Biotoxicity and by-product identification of dye wastewaters

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Abstract

Dyeing effluent is a typical refractory wastewater containing toxic pollutants. It is difficult to treat it to meet discharge regulations. The biotoxic effects of pollutants on microorganisms are one of the main constraints on efficient wastewater degradation. The aim of this study was to evaluate biotoxic effects and try to screen toxic substances from dye wastewater. The toxic effects of wastewaters collected at different treatment stages from a wastewater treatment plant in a dye industrial park were determined using bioluminescent bacteria (*Vibrio qinghaiensis sp.*-Q67). Toxic substances from both influent and effluents were identified by gas chromatography-mass spectrometry. Spearman's rank correlation coefficients indicated significant positive correlations between the toxicity values, and chemical oxygen demand (COD), NH_3-N, and total nitrogen (TN). After identifying the toxic levels, phenol, pyridine, and esters were confirmed as the principal organic pollutants in dye wastewater, and increase its toxicity.

Key words: biochemical treatment unit, bioluminescent bacteria test, dye wastewater, microbial toxicity

INTRODUCTION

Dye wastewater is a major industrial issue in developing countries, often containing high concentrations of unfixed dyes and other organic compounds (Rane *et al.* 2015). Contaminated wastewater from textile dyeing is also a substantial source of environmental pollution. Anaerobic/aerobic biochemical processes have been widely applied for the simultaneous removal of organic contaminants and nitrogen in wastewater treatment. Dye wastewater resists biological degradation and has become one of the most problematic industrial effluents to treat due to its inherent biotoxic constituents. Although traditional biological treatments are effective in reducing chemical pollutants, the treated effluent still remains toxic. Biotoxic pollutants are difficult to remove effectively by traditional treatment methods and can inhibit any further biodegradation. The biotoxic effects of pollutants on microorganisms are one of the main constraints on wastewater degradation efficiency. The toxic species in dye wastewaters could prevent the normal operation and inhibit the metabolic activity of microorganisms. While basic physicochemical parameters are available for dye wastewater, its toxicants remain largely unknown (Punzi *et al.* 2015). Thus, monitoring the solution's biotoxicity and toxic pollutant content is necessary during treatment.

Little attention has been paid to assessing the acute toxicity of dye wastewater when the chemical parameters meet the discharge limits (Sun *et al.* 2015). Karci *et al.* (2014) showed that despite the reduction in total suspended solids (TSS), color, chemical oxygen demand (COD), total nitrogen (TN), ammonia (sum of NH_4^+ and NH_3, referred to hereafter as NH_3-N), and total phosphorus

(TP), the wastewater acute toxicity cannot be reduced significantly, but can, potentially, be increased. Therefore, it is essential to evaluate the dye effluent acute toxicity and identify possible toxic pollutants for their potential risks.

Toxicity evaluations have been adopted for wastewater management in many wastewater treatment plants (WWTPs), where various test methods are used to characterize wastewater toxicity (Manenti et al. 2015). Bioassays can provide valuable information to reflect the toxicity of mixed solutions on living organisms (Ma et al. 2013; Chen et al. 2016). *Vibrio qinghaiensis sp.*-Q67 (Q67), a freshwater luminescent bacterium, can be used to detect a variety of toxic substrates rapidly and sensitively. Toxic chemicals can interfere with the respiratory electron transfer system in Q67 and inhibit its light production.

In an attempt to identify toxic pollutants, toxicity identification evaluation (TIE) has been developed by the United States Environmental Protection Agency (USEPA), and is now widely used to identify and reduce major toxicants in industrial effluents (Deng et al. 2017). TIE methods combine chemical and physical fractionation techniques with the response of test organisms to identify contaminants (Ahn et al. 2015; Ra et al. 2016; Ferraz et al. 2017). The advantage of performing whole-effluent toxicity testing together with chemical analysis of industrial wastewaters is that their results can be used to determine the substances responsible for toxicity and devise strategies to eliminate them from wastewaters (Melo et al. 2013).

The main objective of this work is to assess the physicochemical parameters and acute toxicity from each treatment stage in an industrial dye WWTP, and identify possible toxic contaminants from the influent and effluents. Several conventional sample parameters were analyzed: pH, dissolved oxygen (DO), total suspended solids (TSS), color, COD, TN, NH_3-N, and TP. The toxicity levels of the wastewaters were tested using determinations of luminescent bacteria Q67. The study was designed to determine water quality and toxicity in different wastewater treatment stages. The combination of chemical parameters and toxic effects can provide a comprehensive evaluation of dye effluents to minimize potential risks. Hazardous substance identification in effluents and influents can provide a guideline for substance screening.

METHODS

Wastewater treatment system and sampling

Wastewater samples were collected from the industrial dye WWTP (ID-WWTP) of many textile plants producing towels, sheets and cloths. The wastewater was treated in sequence in tanks for hydrolysis acidification (HAT), aeration (AT), secondary sedimentation (SST), and reaction sedimentation (RST), and biological aerated (BAF) and activated sand (ASF) filters – see Figure 1. The samples were stored at 4 °C in the dark until analysis. All physicochemical analyses and the toxic effects on Q67 were determined within 48 hours after the samples arrived at the laboratory.

Physicochemical analysis

All samples were characterized by quantifying pH, color, DO, TSS, COD, TN, NH_3-N, and TP according to Standard Methods for the Examination of Water and Wastewater (APHA 2005). The pH and DO were measured at the time of collection at the sampling site. COD was determined using the potassium dichromate method. NH_3-N and TN were quantified by Nessler reagent colorimetric determination (Tianjin kermel, China) and the potassium persulfate digestion ultraviolet spectrophotometric method, respectively. TP was determined by ammonium molybdate spectrophotometry, and TSS by gravimetry. Color measurement was conducted multiple dilution. All samples were tested in triplicate.
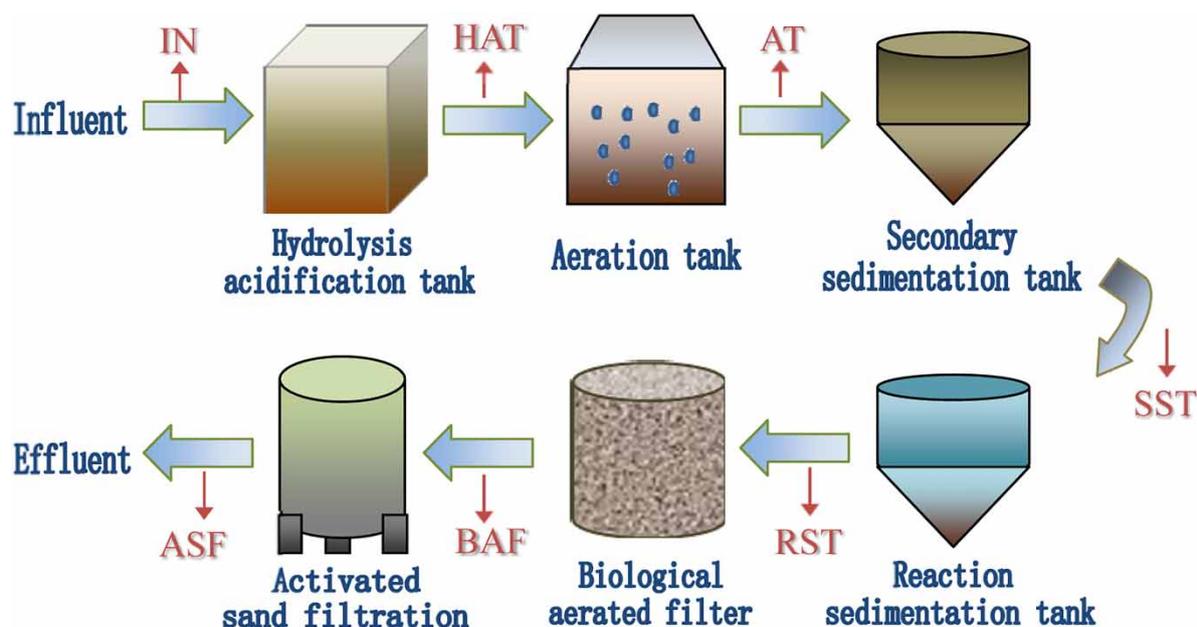
Figure 1 | Flow-process diagram for the industrial dye wastewater treatment (ID-WWTP).

Toxicity tests by bioluminescence inhibition assays

The acute toxicity of the wastewater was analyzed with Q67 according to ASTM D5660-96 via a bioluminescence inhibition assay, using a BHP9511 water quality toxicity analyzer (Beijing HAMAMATSU, China). Analytical samples were prepared by mixing 2 mL of the original water sample thoroughly in a test tube with an osmotic pressure conditioning solution (Beijing HAMAMATSU, China) and a suspension of 0.05 mL of luminescent bacteria. The relative light intensity was recorded after 15 minutes and all samples were tested in triplicate (Ma *et al.* 2016).

Toxic substance identification evaluation

Sample extraction

The 500 mL water samples were filtered through a 0.45 μm cellulose acetate membrane and passed separately through Waters Oasis HLB cartridges (500 mg). Before solid phase extraction, the HLB cartridges were preconditioned with 5 mL each of methanol and ultra-pure water. After extraction, they were eluted with 1.5 mL each of a series of methanol/water solutions with increasing mixing ratios (methanol:water – 25:75, 50:50, 75:25, 80:20, 85:15, 90:10, 95:5 and 100:0, v/v) (Melo *et al.* 2013). Each component's toxicity was tested by bioluminescence inhibition assay, using Q67 and a merger toxic component. The combined toxic component was passed through Waters Oasis HLB cartridges (500 mg) again and eluted with methanol (10 mL), before being dried by anhydrous Na_2SO_4 and readjusted to a final volume of 0.5 mL by rotary evaporation.

Toxic substance identification from the influent and effluent

The enrichment components demonstrating toxicity were identified by gas chromatography-mass spectrometry (GC-MS) using an Agilent 5977 system equipped with a DB-5-MS column. The temperature ramp was 60 °C for two min, 10 °C/min up to 110 °C, 7 °C/min up to 140 °C, 140 °C for three min, 7 °C/min up to 230 °C, 230 °C for one min, 5 °C/min up to 280 °C, and then 280 °C for one min. The injection part and transfer line temperature was 280 °C, and helium was used as the carrier gas at a flow rate of 1.0 mL/min. The mass spectrometer was operated in full-scan mode (35–650 AMU).

Statistical analysis

All statistical analyses were conducted using Origin Pro 8.5 (Origin Lab, USA) and SPSS 20.0 (IBM, USA). Spearman's rank correlation tests ($p < 0.05$) were used for correlation analyses.

RESULTS AND DISCUSSION

Physicochemical characterization

The dye wastewater was removed by conventional physical, chemical, and biological WWT processes. The standard physicochemical characteristics collected from different WWTP stages and the state effluent discharge limits are listed in Table 1. In summary, the influent sample contained high concentrations of color, TSS, COD and TN. After full treatment, the removal efficiencies reported for color, TSS, COD, TN, NH_3-N and TP, were 87, 98, 86, 82, 82, and 70%, respectively. Thus, the WWTP effluent color, TSS, TN, NH_3-N and TP met the discharge limits. COD exceeded the discharge limit by nearly 40%, however.

Table 1 | Physicochemical characteristics of wastewater from different WWTP treatment stages

Sample	pH	Color	water quality index (mg/L, n = 3)					
			DO	TSS	COD	TN	NH_3-N	TP
IN	9.49	32	0.37 ± 0.14	138 ± 8	501.7 ± 5.3	14.14 ± 0.61	7.21 ± 0.14	2.56 ± 0.12
HAT	9.24	16	0.51 ± 0.23	67 ± 12	465.8 ± 13.0	13.24 ± 0.59	9.77 ± 0.42	2.93 ± 0.23
AT	7.88	8	2.37 ± 0.22	2348 ± 77	151.5 ± 7.4	5.84 ± 0.18	2.59 ± 0.10	1.90 ± 0.05
SST	8.05	8	5.68 ± 0.04	12 ± 3	105.1 ± 2.7	3.66 ± 0.20	1.81 ± 0.02	1.66 ± 0.07
RST	8.05	4	5.76 ± 0.04	10 ± 2	105.0 ± 2.2	3.76 ± 0.32	1.96 ± 0.10	1.58 ± 0.03
BAT	8.41	8	8.41 ± 0.41	8 ± 1	95.5 ± 0.3	2.81 ± 0.04	1.64 ± 0.10	0.97 ± 0.05
ASF	6.51	4	7.53 ± 0.43	3 ± 1	68.7 ± 3.3	2.51 ± 0.09	1.31 ± 0.07	0.76 ± 0.03
Standard limits	6-9	30	/	10	50	15	5	1

The removal efficiencies of conventional physicochemical indicators from different WWTP treatment stages are given in Table 2. The highest removal efficiencies for TSS, COD, TN, NH_3-N, and TP, were 61%, 67%, 56%, 73% and 39%, respectively, detected in the ASF, AT, AT, AT, and ASF samples. This shows that AT was the most effective treatment stage for COD, TN, NH_3-N and organic matter, and compounds containing nitrogen and phosphorus were mostly degraded. Of the other stages, HAT removed approximately half of the TSS, but was inefficient at treating COD, TN, NH_3-N, and TP.

Table 2 | Removal efficiencies in different WWTP treatment stages

Sample	Removal efficiency (%)				
	TSS	COD	TN	NH3-N	TP
HAT	52	7	6	−36	−14
AT	/	67	56	73	35
SST	/	31	37	30	13
RST	17	0.1	−3	−8	4
BAT	23	9	25	16	39
ASF	61	28	11	20	22

After SST, the removal efficiencies for COD, TN, and NH$_3$-N were approximately 30%, and organic compounds containing nitrogen were degraded. BAT and ASF degraded the contaminants further.

On the basis of the above it is clear that dye wastewater discharge is affected mainly by organic and nitrogenous pollutants. According to previous reports (Bae *et al.* 2015), the chemical structures of organic substances in dye wastewaters are complex and they resist degradation. This study has confirmed that organic substance removal is quite difficult by conventional biological wastewater treatment methods.

Acute toxicity test results

To clarify the potential risks in dye wastewater, the evolution of acute toxicity was investigated. The acute toxicity results, expressed as equivalent HgCl$_2$ concentration values, revealed that the toxic effects on Q67 varied between the wastewater treatment stages (Table 3). Dye wastewaters from different treatment stages had different toxic effects on Q67 and the toxic substance concentrations were reduced after most treatment stages. The highest toxicity removal efficiency was 37.44% in the SST, whereas the RST increased the treated wastewater's toxicity. Compared to conventional parameters, the efficiency of acute toxicity removal was relatively low, and the final effluent still exerted significant inhibitory effects on Q67.

Table 3 | Wastewater toxic effects on Q67, expressed as relative inhibition rates and HgCl$_2$ equivalent concentrations

Samples	Relative inhibition rate (%)	Removal efficiency (%)	HgCl$_2$ equivalent concentration (mg/L)
IN	59.8	/	0.097
HAT	51.6	13.71	0.087
AT	39.8	22.87	0.073
SST	24.9	37.44	0.045
RST	35.1	-40.96	0.066
BAT	32.0	8.83	0.061
ASF	25.8	19.38	0.048

Ma *et al.* (2013) suggested that samples should be considered toxic when the relative inhibition rate (RI) on Q67 exceeds 15%. The toxicity test results show that the effluents from all treatment stages exceeded this value and had significant acute toxic effects. In terms of the HgCl$_2$ equivalent concentrations, the effluent was below the wastewater discharge limit, whereas the influent exceeded it at 70% (0.057 mg/L in terms of HgCl$_2$ equivalent concentration). The high toxicity of the influent affected the treatment process efficiencies, leading to the results cited.

Correlation analyses of conventional parameters and toxicity indicators

The correlations between the acute toxic effects and the conventional parameters were investigated. Figure 2 shows the conventional wastewater parameters and wastewater sample toxicity indicators from different treatment stages. Correlations were also found between the conventional parameters and the toxicity indicators based on Spearman's rank correlation analyses.

The HgCl$_2$ equivalent concentration showed significantly positive correlations with COD, TN, and NH$_3$-N. DO was also found to be correlated with toxic effects on Q67. Although the toxicity was greatly reduced after a series of treatment processes, the RI of Q67 still exceeded the discharge limit (15%). The high toxicity was mainly caused by the presence of NH$_3$-N, TN and organic substances (Lu *et al.* 2018). As these species are the targets for removal by dye wastewater treatment
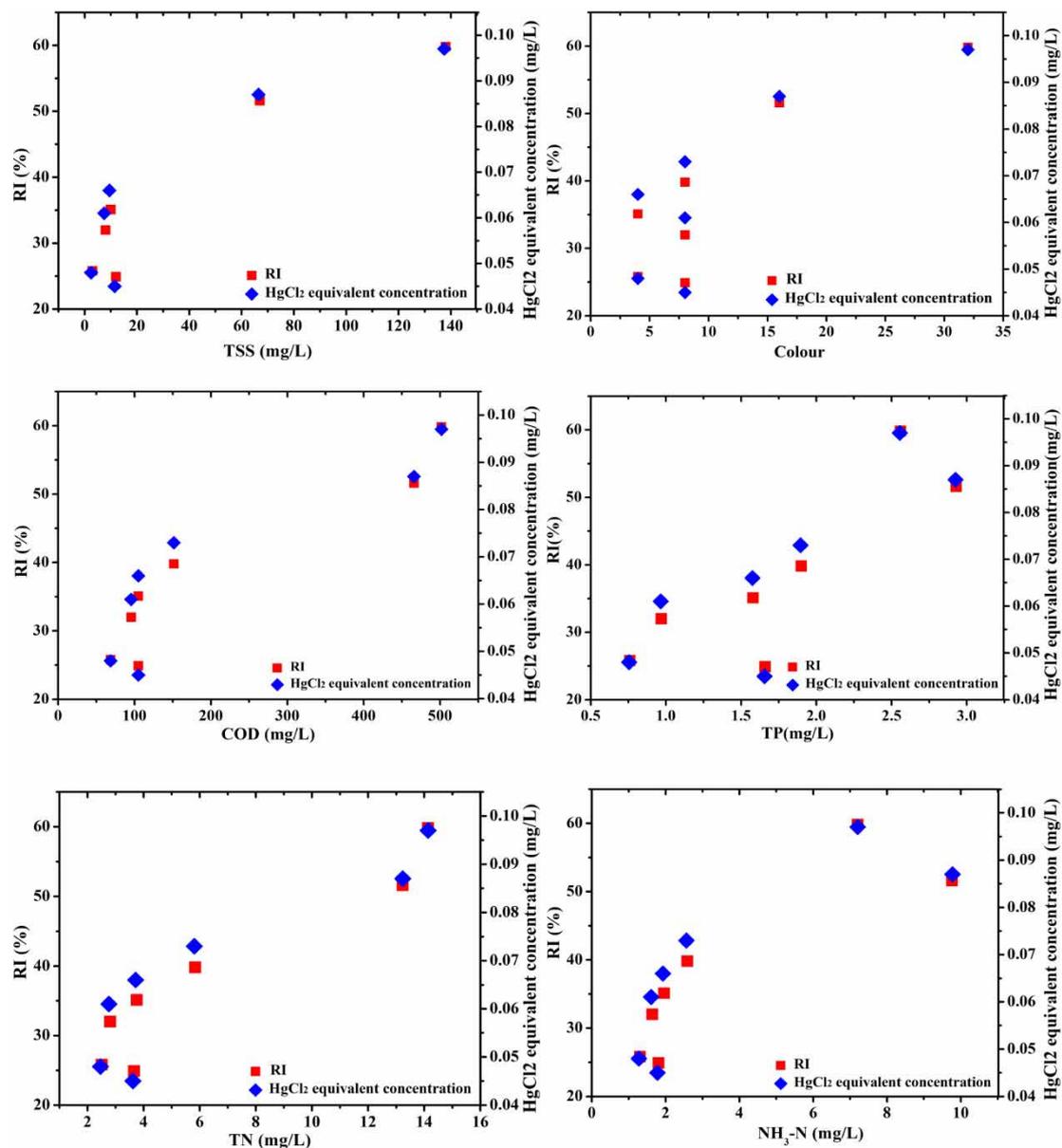
Figure 2 | Correlation between wastewater quality indicators and toxicity to Q67, expressed as RI and HgCl₂ equivalent concentrations.

processes, the obvious correlations between toxicity indicators and conventional parameters explain the high toxicity removal efficiencies in dye WWTPs.

Toxic substance identification

The degradation byproducts in the influent and effluents were identified by qualitative GC-MS scans, the peaks with relatively high abundance being identified as potential contaminants. The classes of organic compounds identified are summarized in Table 4.

Organic compounds including 2-pyridyl acetic acid, phthalates, 2-chloro-5-isocyanatitrobenzene, 2,2′-methylenebis-(4-methyl-6-tert-butylphenol), and metaclazepam were detected in the raw influent and treatment stage effluents. As shown in Table 4, the concentrations in the various effluents were much lower than those in the raw influent. Analysis of the influent and effluents showed that most organic pollutants were degraded in treatment, and only a small portion (particularly silicon oxide)

Table 4 | By-product identification in the influent and effluents from the WWTP

No.	Identified by-products	Samples	
		IN	EF
1	benzamine	+	−
2	amides	+	−
3	phenols	+	+
4	pyridines	+	−
5	anilines	+	−
6	quinolines	+	−
7	siloxanes	+	+
8	alkane	+	−
9	carboxylic acids	+	−
10	esters	+	+
11	aldehydes	+	+
12	alcohols	+	−
13	2-pyridylacetic acid	+	+
14	phthalate	+	−
15	2-chloro-5-isocyanatitrobenzene	+	−
16	2,2'-methylenebis-(4-methyl-6-tert-butylphenol)	+	−
17	phthalate	−	+
18	2-chloro-5-isocyanatitrobenzene	−	+
19	2,2'-methylenebis-(4-methyl-6-tert-butylphenol) Methyl esters	−	+
20	benzyl amines	−	+
21	alcohols	−	+
22	anilines	−	+
23	pyrimidines	−	+
24	alkanes	−	+

IN- influent; EF- effluent; "+" detected; "−" not detected.

could not be degraded completely. This suggests removal of these organic compounds from the raw dye wastewater by the treatment processes is effective.

It is recognized that wastewater toxicity is related to the toxicant concentrations. Previous studies have investigated organic species present in dye wastewaters. Benzamides, amides, phenols, pyridines, anilines, quinolines, siloxanes, alkanes, carboxylic acids, esters, aldehydes, alcohols and some other heterocyclic compounds have been found in influents using GC-MS (López-Montes *et al.* 2013; Moschet *et al.* 2017; Nouren *et al.* 2017), and might contribute to the raw influent toxicity. The influent used in this study was highly toxic, and the RI of Q67 (59.8%) exceeded the emission standards substantially. After a series of treatment stages, the number of organic species was greatly reduced, and the effluent did not show significant acute toxicity. The results indicate effective removal of these organic compounds and their related acute wastewater toxicity by the treatment processes.

CONCLUSIONS

In this study the conventional parameters and toxic effects of samples from different treatment stages in a dye WWTP were evaluated. The concentrations of TSS, color, COD, NH_3-N, TN and TP were all reduced effectively. The main pollutants in the samples were organic and nitrogenous compounds. The dye wastewater's acute toxic effects on Q67 were greatly reduced after treatment. Spearman's rank correlation analysis showed that the toxicity indicators were significantly and positively correlated with the COD,

NH$_3$-N and TN concentrations. Qualitative relationships between the toxic effects and the organic pollutants in both influent and effluents were established in this study. Toxicity identification showed that some organic species might contribute to the toxicity of raw influents, specifically, phenol, pyridine, esters.

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