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# Performance of Anaerobic Process on Toxicity Reduction During Treating Printing and Dyeing Wastewater

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**Abstract** The paper was to evaluate anaerobic treatment efficiency of reducing toxic compounds by gas chromatography mass spectrometry (GC-MS) analysis combined with biological toxicity test during the treatment process of printing and dyeing wastewater. There had an obvious decrease trend in the response abundance of GC-MS chromatograms between raw influent and anaerobic effluents with the removal of COD. A main component of the raw effluent was long-chain *n*-alkanes. Alkanes in the expanded granular sludge bed (EGSB) categories could be reduced by 75%. EGSB had a better degradation performance on some complicated pollutants and toxicity. The most sensitive bioassay was Microtox® bioassay.

**Keywords** Printing and dyeing wastewater · Toxicity test

Secondary wastewater treatment plants (WWTPs) are being built rapidly throughout the world. The majority of industrial wastewaters are treated at the WWTP before being discharged to the water environment. However, toxicants in WWTP influent may inhibit the biological activity of the activated sludge and cause treatment plant process upsets (Grau and Da-Rin 1997). With the development of industry, reality effluents become more complex mixtures of chemicals with interactive effects. Hence, it is necessary to give a realistic estimate of their toxicity on biota. Toxicity assessment methods were used as microcalorimetry, titration bioassays, respirometry, the Microtox® assay, whole-cell sensors, and molecular-based biosensors and assays (Dalzell et al. 2002; Ren 2004; Prato et al. 2006). However, there is little correlation between bacteria toxicity tests based on different principles or species. On the other hand, it is more important and practical for the WWTP to learn what toxicants are and how to improve its ability of removing toxicity from input wastewater. Although the majority of the organic pollutions were removed from the wastewater through a combination process unit, there were still a significant amount of toxic contaminants present in the final effluents. So, more information was required for the unequivocal identification of components responsible for the harm to obtain higher wastewater treatment performance and environments.

The main aim of this study was to evaluate the anaerobic treatment efficiency of reducing toxic compounds by gas chromatography mass spectrometry (GC-MS) analysis combined strategy with biological toxicity test during the treatment process of municipal sewage, mainly containing industrial wastewater from printing and dyeing industries at Shaoxing WWTP (SWWTP).

## Materials and Methods

SWWTP's wastewater was composed of 8% municipal sewage, 90% dyeing and printing wastewater and 2% other

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industrial wastewater. Terephthalic acid (TA) accounted for 40%–60% chemical oxygen demand (COD) of the wastewater. SWWTP was responsible to treat municipal and industrial wastewater with a total treatment capacity of 700,000 $m^3$ $day^{-1}$. In order to optimize the treatment efficiency of anaerobic process unit of SWWTP, a pilot-scale expanded granular sludge bed (EGSB) reactor with a total volume of 0.777 $m^3$ was operated for 400 days as the control. The EGSB reactor was kept at short hydraulic retention time (HRT) of 15 h, which was controlled according to the design of ponds at SWWTP.

Standard Methods (APHA 1999) were used for analysis of the following parameters: pH, SS (suspended solids), VSS (volatile suspended solids) and COD.

Organic constituents of the wastewater samples with a volume of 1.0 μL were extracted according to USEPA Method 3510C (USEPA 1996a) by methylene dichloride into acid, base and neutral fractions, and then qualitatively analyzed by USEPA Method 8270C (USEPA 1996b) with an Agilent 6890/5973 GC-MSD equipment. The analytical conditions were: a mass spectrometer detector (MSD), the capillary column model number was Agilent 19091S-433, which was made of quartz with nominal diameter of 0.25 mm, nominal film thickness of 0.25 μm and length of 30 m packed with 5% phenyl methyl siloxane; front inlet was splitless; total flow was 4.3 mL $min^{-1}$; and the temperature for the gasification compartment was 280°C. The temperature control program registered initial 40°C, retaining for 4 min, then increasing to 300°C with an increment of 8°C $min^{-1}$, total run time was 46 min; the temperature for MS quad was 150°C and for MS ion source was 230°C, electron energy was 70 eV, resulting electron multiplier (EM) voltage was 2105.9 V, scan parameters was 35–500 μm. Compounds were identified from their molecular fragmentation and quantified from the peak area of their major fragment ions, according to the instrument library (NIST 98.L) database.

Three biological toxicity test methods were adopted in this paper. Anaerobic toxicity assay (ATA) (Sponza and Işık 2005) was performed at 37°C using serum bottles with a capacity of 150 mL. The modified Nitrification inhibition was used according to Dalzell et al. (2002). Microtox® test was performed by Microtox® test instrument (Model toxicity analyzer DXY-2) (Ai-Ju et al. 2006) made by the Institute of Soil Science, Academic Sciences, Nanjing, PRC. Every sample was measured in triplicate.

## Results and Discussion

The COD concentration average of raw influent was 1339.9 mg $L^{-1}$, and that of anaerobic effluent still remained 1283.2 mg $L^{-1}$ at SWWTP. The value of effluent from aerobic unit was 233.5 mg $L^{-1}$. The total COD removal ratio was 90.94% after flocculation-precipitation. Final effluent could be discharged below the discharging limit. As a control, there was a more considerable removal of COD in the pilot-scale EGSB compared with the anaerobic unit of SWWTP. The average of COD decreased from 1339.9 to 869.1 mg $L^{-1}$ with the COD removal ratio of 35.14%. Because the printing and dyeing wastewater contained lots of toxic compounds with high pH (9.14–10.21) and high sulfate concentration (about 500mg/L), in addition to, the hydraulic retention time (HRT) of 15 h was short which was confined by the future renovation of the ponds at SWWTP, the diminished COD of 470.8 mg/L should be better and steady result of the reactor.

Terephthalic acid (TA) was not toxic pollution to activated sludge system (Guan et al. 2003) as a main component of SWWTP's wastewater, and it wasn't extracted and determined by GC-MS analysis because of its insolubility with methylene dichloride. The aim of this paper was to discuss the degradability of the main toxic pollutions except TA in SWWTP's wastewater. It could be seen from the GC-MS chromatograms that the raw influent had the largest number of spectra registering high organic component loading (with greater than 3.5e+7 response abundance), while the effluent of the EGSB reactor had only one peak with more than 3.5e+7 abundance. There was an obvious decrease trend in the response abundance between raw influent and anaerobic effluents with the removal of COD.

Table 1 showed that the majority of organics presented in samples were aliphatic, halogenated and aromatic hydrocarbons. The main components of raw effluent were

**Table 1** Summary of GC-MS analyses for categories (mass percentage, %) of organic compounds in SWWTP's wastewater after different anaerobic reactors

| Organic compounds | Raw influent | Effluent of anaerobic pond | Effluent of the pilot-scale EGSB reactor |
|---|---|---|---|
| Alkanes | 69 (71.26) | 74 (65) | 17 (19.57) |
| Alkenes | 10 (6.75) | 6 (8.49) | 6 (8.54) |
| Phenols | 4 (3.31) | 3 (1.59) | 4 (4.84) |
| Anilines | 2 (0.26) | 3 (0.63) | 2 (4.08) |
| Amides | – | 2 (0.21) | 2 (0.90) |
| Enals | – | – | 2 (1.79) |
| Carboxylic acids | – | 6 (1.41) | 5 (13.62) |
| Quinolines and isoquinolines | 8 (1.74) | 6 (0.73) | 5 (9.78) |
| Esters | 7 (3.45) | 4 (2.74) | 6 (9.69) |
| Alcohols | 5 (6.45) | 7 (5.91) | 9 (7.77) |
| Others | 7 (6.78) | 13 (13.29) | 8 (19.42) |
| Total | 112 (100.00) | 124 (100.00) | 66 (100.00) |

long-chain n-alkanes ($C_{10}$–$C_{44}$), which included 69 kinds of different alkanes and accounted for 71.26% of total organic compounds. After anaerobic process unit at SWWTP, the ratio of alkanes slightly decreased to 64% of the total organic components, but their categories increased to 74. Nevertheless, the ratio of alkanes in the effluent of the EGSB reactor decreased to 19.57% of the total organic components, and the categories of alkanes reduced 75% of influent to 17. Many studies demonstrated that the abundance of alkanes degraders involved in microbial degradation of alkanes (Hasanuzzaman et al. 2007; Van Beilen and Funhoff 2007). A conclusion can be drawn that alkanes also can be well removed or degraded in the advanced anaerobic process.

The categories of organic components in the effluent of the EGSB reduced from raw influent 112 to EGSB effluent 66, though its COD removal ratio was only 35.14%. That proved that under advanced anaerobic operation conditions (i.e. EGSB reactor) some refractory compounds could be decomposed and resulted in the production of some new intermediates, which were more readily degraded in the subsequent aerobic stage. Hence, further effort should be placed on optimizing the anaerobic treatment process unit at SWWTP.

Internal standard method was employed for quantitative analysis. Table 2 presented the result of quantitative analysis. 4-Methylphenol was reduced from 34.58 μg $L^{-1}$ of raw influent to 0.07 μg $L^{-1}$ of effluent of the EGSB, while the effluent of anaerobic at SWWTP was not almost reduced and still remained at 31.74 μg $L^{-1}$. Nevertheless, the phenol concentration of the anaerobic effluent at SWWTP was the highest, and it should be the intermediate of partial anaerobic conversion. It was interesting that the concentration of azobenzene, 2,6-dinitrotoluene and 4-nitrophenol in the effluent of the EGSB dramatically soared to 89.22, 189.37 and 45.07 μg $L^{-1}$, respectively, while all of them could not be detected in the raw influent. These compounds could be generally regarded as the intermediate of some complicated structure or large molecule, which were always mutagenic and cancerogenic compounds, e.g. dyestuffs. The critical steps in anaerobic degradation of these compounds include partial scission of polycyclic or heterocyclic rings, cleavage of long chains, and degradation of these organics through anaerobic fermentation (McMullan et al. 2001). Many persistent organic compounds, such as di-*n*-butyl phthalate listed as priority pollutants by the USEPA and bis (2-chloroethyl) ether, were found to degrade rapidly under anaerobic conditions than under aerobic conditions. The EGSB reactor had a better degradation performance on these toxic priority pollutants than the anaerobic even aerobic process of SWWTP. Anaerobic, as a pretreatment process to partially convert complicated pollutions to intermediates which are more readily degradable in subsequent treatment processes, is attractive for refractory wastewater treatment. It is necessary to introduce advanced anaerobic process to remove these toxic substances from municipal sewage and industrial wastewater for reducing their harm to environments.

**Table 2** Quantitative analysis (concentrations in μg $L^{-1}$) of different components probed with GC-MS in SWWTP's wastewater after different process units

| Target compounds | Raw influent | Effluent of anaerobic | Effluent of the EGSB reactor |
|---|---|---|---|
| Bis(2-chloroethyl)ether | 14 | 16 | ND |
| Di-n-butyl phthalate | 2.1 | 1.3 | ND |
| Bis(2-ethylhexyl)-phthalate | 5.3 | 5.1 | ND |
| 4-Methylphenol | 34 | 31 | 0.07 |
| Phenol | 6.3 | 31 | 0.01 |
| 2,4-Dimethyl phenol | 0.31 | 0.84 | 1.2 |
| *N*-nitroso-diphenylamine | 1.3 | 0.61 | 1.3 |
| Aniline | 9.1 | 10 | ND |
| 2-Methylphenol | 1.1 | 1.3 | ND |
| Naphthalene | 0.29 | 0.19 | ND |
| 4-Chloroaniline | 0.95 | 1.0 | ND |
| 4-Chloro-3-methyl phenol | 1.6 | 1.9 | ND |
| 2-Methylnaphthalene | 0.25 | 0.17 | ND |
| Pyrene | 0.06 | 0.09 | ND |
| Phenanthrene | 0.10 | ND | ND |
| Azobenzene | ND | ND | 89 |
| Diethyl phthalate | ND | ND | 5.7 |
| Isophorone | ND | ND | 0.11 |
| Benzoic acid | ND | ND | 2.8 |
| 2,6-Dinitrotoluene | ND | ND | 189 |
| 4-Nitrophenol | ND | ND | 45 |
| 2-Nitroaniline | ND | ND | 6.3 |
| *N*-nitrosodimethylamine | ND | ND | 1.9 |
| Nitrobenzene | ND | ND | 0.22 |
| *N*-nitroso-di-*n*-propylamine | ND | ND | 0.17 |
| Bis(2-chloroethoxyl)-methane | ND | ND | 0.14 |

*ND* (not detected) means the concentration was below detection limit

Although some of the organic pollutants including lots of toxic pollutants were removed from the wastewater after anaerobic treatment processes, there were still a significant amount of toxic pollutions present in the anaerobic effluents. According to Table 3, the toxicity index of anaerobic effluent at SWWTP had only slight decrease, while that of effluent from the EGSB reactor dramatically reduced, especially the index of Nitrification inhibition becoming negative value, which meant that the effluent had a slight promotion to nitrification instead of inhibition. The different inhibition ratio for the effluent of the EGSB reactor between these toxicity assessment methods was due to these toxicity tests based on different principles or species.

**Table 3** Percent inhibition results in different toxicity tests

| Index | Raw influent | Effluent of anaerobic | Effluent of the pilot-scale EGSB reactor |
|---|---|---|---|
| ATA inhibition (%) | 63.5 ± 6.1 | 57.9 ± 6.4 | 21.5 ± 2.9 |
| Nitrification inhibition (%) | 59.2 ± 4.8 | 55.6 ± 6.3 | –9.8 ± 1.5 |
| Microtox® inhibition (%) | 71.3 ± 8.2 | 65.2 ± 7.1 | 37.6 ± 4.4 |

All data are shown as means ± standard deviation of three replicates

Possible factors linked to sample preparation (e.g. suspended particles in samples) or to bacteria (e.g. conservation and time to equilibration to test temperature) could all alter the results. Here, the most sensitive bioassay was Microtox® bioassay. Therefore, it is recommended that during assessment of wastewater toxicity a suite of tests should be used rather than reliance on one particular test.

In addition, according to Tables 1 and 3, long-chain *n*-alkanes as the main components of wastewater usually could make a major contribution to the toxicity though alkanes also could be well removed or degraded by the advanced anaerobic process. Because of very low $IC_{50}$ (toxicant concentration eliciting a 50% inhibitory effect) (Speece 1996), the toxicity of long-chain *n*-alkanes should be noticed during wastewater treatment. According to Tables 2 and 3, some complicated pollutants such as azo dyes themselves or their metabolic productions might contribute to the toxicity. The trend of toxicity tests results were in agreement with GC-MS analyses. Integrated strategy with biological and chemical analysis for water quality control and evaluation potential risk linked with the presence of chemicals was a recognized complementary protocol.

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