analysis

The derived TUs were used to assess the acute and embryo toxicity of the wastewater samples, which were calculated based on the formulas below (Karaouzas et al., 2011):

$$(LC_{50}) \quad 2$$

$$(EC_{50}) \quad 3$$

LC₅₀ or EC₅₀ were determined from the dose-response curve by probit analysis with their 95% confidence intervals using the SPSS (version 13.0). The acute toxicity

results were classified according to the following toxicity classification system applied for wastewaters discharged (Tonkes et al., 1999; Persoone et al., 2003): TU < 0.4, no acute toxicity; 0.4 < TU < 1, slight acute toxicity; 1 < TU < 10, acute toxicity; 10 < TU < 100, high acute toxicity; TU > 100, very high acute toxicity.

3. Results and discussion

3.1. Wastewater quality analysis

The COD, TOC and NH₄⁺-N of ACN wastewater samples at different stages from the treatment process are shown in **Table 1**. The other physicochemical parameters are summarized in **Table S1** in the supplement data.

Table 1

Physicochemical parameters of the ACN wastewater from different treatment stages

Parameters (mg L ⁻¹)	Standard ^a (mg L ⁻¹)	A/O process				ABFT process system			
		RI	A	B	AOE	C	D	C	FE
COD	50	378	108	88.1	81.5	73.6	53.5	40.9	39.6
TOC	- ^b	411	206	190	173	150	38.9	21.3	18.1
NH ₄ ⁺ -N	8	47.6	88.1	88.0	84.6	80.6	53.2	40.2	39.2

^a the first graded of integrated wastewater discharge standard in China (GB8978-2002). ^b does not

exist. Refer to Figure for the samples A–FE.

As shown in **Table 1**, the COD and TOC of raw influent were 378 mg L⁻¹ and 411 mg L⁻¹, respectively. The COD and TOC were gradually decreased to 81.5 mg L⁻¹ and

173 mg L⁻¹ after going through the A/O process, respectively. The effluent COD didn't meet the integrated wastewater discharge standard. After the treatment process upgrading, the COD and TOC were further reduced to 39.6 mg L⁻¹ and 18.1 mg L⁻¹ by the ABFT process system, respectively. The final effluent COD met the integrated wastewater discharge standard. The results indicated that the ABFT process system was effective for the COD and TOC removal. As shown in **Table 1**, the NH₄⁺-N was increased from 47.6 mg L⁻¹ to 84.6 mg L⁻¹ after going through the A/O process due to the ammonification of organic nitrogen (de Guardia et al., 2008). The effluent NH₄⁺-N didn't meet the integrated wastewater discharge standard. After the treatment process upgrading, the NH₄⁺-N was gradually decreased from 84.6 mg L⁻¹ to 39.2 mg L⁻¹ by the treatment of ABFT process system. It indicated that the ABFT process system was effective for the NH₄⁺-N removal. But the effluent NH₄⁺-N still didn't meet the integrated wastewater discharge standard. The result illustrates the need of further wastewater treatment to efficiently remove the NH₄⁺-N.

3.2. Acute toxicity

The results of acute toxicity to *D. magna* and *D. rerio* of the ACN wastewater samples from different treatment stages in the process are shown in **Fig. 2** (LC₅₀ values were given in supplementary material).

Fig. 2. Acute toxicity results of ACN wastewater samples from different treatment stages. Dotted lines represent the boundary between the A/O process and the ABFT process system. Refer to Figure or the samples A–FE.

The toxic units (TUs) of *D. magna* acute toxicity test results of the wastewater samples from different treatment stages are shown in **Fig. 2A**. The raw influent of ACN wastewater showed very high acute toxicity to *D. magna* (48 h LC_{50} = 0.80%, 48 h TU = 125). The A/O process reduced the 48 h TU of *D. magna* from 125 to 2.40 with 98% removal efficiency. However, the A/O process effluent still showed slight acute toxicity to *D. magna* (48 h LC_{50} = 41.7%, 48 h TU = 2.40). After going through the ABFT process system, the 48 h TU of *D. magna* was decreased to less than 0.4. It suggested that the final effluent displayed no acute toxicity to *D. magna*.

D. rerio acute toxicity test results of the wastewater samples from different treatment stages are shown in **Fig. 2B**. The raw influent of ACN wastewater showed very high acute toxicity to *D. rerio* (96 h LC_{50} = 3.38%, 96 h TU = 29.6). The previous A/O process reduced the 96 h TU of *D. rerio* from 29.6 to 5.67 with 81% removal efficiency. However, the effluent from A/O process still displayed slight

acute toxicity to *D. rerio* (96 h LC₅₀ = 17.6%, 96 h TU = 5.67). After going through the ABFT process system, the 96 h TU of *D. rerio* was decreased to less than 0.4. It indicated that the final effluent showed no acute toxicity to *D. rerio*.

The results indicated that A/O process effectively removed the toxicants that responsible for the acute toxicity to *D. magna* and *D. rerio*. However, the A/O process effluent still contained some refractory toxicants and displayed slight acute toxicity to *D. magna* and *D. rerio*. The ABFT process system further removed the toxicants in the wastewater. The final effluent displayed no acute toxicity to both *D. magna* and *D. rerio*, which suggested that the compounds in the final effluent were not toxic to *D. magna* and *D. rerio*.

3.3. Fish Embryo toxicity

The zebrafish embryo toxicity test results are shown in **Fig. 3**. The hatching rate, total survival rate, survival rate after hatching, and survival index of the raw influent were 12.4, 14.1, 13.1 and 14.3 TUs, respectively. The EC₅₀ values of raw influent for the hatching rate and the survival rate after hatching of the zebrafish embryo were found as 8.04% and 7.66%, respectively (**Table S2**). It indicated that the raw influent caused significant inhibition in zebrafish embryo hatching and the larvae survival after hatching. The previous A/O process reduced the TUs of these four parameters to 4.73, 11.2, 11.2 and 11.4, respectively. The EC₅₀ values of A/O process effluent for the hatching rate and the survival rate after hatching were increased to 21.1% and 8.96%, respectively (**Table S2**). However, the effluent from the A/O process was shown to be toxic to zebrafish eggs. The TUs for these four parameters were further

reduced by the ABFT process system. And since no effects were found in the final effluent for the highest exposure concentration for zebrafish embryo EC_{50} values could not be ascertained. It indicated that the final effluent exhibited no observable toxicity to the zebrafish embryo and larvae. Moreover, the TUs of hatching rate were always lower than that of survival rate after hatching in each wastewater samples, which indicated that newly hatched larvae are more sensitive than the embryo to the ACN wastewater exposure. The possible reason was that the intact chorion could protect the zebrafish embryo from the toxicants in the wastewater (Hassan et al., 2008; Ruyra et al., 2013). In zebrafish embryos, pore channels (500-700 nm) in the three-layered chorion could act as an efficient barrier against the uptake of particles into the embryo (Felix et al., 2013; Weil et al., 2015). Shang and Zhdanova found that an intact chorion could protected the zebrafish embryos from cocaine (Shang and Zhdanova, 2007). Among these four parameters, the TU of survival index which were calculated based on the hatching rate and survival rate after hatching could characterize the embryo toxicity of the wastewater comprehensively.

Fig. 3. Embryo toxicity results of ACN wastewaters from different treatment stages. (A) TUs for hatching rate, total survival rate, survival rate after hatching and survival index. Dotted line represents the boundary between the A/O process and the ABFT process system. Refer to Figure 1 or the samples A–FE. (B) Effects of exposure to the raw influent, the A/O process effluent and the final effluent on embryonic phenotypes. a,b = normal phenotypes in blank; c~f = exposure to raw influent in 2.8%, 4.0%, 5.6% and 8.0% dilution times; g,h = exposure to A/O effluent in 7.0% and 10% dilution times; i,j = exposure to final effluent in 60% and 80% dilution times.

In addition, the exposure to ACN wastewater samples could result in the effects on zebrafish larvae phenotypes. Prevalent morphological effects of zebrafish larvae observed after exposure included pericardial and yolk sac edema, opaque tissues (particularly in the head region), and spinal curvature (McGee et al., 2012; Wiecinski et al., 2013). **Fig. 3B** shows the representative images of the relative severity of the malformations following 9 days exposure to the raw influent, A/O process effluent

and final effluent after treatment process upgrading. Images (a,b) show the normal phenotypes larvae in control. For the raw influent of ACN wastewater, spinal curvature was observed after exposure in 2.8% dilution (c), the exposure in 4.0% dilution times resulted in yolk sac edema and opaque tissues in the head region obviously (d), and the exposure in 5.6% and 8.0% dilution led to significant pericardial edema, yolk sac edema, opaque tissues in the head region and body length decrease (e,f). For the A/O process effluent, we observed no significant abnormal phenotypes after exposure in 7.0% dilution (g), but yolk sac edema and spinal curvature were observed with the exposure concentration increased to 10% dilution (h). In addition, the final effluent displayed no any significant effects on the zebrafish larvae phenotypes, apart from the little spinal curvature that was observed in the exposure in 60% and 80% dilution (i,j).

The embryo toxicity test results indicated that A/O process could reduce some toxicants that were responsible for the embryo toxicity in ACN wastewater. However, the effluent from the A/O process showed toxicity to zebrafish eggs. The dramatically decrease in the embryo toxicity of the ACN wastewater during the ABFT process system suggested that the refractory toxicants were effectively removed by the ABFT process system.

3.4. Chemical analysis

To investigate the potential toxic source of ACN wastewater, GC-MS was performed and the total ion chromatograms and the main organic compounds of the ACN wastewater samples are shown in the **Fig. 4**.

Fig. 4. Total ion chromatograms (A) and the main possibly organic compounds (B) of ACN wastewaters from different treatment stages obtained by GC-MS. Vertical coordinates for all chromatograms were in the same range except for the RI, which was compressed 5 times. Refer to

Figure or the samples A–FE. ■ detected. □ not detected. R.T. = Retention time

As shown in **Fig. 4A**, the total ion chromatograms for the effluents during A/O treatment process were different from that for the raw influent, which suggested that the organic compounds in raw influent had been removed or transformed into other compounds by the treatment of A/O process. The number and abundance of the peaks in the total ion chromatogram were decreased after going through ABFT treatment process, which indicated that the organic compounds in the wastewater were further removed or transformed by ABFT treatment process.

The potential organic compounds with relatively high peak abundance were identified by comparison with National Institute of Standards and Technology (NIST) mass spectral library database, and the details of organic pollutants identification in all the wastewater samples are supplied in **Table S2** in the supplemental data. The main toxic organic compounds existed in the wastewater samples are summarized in **Fig. 4B**. 3-cyanopyridine and succinonitrile were detected in the raw influent with high abundance corresponding to the highest peaks at retention time 6.950 min and 7.318 min. It was reported that the oral LD₅₀ values of 3-cyanopyridine were 1185–1680 mg kg⁻¹ and 1225 mg kg⁻¹ in rats and mice, respectively (Majka et al., 1979). The no observed adverse effect level (NOAEL) of 3-cyanopyridine for reproductive and developmental toxicity was 30 mg kg⁻¹ d⁻¹ to rats (Takahashi et al., 2013). They could be the main toxicants in raw influent of ACN wastewater. Besides that, many other toxic compounds were detected in the raw influent, such as

331 fumaronitrile, 3-anilinopropionitrile, 2,3-dimethyl butanedinitrile, imidazole,
 332 benzamidine, 5-ketohexanenitrile, 3-(diethylamino) propionitrile,
 333 2-methyleneglutaronitrile, nicotinamide, 1,2,4-benzenetricarbonitrile,
 334 1,4-dihydropyridine-3-carbonitrile, N-(2-cyanoethyl)-imidazole, 2-cyanopyridine.
 335 Suedel et al., found that the EC₅₀ and LC₅₀ values of fumaronitrile for freshwater
 336 species ranged from 7.7 to 187 µg L⁻¹, such as the 48 h LC₅₀ values to *D. magna* was
 337 105 µg/L (Suedel et al., 1993; Suedel, 1996), the EC₅₀ (growth rate) values to alga,
 338 *Pseudokirchneriella subcapitata*, was 190 µg L⁻¹ (Šepič et al., 2003). And the nitriles
 339 were belonged to the category of reactive toxicants, which could release cyanide ions
 340 through hydrolysis (Lipnick, 1991). Huang et al., found that various nitriles,
 341 especially halogen-substituted nitriles, showed toxicity to *P. subcapitata* (Huang et al.,
 342 2007). Some toxic organic compounds in the raw influent, such as 3-cyanopyridine
 343 and succinonitrile, were removed completely by the A/O process. However, the A/O
 344 process effluent still contained many toxicants and some were suggested to be the
 345 byproducts generated in A/O process, such as phenol, 3-methylhexanenitrile and
 346 4-cumylphenol. It has been reported that the 96 h LC₅₀ values of phenolic compounds,
 347 containing phenol and 4-cumylphenol, to fish species ranged from 5 to 70 mg L⁻¹
 348 (Brown et al., 2004). This could be related to the acute toxicity of the previous A/O
 349 effluent to *D. magna* and *D. rerio* and embryo toxicity to zebrafish eggs in this study.
 350 Moreover, the residual organic pollutants in the effluent from A/O process were
 351 further removed by ABFT process system. The organic chemicals detected in the final
 352 effluent seem to be little toxicity to the organisms, corresponding to the bioassay

results in this study.

4. Conclusion

The raw influent of ACN wastewater exhibited acute toxicity to *D. magna* and *D. rerio* and embryo toxicity to zebrafish eggs. After going through the A/O process, the acute and embryo toxicity were reduced, but the A/O process effluent still showed significant acute and embryo toxicity. After going through the subsequently ABFT process system, the acute and embryo toxicity were further reduced and the final effluent displayed no observable acute and embryo toxicity. The bioassay results suggested that ABFT process system could effectively remove the toxicants that weren't removed by A/O process. Through GC-MS analysis we found that the toxicity of raw influent was attributed by a variety of toxic organic compounds, such as 3-Cyanopyridine, succinonitrile and a series of nitriles. Many of these compounds were removed after going through the A/O process. The undegraded and the newly generated compounds in the A/O process effluent were effectively removed by the ABFT treatment process. The organic compounds detected in the final effluent seem to be little toxicity to the organisms. It was further demonstrated that that A/O-ABFT process was an effective treatment for the toxicants removal of ACN wastewater, corresponding to the bioassay results in this study. Moreover, this study revealed that *D. magna* and *D. rerio* acute toxicity and zebrafish embryo toxicity tests were effective for ACN wastewater biotoxicity assessment. This study also highlights that emission limit values on biotoxicity of wastewater need to be set before discharging

of the final effluent considering water environmental security.

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