

# DEGRADATION OF ORGANIC POLLUTANTS AND CHARACTERISTICS OF ACTIVATED SLUDGE IN AN ANAEROBIC/ANOXIC/OXIC REACTOR TREATING CHEMICAL INDUSTRIAL WASTEWATER

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(Submitted: June 6, 2013 ; Revised: November 10, 2013 ; Accepted: November 19, 2013)

**Abstract** – A laboratory-scale anaerobic/anoxic/oxic system operated at the hydraulic retention times (HRT) of 20, 40, and 60 h with mixed liquor suspended solids (MLSS) concentrations of 3 g/L and 6 g/L was considered for treating chemical industrial wastewater rich in complex organic compounds and total dissolved solids. Extending the HRT and increasing the MLSS concentration resulted in higher removal efficiency for chemical oxygen demand at 72%. Organic compounds in wastewater could be classified into easily-removed and refractory compounds during treatment. The easily-removed compounds consisted primarily of ethers, alcohols, and aldehydes, whereas the refractory compounds included mainly oxygen-containing heterocyclic and benzene-containing compounds. Results from energy-dispersive X-ray spectroscopy showed that several metal ions accumulated in activated sludge, particularly Fe(III). Fe accumulated mainly on the surface of sludge floc pellets and resulted in the compactness of activated sludge, which caused the values of mixed liquor volatile suspended solids /MLSS and sludge volume index to decrease.

**Keywords:** Anaerobic/anoxic/oxic process; Chemical industrial wastewater; Fe(III) accumulation; Organic matter classification; Hydraulic retention time.

## INTRODUCTION

Treatment of chemical industrial wastewater by microbial degradation is one of the most important waste management processes (Kim *et al.*, 2013). However, chemical industrial wastewater frequently exhibits characteristics that adversely affect microbial degradation processes, such as complex components, toxicity, inhibition of biological treatment systems, and refraction to biodegradation (Lei *et al.*, 2010; Motteran *et al.*, 2013; Wang *et al.*, 2014). Further-

more, because of industrial production processes and water salinity in coastal areas, several sources of chemical industrial wastewater are saline, which also adversely affects biological processes (Lefebvre *et al.*, 2005).

To resolve problems caused by complex components, toxicity, inhibition, recalcitrant compounds, and salinity, optimal operating conditions such as mixed liquor suspended solids (MLSS) concentration and hydraulic retention time (HRT) should be considered (Ludzack and Noran, 1965). Extending the

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HRT and increasing biomass can improve the removal of several refractory organic compounds which can be used slowly as carbon and energy sources by microorganisms, thus mitigating the adverse effects of industrial wastewater treatment (Park *et al.*, 2001; Rempel *et al.*, 1992). Kim *et al.* (2008) used an anaerobic/aerobic reactor for treating coke wastewater, 100% phenol, 100% free cyanide, and 100% thiocyanate  $[\text{SCN}]^-$ ; approximately 85% of the chemical oxygen demand (COD) were removed at HRT longer than 11.9 h. Wang *et al.* (2012) employed an anaerobic/anoxic/aerobic ( $\text{A}^2/\text{O}$ )–membrane bioreactor with an HRT of 48 h for treating coal gasification wastewater and obtained 97.4% COD removal efficiency. Maranon *et al.* (2008) reported that 85% COD, 98%  $[\text{SCN}]^-$ , and 99% phenols were removed when the HRT of 66 h was adopted for treating coke wastewater with a sequencing batch reactor. Hence, to achieve the best possible removal performance of organic compounds, optimal HRT and biomass concentration should be considered. Moreover, the removal characteristics of organic compounds in wastewater are also important factors in assessing the performance of biological treatment systems (Wang *et al.*, 2012).

In addition, industries generate saline effluent because of the addition of various chemicals during the different stages of the production process (Wiegant *et al.*, 1999). Complex saline metal ions have different effects on activated sludge, which are described as follows. (1) They can be absorbed by activated sludge and inhibit its action (Quesnel and Nakhla, 2005). (2) They can be absorbed by activated sludge, but will have no adverse effect on its action. (3) They cannot be absorbed by activated sludge in huge amounts, but they can have adverse effects on its action (Dincer and Kargi, 2001). They can accumulate on and flocculate with activated sludge.

In this study, a laboratory scale  $\text{A}^2/\text{O}$  system was used to treat chemical industrial wastewater in Tianjin, China. The removal performance of the organic compounds in the system upon extending HRT and increasing MLSS was investigated. Removal characteristics of the organic compounds in the influent and effluent were also considered. Furthermore, changes in the characteristics of activated sludge, such as mixed liquor volatile suspended solids (MLVSS)/MLSS and sludge volume index (SVI), were considered. The effects of metal ion accumulation on activated sludge were also determined. The results of the analyses could provide economical and effective technical support to full-scale industrial wastewater treatment plants.

## MATERIALS AND METHODS

### Wastewater Source and Characteristics

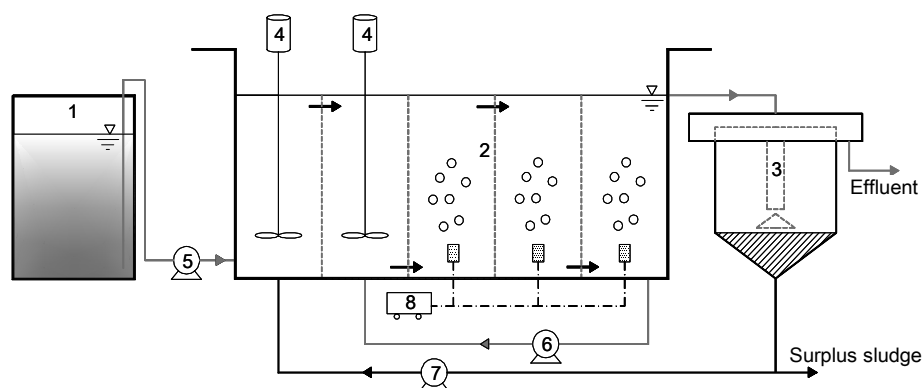
Wastewater was obtained from the influent of a full-scale wastewater treatment plant (an  $\text{A}^2/\text{O}$  system) in a chemical industrial park in Tianjin, China. More than 50 types of industrial plants are located in this area, including pharmaceutical, manufacturing, petroleum chemical, electronics, chemical reagent, household, and personal care product plants. The wastewater contains the following (all values are in mg/L): COD, 190 to 574; 5 day biochemical oxygen demand ( $\text{BOD}_5$ ), 55 to 243; total nitrogen (TN), 23.50 to 28.78; ammonia nitrogen ( $\text{NH}_4^+-\text{N}$ ), 17.65 to 24.20; total phosphorus, 0.9 to 1.2, and total dissolved solids, 10570 to 11480 ( $\text{Na}^+$ , 2369.8 – 2464.3;  $\text{Mg}^{2+}$ , 378.9 – 470.8;  $\text{K}^+$ , 67.5 – 70.1;  $\text{Ca}^{2+}$ , 34.4 – 36.6;  $\text{Fe}^{3+}$ , 12.7 – 23.5).

### Reactor Setup

A schematic illustration of the reactor system is shown in Figure 1. Two sets (#1 and #2) of the  $\text{A}^2/\text{O}$  reactor were made of Plexiglas and had working volumes of 12.8 L (Anaerobic tank: 8 cm × 8 cm × 40 cm; Anoxic tank: 8 cm × 8 cm × 40 cm; oxic tank: 24 cm × 8 cm × 40 cm). Motor-driven mixers were employed in the anaerobic and anoxic reactors. An air pump (electromagnetic air pump) and a set of diffusion aerators (multiaperture pottery diffusers) were employed to supply air to the oxic reactor. Peristaltic pumps were used to automatically furnish the system from the feed tank and recycle the mixed liquor and sludge. The sludge was returned from the bottom of the settling reactor to the anaerobic reactor. The mixed liquor was recycled from the oxic reactor to the anoxic reactor. The circulation ratios for the sludge and mixed liquor were 0.3-1 and 2, respectively.

### Operational Conditions

The details of the experimental conditions are shown in Table 1. The HRT of the #1 reactor system (from stage I to stage III) was varied to consider the effects of HRT on the removal performance; the MLSS (from stage IV to stage VI) was increased to consider the effects of the MLSS on the removal performance. The HRT and MLSS concentration of the #2 reactor system were maintained constant to serve as control. The seed sludge was obtained from a full-scale municipal wastewater treatment plant (an



**Figure 1:** Schematic diagram of the A<sup>2</sup>/O process : 1) influent tank; 2) A<sup>2</sup>/O reactor system; 3) secondary sedimentation tank; 4) stirring apparatus; 5) influent feeding pump; 6) mixed liquid recycle pump; 7) activated sludge recycle pump; 8) aeration pump.

**Table 1: Operational conditions of the reactor system.**

| Stage | 1#      |            |                               | 2#(control) |            |                               | Days covered (d) |
|-------|---------|------------|-------------------------------|-------------|------------|-------------------------------|------------------|
|       | HRT (h) | MLSS (g/L) | OLR (kgCOD/m <sup>3</sup> .d) | HRT (h)     | MLSS (g/L) | OLR (kgCOD/m <sup>3</sup> .d) |                  |
| I     | 20      | 3.2 ± 0.2  | 0.56                          | 20          | 3.0 ± 0.4  | 0.56                          | 32               |
| II    | 40      | 4.0 ± 0.6  | 0.25                          | 20          | 3.7 ± 0.8  | 0.52                          | 36               |
| III   | 60      | 4.2 ± 0.9  | 0.13                          | 20          | 3.8 ± 0.3  | 0.41                          | 30               |
| IV    | 60      | 6.0 ± 0.4  | 0.13                          | 20          | 3.2 ± 0.2  | 0.39                          | 22               |
| V     | 40      | 6.0 ± 0.3  | 0.14                          | 20          | 3.6 ± 0.5  | 0.30                          | 30               |
| VI    | 20      | 5.9 ± 0.4  | 0.24                          | 20          | 3.4 ± 0.2  | 0.24                          | 30               |

A<sup>2</sup>/O system) in Tianjin. The sludge retention time was kept at 40-50 d because of low growth rate of the sludge and the excess sludge was discharged from the bottom of the settling reactor each 3 days. The temperature of the treatment system was kept constant at 25 ± 2 °C.

### Determining the Composition of the Wastewater Organic Compounds

The composition of organic compounds in the wastewater was analyzed via gas chromatography–mass spectrometry (GC-MS). The samples were pretreated by CH<sub>2</sub>Cl<sub>2</sub> (Fisher, USA) liquid–liquid extraction and extracted under acidic, neutral, and alkaline conditions using the extraction procedure proposed by Lai *et al.* (2008). Then, 1 µL of pretreated sample was injected into the GC-MS system (Agilent, USA). The carrier gas was pure helium (99.999%) at a flow rate of 1 mL/min. A 30 m long HP-5MS capillary column with an inner diameter of 0.25 mm was used in the separation system. The temperature control program was implemented as

follows. The initial temperature of 40 °C was maintained for 4 min. Afterward, the temperature in the oven was increased to 250 °C at a rate of 5 °C/min, and then to 300 °C at a rate of 10 °C/min and maintained for 10 min. Organic compound analysis was conducted according to the National Institute of Standards and Technology 05 mass spectral library database.

### Morphologies of and Metal Ion Accumulation in the Activated Sludge

Morphologies of and metal ion accumulation in the activated sludge were analyzed by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The pretreatment procedure for the sludge samples was conducted by the method presented by Zhang *et al.* (2008). The samples were gently washed with distilled water, allowed to settle naturally, and then fixed with 2% glutaraldehyde for 4 h. The fixed samples were washed thrice with 0.1 M sodium cacodylate buffer and left for 20 min each time. Afterward, they were

dehydrated by successively passing them through 50%, 70%, 85%, 95%, and 100% ethanol. The dehydrated samples were then transferred to a freeze-dryer. The dried samples were sputter coated with gold and observed by SEM (Hitachi S-4800, Japan).

### Determining the Concentrations of Accumulated Metal Ions in Activated Sludge Flocs

Extracted extracellular polymeric substances (EPS) were measured using a modified EPS extraction procedure presented by Yu *et al.* (2008). The EPS in sludge flocs were composed of soluble (i.e., slime) and bound EPS. Bound EPS exhibited a dynamic double-layer-like structure composed of loosely bound (LB-EPS) and tightly bound (TB-EPS) EPS (Poxon and Darby, 1997; Ramesh *et al.*, 2006; Li and Yang, 2007). During EPS extraction, sludge was initially allowed to settle for 1.5 h at 4 °C, with the supernatant being collected as bulk solution. The sediments were then centrifuged at  $2000 \times g$  for 15 min. The supernatant was collected as slime, which represented the part being removed by soft centrifugation. The collected bottom sediments were resuspended to their original volume using a pH 7 buffer solution consisting of 1.3 mM  $\text{Na}_3\text{PO}_4$ , 2.7 mM  $\text{NaH}_2\text{PO}_4$ , 6 mM NaCl, and 0.7 mM KCl. The suspensions were centrifuged again at  $5000 \times g$  for 15 min with the supernatant, and the solid phases were collected separately. The organic matter in the supernatant consisted of LB-EPS of the sludge samples. Collected sediments were resuspended with the buffer solution to their original volumes, and then treated by ultrasound at 20 kHz and 480 W for 10 min. Extracted solutions were centrifuged at  $20,000 \times g$  for 20 min. The organic matter in the supernatant consisted of TB-EPS, whereas the residues (solid phase) resuspended with the buffer solution to their original volumes were pellets.

The concentrations of accumulated metal ions in the pellets were measured via ethylenediaminetetraacetic acid (EDTA) washing and concentrated nitric acid digestion, as presented by Hu *et al.* (2003). Washing was conducted as follows. Microbial cell pellets which remained after centrifugation were resuspended in 30 mL of washing solution (1 mM EDTA, pH 7.0, and 0.1 M NaCl) to prevent osmotic shock, and agitated at 150 rpm for 30 min to wash surface-bound metals. Then, the pellets were further centrifuged twice for 5 min at  $1600 \times g$ . Between each centrifugation, the supernatant was removed and the pellets were resuspended in a washing solution for 10 min. Concentrated nitric acid (trace metal grade) was added to the pellets, and the contents

were quantitatively transferred to a glass reaction tube. The suspension containing 4 M nitric acid was digested at 150 °C for 24 h. The cooled digest was filtered (0.45  $\mu\text{m}$ , glass fiber filter membrane). Finally, the EPS samples and the pellets were used to test nonpurgeable organic carbon (NPOC) and metal ion concentrations by inductively coupled plasma-mass spectrometry.

### Methods for Analyzing Wastewater Quality

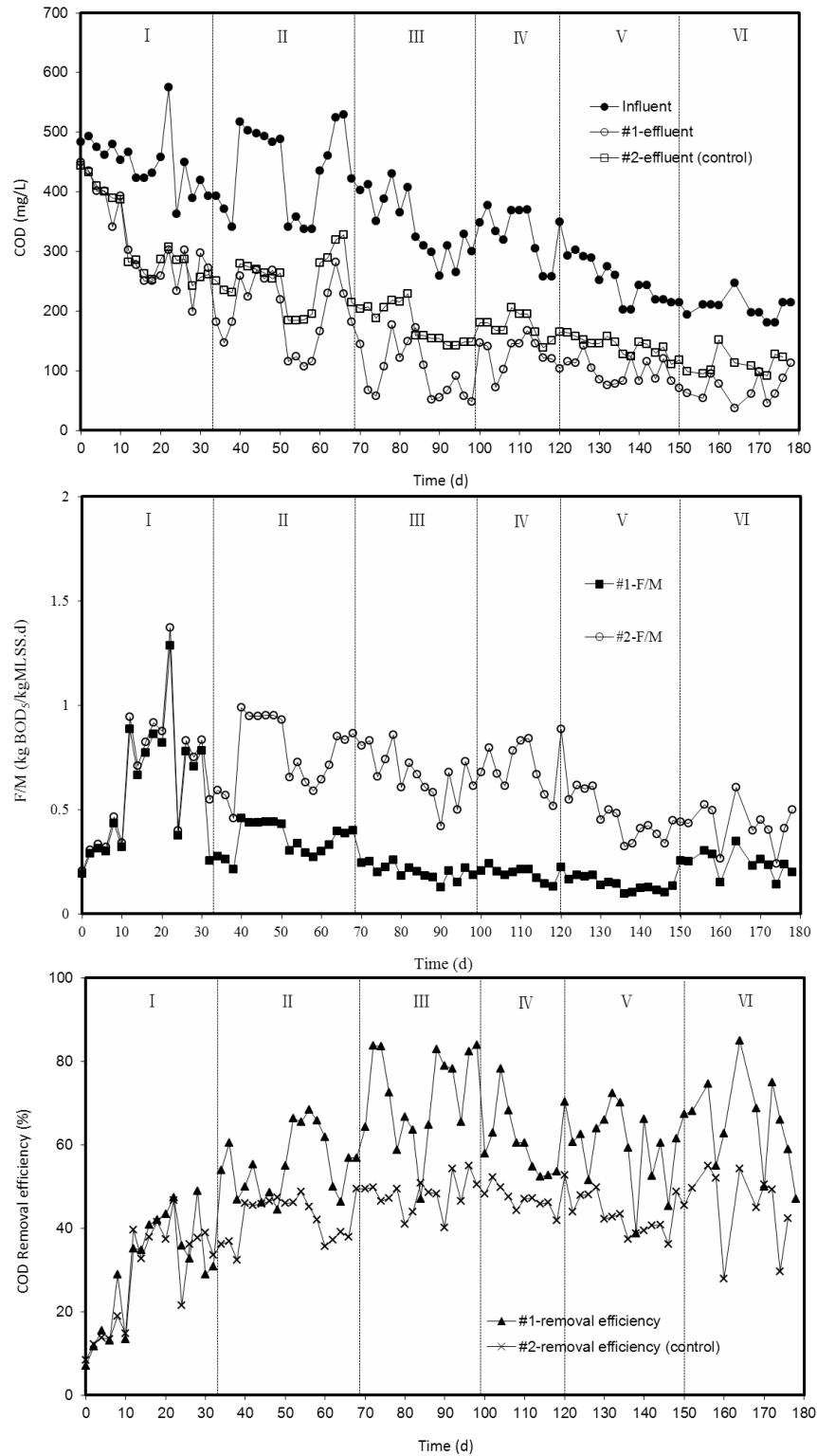
The influent and effluent of the system were collected and tested for parameters following standard methods. COD was measured using the potassium dichromate oxidation method. Five-day biochemical oxygen demand ( $\text{BOD}_5$ ) was determined using rapid BOD measuring devices (OxiTOP IS6, Germany).  $\text{NH}_4^+\text{-N}$  was measured with Nessler's reagent by spectrophotometry. Total nitrogen (TN) concentrations were measured by ultraviolet spectrophotometry at 220 nm and 275 nm after digestion. Dissolved oxygen and pH levels were measured daily (HACH sension156, USA). MLSS, MLVSS, settling velocity values, and SVI were also investigated.

## RESULTS AND DISCUSSION

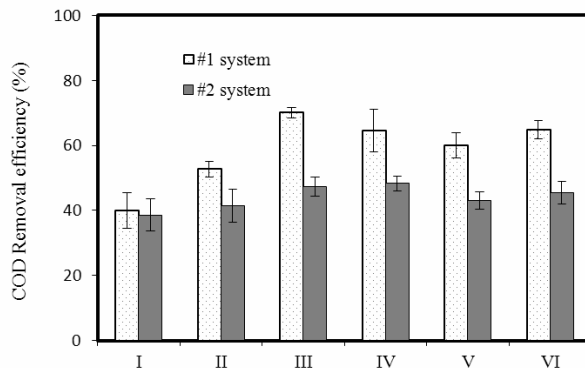
### COD Removal Performance of the System

The profile of COD removal performance of the system at different stages of the experiment is shown in Figure 2 and 3. In the #1 A<sup>2</sup>/O reactor system, COD removal efficiency increased from  $38.5\% \pm 8.8\%$  to  $71.5\% \pm 9.6\%$  as the HRT increased from 20 h (stage I) to 60 h (stage III). The results showed that COD removal efficiency might be improved by extending the HRT. In the #2 A<sup>2</sup>/O reactor system (the control reactor, HRT = 20 h), the COD removal efficiency varied from 34.1% (stage I) to 50.2% (stage III). Removal efficiency increased slightly from stage I to stage III primarily because of the acclimation and enrichment of activated sludge. However, the COD removal efficiency in the #1 reactor was higher than that in the #2 reactor during stage III. This result proved that the COD removal efficiency was improved by extending the HRT. Hence, this condition (HRT = 60 h) may contribute to a longer contact time between activated sludge and organic materials, thus resulting in better COD removal performance.

During stages IV to VI, the MLSS concentration was maintained at  $6.0 \pm 0.4\text{g/L}$ , and the HRT decreased from 60 h to 20 h in the #1 reactor. COD



**Figure 2:** Profile of COD removal performance of the system at different stages of the experiment.



**Figure 3:** Average COD removal efficiency at different stages of the experiment (error bar is the standard deviation).

removal efficiencies were 59.5% (stage IV), 58.75% (stage V), and 64.6% (stage VI), which varied insignificantly. However, in the #2 reactor, the COD removal efficiency was 38.5% (HRT = 20h, MLSS = 3.2 g/L to 3.6 g/L) during stages IV to VI. COD removal efficiency in the #1 reactor was higher than that in the #2 reactor because of higher MLSS concentration during stage VI. This result indicated that higher biomass concentration could improve COD removal, and that the HRT effect was insignificant at higher MLSS concentrations. The same observation was reported by Kim *et al.* (2013), that is, higher biomass could improve removal efficiencies of recalcitrant compounds because of the increase in the amount of degrader.

### Degradation Characteristics and Classifications of Organic Compounds

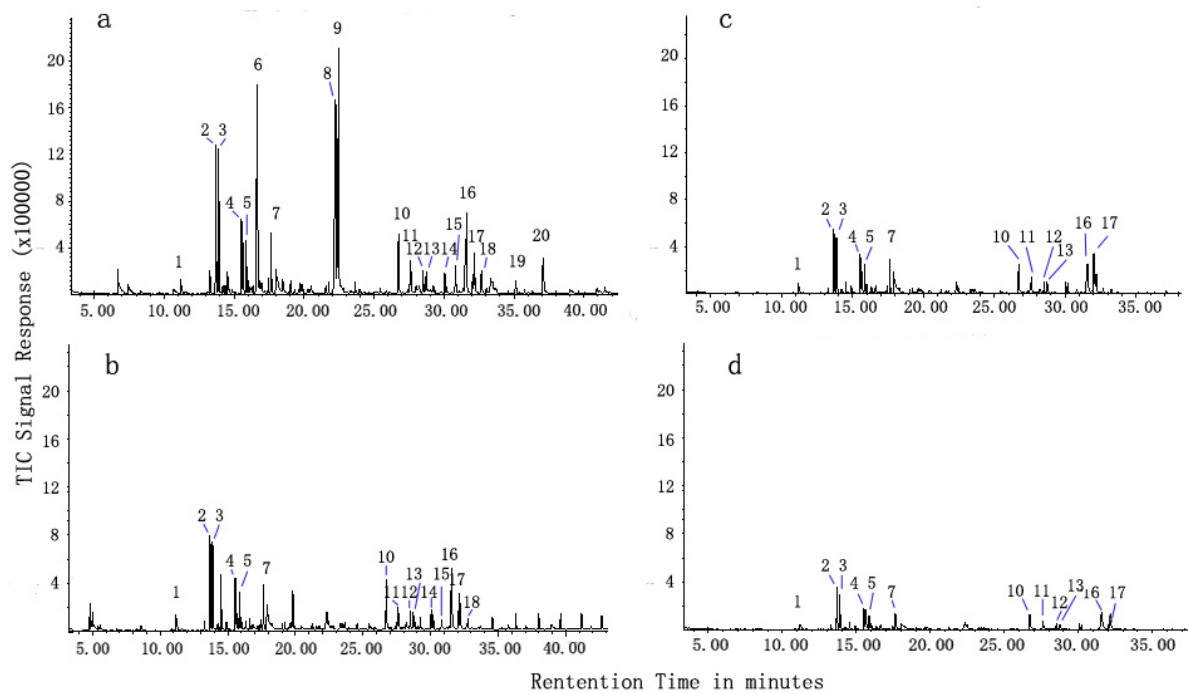
The results of the GC-MS analysis of the influent and effluent in the system are shown in Figure 4 and Table 2. The peaks of the organic compounds decreased significantly with HRT extension. The organic compounds were marked by numbers 1 to 20, as shown in Figure 4. Compounds 6, 8, and 9 disappeared when the system HRT was 20 h, whereas the rest of the compounds degraded insignificantly. When the HRT was prolonged to 40 h, organic compounds 1 to 7 and 10 to 20 degraded remarkably, whereas compounds 15, 19, and 20 disappeared. When the HRT was prolonged to 60 h, organic compounds 1 to 5, 7, 10 to 13, 16, and 17 remained, but their peaks became significantly lower.

Based on Table 2, the organic compounds in wastewater could be classified into easily removed and refractory compounds. The easily removed compounds can be removed immediately during treatment and consist mainly of ethers, alcohols, and

aldehydes. Benzyl alcohol can be considered as an easily removed organic matter because of its active hydroxyl. By contrast, the refractory compounds are degraded slowly by microbes and consist mainly of oxygen-containing heterocyclic and benzene-containing compounds.

Operating parameters of the system (e.g., HRT, solid retention time) will strongly affect the performance of a system. The HRT is the average length of time that soluble compounds remain in the reactor (Radjenovic *et al.*, 2007). These soluble compounds, such as benzyl alcohol, phenol, phenylacetic acid, and benzenediamine, in wastewater, may be sufficiently degraded in the system by extending the HRT.

The most important removal pathways of organic compounds during wastewater treatment are biotransformation/biodegradation, abiotic removal by adsorption in sludge (excess sludge removal), and stripping by aeration (volatilization) (Radjenovic *et al.*, 2007). In the industrial wastewater samples used in this study, some of compounds, such as haloid propane, could be removed by volatilization and biodegradation (Cheng *et al.*, 2008). However, haloid propane was mainly removed by biodegradation, while volatilization could be neglected due to the low concentration in wastewater (Galapate *et al.*, 1999; Moura *et al.*, 2007). Ether, alcohol, and aldehydes were considered to be soluble in water and biodegradable. Phthalates could be removed by biodegradation and adsorption in activated sludge processes (Huang *et al.*, 2010; Huang *et al.*, 2008), whereas the adsorption of the contaminants could be neglected due to continuous operation of the system. The adsorption capacity of the sludge was saturated due to the low growth rate of the sludge. Hence, the main removal pathway of organic matter in the wastewater was biodegradation in the system.



**Figure 4:** The results of the GC-MS analysis of the influent and effluent in the system: (a) influent; (b) effluent of HRT=20h; (c) effluent of HRT=40h; (d) effluent of HRT=60h

**Table 2: Degradation characteristics and classifications of the organic compounds.**

| Category                 | No.  | Compounds appellation  | Relative removal percentage (%) <sup>a</sup> |           |           |
|--------------------------|--|--|--|-----------|-----------|
|                          |  |  | 20h  | 40h       | 60h       |
| Easily removed compounds | 6  | Benzyl alcohol   | 94.5±3.1                                     | 96.2±2.2  | 97.6±1.5  |
|                          | 8  | propane, 2,2'-oxybis[1,3-dichloro-   | 87.0±7.3                                     | 88.0±5.6  | 99.1±0.3  |
|                          | 9  | propane, 1,2-dichloro- 3-[2- chloro- 1- (chloromethyl)ethoxy]-               | 91.5±2.6                                     | 98.9±0.7  | 100.0±0.0 |
|                          | 14   | phthalic acid, isobutyl nonyl ester  | 72.7±5.4                                     | 77.5±3.6  | 96.9±1.3  |
|                          | 18   | ethane, 2-chloro-1,1-dimethoxy-  | 76.3±3.9                                     | 79.0±2.8  | 92.5±6.1  |
|                          | 19   | phenol, 4-propyl-  | 75.3±8.1                                     | 100.0±0.0 | 100.0±0.0 |
| Refractory compounds     | 20   | propanedinitrile,[3-(4-methoxyphenyl)-1-methylpropylidene]-                  | 91.8±3.6                                     | 95.3±2.1  | 97.5±2.4  |
|                          | 1  | 3,3-dichloro-propene   | 2.1±1.4                                      | 4.5±3.2   | 7.1±4.7   |
|                          | 2  | phenylacetic acid, 4-methoxy-, methyl ester                                  | 35.1±7.2                                     | 57.4±12.1 | 73.1±6.6  |
|                          | 3  | 1,3-dioxolane, 2-heptyl-4- chloro- methyl-                                   | 12.6±4.3                                     | 38.2±9.1  | 59.3±11.5 |
|                          | 4  | bis[(4-methoxyphenyl)methyl]disulfide  | 20.5±6.2                                     | 28.8±9.7  | 58.0±12.4 |
|                          | 5  | 1,3-benzenediamine, 4-methyl-  | 13.4±3.8                                     | 32.0±10.3 | 65.1±7.4  |
|                          | 7  | 4-(But-2-oxy)benzaldehyde  | 15.7±6.5                                     | 29.0±13.2 | 53.2±9.7  |
|                          | 10   | benzene, [(3-chloro-2-propenyl) oxy]-  | 24.3±9.1                                     | 47.1±12.7 | 67.7±17.3 |
|                          | 11   | 2,6-dichlorobenzaldoxime   | 30.9±11.4                                    | 49.2±13.9 | 68.2±12.7 |
|                          | 12   | 3-Furan-2-yl-acrylicacid 4-[(2-chloro-benzoyl)-hydrazonomethyl]-phenyl ester | 14.7±4.6                                     | 30.4±8.3  | 64.4±14.2 |
|                          | 13   | benzene, 1-methoxy-4-propyl-   | 14.7±3.8                                     | 50.4±13.8 | 70.8±21.2 |
| 15                       | benzene, 1-nitro-2-(p-methylphenoxy)-4-fluoro- | 57.6±5.3   | 100±0.0                                      | 100±0.0   |           |
| 16                       | dibutyl phthalate                              | 31.1±6.7   | 76.7±9.2                                     | 88.5±5.3  |           |
| 17                       | benzene, 1,1'-(1,2-ethanediyl) bis[4-methoxy-  | 16.3±4.2   | 31.2±6.8                                     | 51.7±11.4 |           |

<sup>a</sup>—Values are given as average value ± standard deviation (n=3), which are estimated according to the peak areas of the compounds.

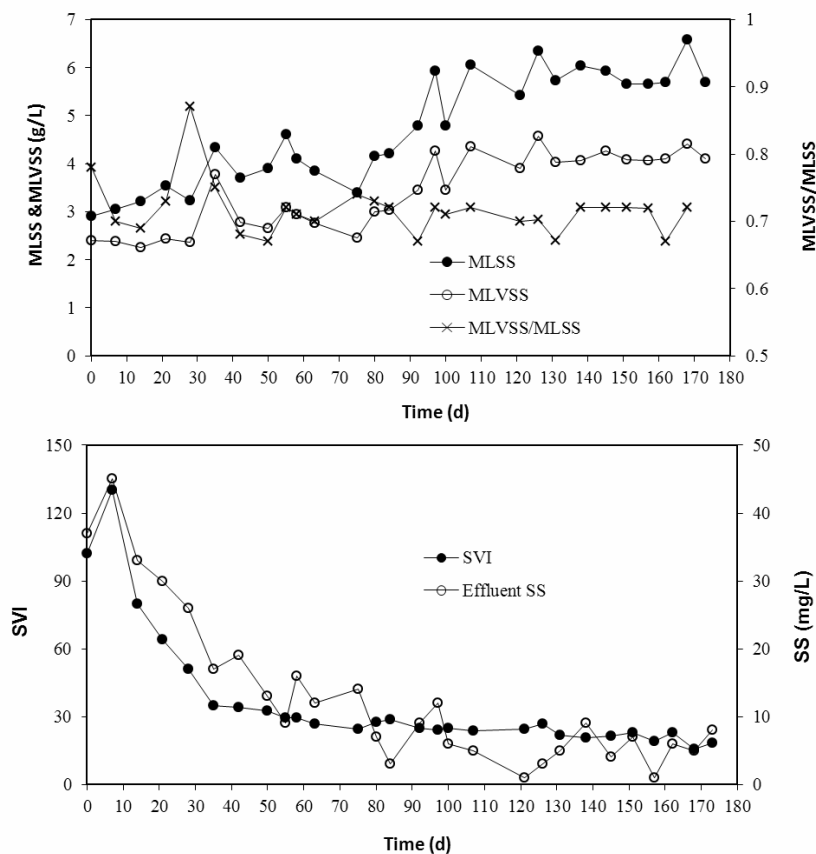
### Changes in the Characteristics of Activated Sludge

The characteristics of activated sludge in the system are shown in Figure 5. The ratio between MLVSS/MLSS was initially 0.75 to 0.80. The ratio decreased to 0.67 to 0.7 during stage VI. This result showed that the inorganic part in activated sludge increased gradually during the long-term operation of the system. This phenomenon could be attributed to the inorganic matter accumulation in activated sludge, such as metal ion accumulation. The SVI value of activated sludge in the system and the concentration of effluent suspended solids (SS) also decreased significantly during long-term operation. Given the change in settling properties, the concentration of effluent SS was maintained under 10 mg/L.

### Elemental Composition of Activated Sludge

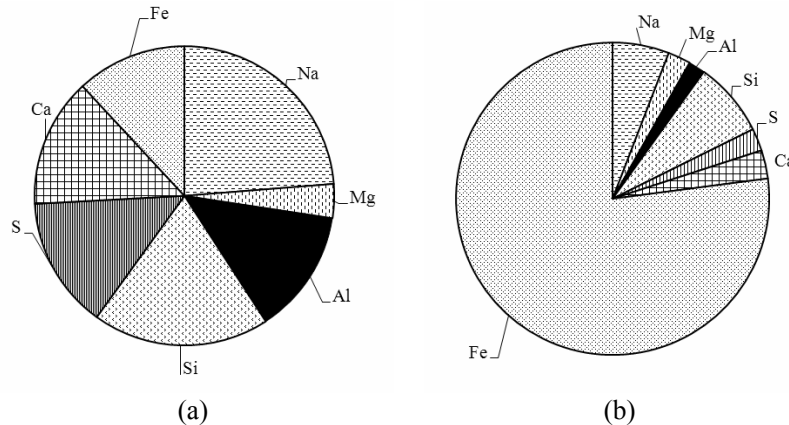
The elemental composition of activated sludge

was obtained by EDX analysis and the percentage of the elements is shown in Figure 6. The main elements in activated sludge were Na, Mg, Al, S, Si and Fe during the initial stage of operation in Figure 6a. The percentage of Fe increased significantly during the stable stage of operation in Figure 6b. Fe in wastewater accumulated in the surface of activated sludge. Fe could interact with the anionic adsorption sites provided by EPS or cell surfaces to a great extent. Iron salts could react with orthophosphate in wastewater and ferric phosphate was produced. Hence, the concentrations of inert solids increased in activated sludge, and reduced the percentage of MLVSS (Gregorio *et al.*, 2011). This result can explain the decrease of MLVSS/MLSS ratio in this experiment. In addition, the SVI value decreased in the present experiment because of Fe(III) accumulation in activated sludge and the flocculation effect, which was also reported by Oikonomidis *et al.* (2010).



**Figure 5:** The characteristics of activated sludge in the system.

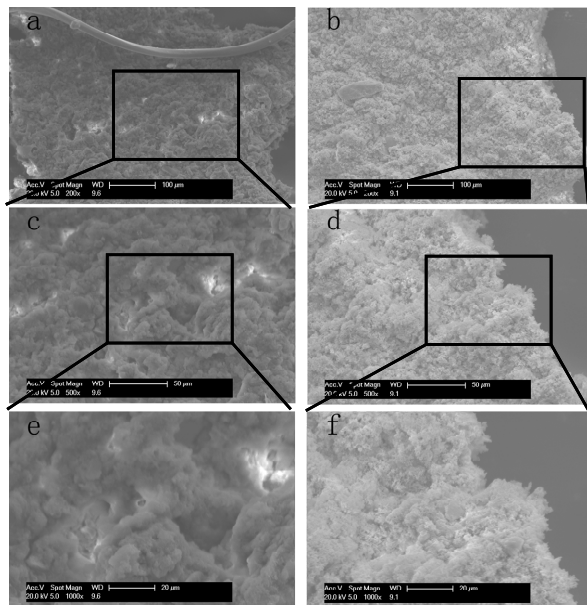




**Figure 6:** The elemental composition percentage of activated sludge as shown by EDX analysis: (a) activated sludge during the initial stage of operation; (b) activated sludge during the stable stage of operation.

### Morphological Analysis of Activated Sludge by SEM

As shown in Figure 7, the properties of activated sludge flocs changed from being fluffy during the initial stage of operation to being dense during the stable stage of operation. Fe accumulation in activated sludge caused the changes in the properties of flocs of activated sludge because of the flocculation effect of Fe.



**Figure 7:** Changes in the properties of activated sludge flocs as shown by SEM: (a), (c), and (e) represent the activated sludge during the initial stage of operation ( $\times 200$ ,  $\times 500$ , and  $\times 1000$ , respectively); (b), (d), and (f) represent the activated sludge during the stable stage of operation ( $\times 200$ ,  $\times 500$ , and  $\times 1000$ , respectively).

Oikonomidis *et al.* (2010) also reported that floc surface properties were modified and settleability was enhanced because of the effective flocculation of Fe. The positively charged Fe species could bridge the anionic chemical groups on the floc surface. The biopolymers and bacteria were compacted and promoted flocculation according to the multivalent cation bridging theory.

### Determining the Source of Accumulated Ferric Ions in Activated Sludge Flocs

Changes in EPS and the location of accumulated Fe during the initial and stable stages of the system are shown in Table 3. Fe was found on the surface and on internal pellets. This illustrated that Fe was more easily accumulated in the pellets. Moreover, the EPS of activated sludge during the stable stage of the system was much thinner than during the initial stage. This indicated that the sludge was compact due to Fe accumulation. This result could also explain the decrease in SVI values in the system resulting from the flocculation effect of Fe.

**Table 3** Changes in EPS and the location of accumulated Fe in activated sludge during the initial and stable stages of the system (Unit: mg/g VSS).

| EPS            | Initial stage |                   | Stable stage  |                   |
|----------------|---------------|-------------------|---------------|-------------------|
|                | Fe            | NPOC <sup>b</sup> | Fe            | NPOC <sup>b</sup> |
| slime          | 0.32 ± 0.12   | 6.56 ± 0.50       | 0.37 ± 0.15   | 1.78 ± 0.15       |
| LB-EPS         | 2.7 ± 0.25    | 2.62 ± 0.22       | 0.068 ± 0.024 | 1.23 ± 0.30       |
| TB-EPS         | 2.39 ± 0.16   | 81.89 ± 0.33      | 0.39 ± 0.078  | 8.70 ± 0.12       |
| pellet surface | 50.38 ± 4.149 | —                 | 604.48 ± 40.3 | —                 |
| pellet         | 11.97 ± 0.985 | —                 | 78.02 ± 5.564 | —                 |

<sup>b</sup> — The EPS was measured by NPOC

## CONCLUSIONS

In this study, a laboratory scale A<sup>2</sup>/O system was utilized to treat chemical industrial wastewater. The COD removal performance was improved by extending the HRT and increasing the MLSS. According to GC-MS analysis, some organic compounds, which consisted mainly of ether, alcohol, and aldehyde, were easily removed during treatment. Some organic matter, which were refractory and consisted mainly of oxygen-containing heterocyclic and benzene-containing compounds, could be partially removed by HRT extending. In addition, the characteristics of activated sludge, such as MLVSS/MLSS ratio and SVI, changed significantly during operation. It was found that Fe in the wastewater accumulated in the activated sludge according to the results of EDX analysis; Fe accumulated in the surface and internal pellets of the flocs. This indicated that Fe could be adsorbed by the anionic chemical groups on the EPS or cell surfaces which caused the decrease of MLVSS/MLSS ratio. Fe could also bridge the flocs and make the sludge compact. Hence, the SVI value decreased due to the flocculation effect of Fe.

## ACKNOWLEDGEMENTS

The authors wish to thank the support from the Science and Technology Support Project of Tianjin, China (No. 12ZCZDSF01800).

## NOMENCLATURE

|                                 |                                     |                              |
|---------------------------------|-------------------------------------|------------------------------|
| BOD <sub>5</sub>                | 5-day biochemical oxygen demand     | mg/L                         |
| COD                             | chemical oxygen demand              | mg/L                         |
| F/M                             | food to microorganism ratio         | kgBOD <sub>5</sub> /kgMLSS.d |
| HRT                             | hydraulic retention time            | h                            |
| K <sub>H</sub>                  | Henry coefficient                   | atm m <sup>3</sup> /mol      |
| log K <sub>ow</sub>             | octanol/water partition coefficient | dimensionless                |
| MLSS                            | mixed liquor suspended solids       | g/L                          |
| NH <sub>4</sub> <sup>+</sup> -N | ammonia nitrogen                    | mg/L                         |
| NPOC                            | nonpurgeable organic carbon         | mg/L                         |
| OLR                             | organic loading rate                | kgCOD/m <sup>3</sup> .d      |
| TDS                             | total dissolved solids              | mg/L                         |
| TN                              | total nitrogen                      | mg/L                         |

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