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Removal of veterinary antibiotics from sequencing batch reactor (SBR) pretreated swine wastewater by Fenton's reagent

Weiwei Ben, Zhimin Qiang*, Xun Pan, Meixue Chen

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuang-qing Road, Hai-dian District, Beijing 100085, China

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ABSTRACT

The large-scale application of veterinary antibiotics in livestock industry makes swine wastewater an important source of antibiotics pollution. This work investigated the degradation of six selected antibiotics, including five sulfonamides and one macrolide, by Fenton's reagent in swine wastewater pretreated with sequencing batch reactor (SBR). The dosing mode and practical dosage of Fenton's reagent were optimized to achieve an effective removal of antibiotics while save the treatment cost. The effects of initial pH, chemical oxygen demand (COD) and suspended solids (SS) of the SBR effluent on antibiotics degradation were examined. The results indicate that the optimal conditions for Fenton's reagent with respect to practical application were as follows: batch dosing mode, 1.5:1 molar ratio of $[H_2O_2]/[Fe^{2+}]$, initial pH 5.0. Under the optimal conditions, Fenton's reagent could effectively degrade all the selected antibiotics and was resistant to the variations in the background COD (0–419 mg/L) and SS (0–250 mg/L) of the SBR effluent. Besides, Fenton's reagent helped to not only remove total organic carbon (TOC), heavy metals (As, Cu and Pb) and total phosphorus (TP), but also inactivate bacteria and reduce wastewater toxicity. This work demonstrates that the integrated process combining SBR with Fenton's reagent could provide comprehensive treatment to swine wastewater.

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1. Introduction

The concern about the occurrence of antibiotics in the environment has been growing due to the potential evolution of antibiotic resistant bacteria. Antibiotics are widely used in concentrated animal feeding operations (CAFOs) around the world to treat the diseases and improve the growth rate of animals. In China, over 8000 tons of antibiotics are currently used as feed additives each year (Henan Animal Husbandry Bureau, 2009). Antibiotics are often added in the feed and water for animal feeding. However, the majority of antibiotics

are excreted in feces and urine without adsorption and metabolism by animals (Sarmah et al., 2006). As a result, the potential environmental risks brought about by antibiotics are increasing along with the rapid advance of livestock industry. In fact, antibiotics have been frequently detected in the slurry and wastewater of CAFOs (Haller et al., 2002; Malintan and Mohd, 2006). Multiple classes of antibiotics with relatively high concentrations, commonly at $>100 \mu\text{g/L}$ levels, were detected in swine waste storage lagoons in the United States (Campagnolo et al., 2002). In our previous work, eight antibiotics including four sulfonamides, three tetracyclines and one

* Corresponding author. Tel.: +86 10 62849632; fax: +86 10 62923541.

E-mail address: qiangz@rcees.ac.cn (Z. Qiang).

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macrolide were detected in the swine wastewaters of three CAFO sites located in Beijing area, with concentrations ranging from 0.62 to 32.67 $\mu\text{g/L}$ (Ben et al., 2008). In many countries, applying animal wastes after composting as fertilizer to agricultural farmlands is a common practice, which may pose a potential threat of transmission of antibiotics as well as antibiotic resistant genes to the soils and surrounding streams (Tolls, 2001; McArdell et al., 2003; Sarmah et al., 2006). For example, Dolliver et al. (2008) has recently reported that although more than 99% of chlortetracycline was degraded during animal manure composting over a period of 22–35 d, monensin and tylosin were only degraded by 54–76%, while sulfamethazine was not degraded at all. In addition, heavy metals such as arsenic (As), copper (Cu) and lead (Pb) have been frequently detected in animal manure at mg/kg concentration levels, indicating that animal waste is also an important source of heavy metals pollution (Nicholson et al., 1999).

Swine wastewater is usually characterized with high concentrations of chemical oxygen demand (COD, 5000–15,000 mg/L), total nitrogen (TN, 800–1300 mg/L), total phosphorus (TP, 100–250 mg/L) and pathogenic microorganisms. Besides, it contains hazardous micro-pollutants such as antibiotics (generally $\mu\text{g/L}$ to occasionally mg/L level) and heavy metals ($\mu\text{g/L}$ to mg/L level) (Han et al., 2000; Cang et al., 2004). In particular, antibiotics may pose a severe threat to human beings and wildlife even present at trace levels if released into the environment due to the formation of antibiotic resistant organisms (Baquero et al., 2008).

In recent years, sequencing batch reactor (SBR) has been successfully employed to remove COD, SS and nutrients from animal wastewaters (Dosta et al., 2008; Lemaire et al., 2008). However, it is reported that the biological technique is often not effective in degrading many antibiotics, for example, sulfonamides, tetracyclines and macrolides (McArdell et al., 2003; Kim et al., 2005; Batt et al., 2007). Therefore, an integrated process that combines biological technique with chemical oxidation technique is necessary for comprehensive treatment of swine wastewater. The foregoing biological technique is mainly applied to reduce COD, TN and TP. The subsequent chemical oxidation technique, as a polishing process, is applied to further remove antibiotics and/or heavy metals. Since a majority of dissolved organic materials (DOM) have been removed in the biological process, which minimizes their competition for chemical oxidants, the subsequent chemical oxidation process will effectively remove the micro-pollutants escaping from the biological treatment.

Fenton's reagent, a reaction system consisting of H_2O_2 and Fe^{2+} ion, is one of the most effective advanced oxidation processes with respect to degrading recalcitrant organic compounds. Under the catalysis of Fe^{2+} , H_2O_2 is rapidly decomposed to hydroxyl radicals ($\cdot\text{OH}$) as follows:

Due to the high oxidation potential of $\cdot\text{OH}$, Fenton's reagent has been commonly applied either as a pretreatment process to increase wastewater biodegradability or as a polishing process to further remove recalcitrant pollutants escaping from the foregoing biological unit (Zazo et al., 2005; Catalkaya

and Kargi, 2007). The effectiveness of Fenton's reagent, assisted with UV radiation, on decomposing antibiotics such as tetracycline and sulfamethoxazole in distilled water has been investigated in recent years (Bautitz and Nogueira, 2007; González et al., 2007). Our previous work has indicated that free chlorine was effective in degrading sulfonamides as well as inactivating bacteria in swine wastewater collected from several CAFO lagoons (Qiang et al., 2006). However, a considerably high chlorine dosage was required to directly remove sulfonamides from lagoon wastewater, which is likely to generate a high concentration of chlorinated disinfection byproducts with potential environmental and/or human toxicity. Therefore, Fenton's reagent may be a better choice to effectively oxidize antibiotics without producing toxic byproducts because the oxidant is $\cdot\text{OH}$ instead of chlorine.

The main objective of this study was to investigate the potential of Fenton's reagent, as a polishing process, for removal of commonly used veterinary antibiotics including five sulfonamides and one macrolide from SBR pretreated swine wastewater. The dosing mode and practical dosage of Fenton's reagent were optimized to achieve an effective removal of antibiotics. The effects of SBR effluent properties including initial pH, COD, and SS on antibiotics degradation were systematically studied. In addition, this work also investigated the secondary benefits of Fenton's reagent treatment of SBR effluent, including the removal of total organic carbon (TOC), heavy metals (As, Cu and Pb) and TP, as well as bacteria inactivation and wastewater toxicity reduction.

2. Materials and methods

2.1. Chemicals

The studied antibiotics were purchased from the following sources: sulfathiazole (STZ, 99%), sulfamethoxazole (SMX), sulfamethizole (SML), sulfadimethoxine (SDM) from Sigma-Aldrich (St. Louis, MO, USA); sulfamethazine (SMN, 99%) from Acros (New Jersey, USA); and tiamulin fumarate (TIA, 98%) from Dr. Ehrenstorfer (Augsburg, Germany). The major physico-chemical properties of the selected antibiotics are described in Table 1 (Qiang and Adams, 2004; Díaz-Cruz et al., 2006). Acetonitrile and methanol of HPLC grade were obtained from Fisher Scientific (Houston, TX, USA). Analytical grade ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), hydrogen peroxide solution (30%, w/w), H_2SO_4 and NaOH were all purchased from Beijing Chemical Reagents Company (Beijing, China). The stock solution of five sulfonamides was prepared in a mixture at an individual concentration of 100 mg/L in deionized (DI) water. It could keep stable for more than 1 month at 4 °C. The stock solution of TIA was freshly prepared at a concentration of 100 mg/L in DI water prior to each experiment to minimize hydrolysis. DI water was purposely used to dissolve the antibiotics instead of organic solvent considering that organic solvent may compete for $\cdot\text{OH}$ during Fenton's reaction.

2.2. Analysis

The concentrations of COD, free ammonia ($\text{NH}_3\text{-N}$), TN and TP were determined by Hach Methods 8000, 10031, 10072 and

Table 1 – Major physico-chemical properties and MS parameters of the selected antibiotics.

Antibiotics	Molecular weight	Log K_{ow}^a	pK_a^b	Precursor ion (m/z)	Qualifier ion (m/z)	Retention time ^c (min)	Cone voltage (V)
SMX	253.3	0.9	1.85 ± 0.30 5.60 ± 0.04	254	156	12.38	20
STZ	255.3	0.72	2.01 ± 0.30 7.11 ± 0.09	256	156	6.47	20
SML	270.3	0.54	1.86 ± 0.30 5.29 ± 0.04	271	156	9.51	20
SMN	278.3	0.89	2.07 ± 0.30 7.49 ± 0.13	279	156	8.73	30
SDM	310.3	1.63	2.13 ± 0.30 6.08 ± 0.09	311	156	14.27	30
TIA	493.7	–	7.6 ^d	494	192	13.04	30

a Reference, Díaz-Cruz et al. (2006).

b Reference, Qiang and Adams et al. (2004).

c Obtained from the LC-MS total-ion SIR chromatogram of the selected antibiotics prepared in the SBR effluent.

d <http://www.tiamutin.com/vet-pig/respiratory/app/en/microbio.shtml>.

10127 with a DR/5000 spectrophotometer (Hach Co., Loveland, USA), respectively. The concentration of suspended solids (SS) was analyzed in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Total organic carbon (TOC) was analyzed with a Total Organic Carbon Analyzer (Phoenix 8000, Tekmar-Dohrmann Co., USA). The concentration of H_2O_2 was determined by the peroxidase-DPD method (Bader et al., 1998). The concentration of Fe^{2+} was determined by HACH DR/5000 at 510 nm after complexing with 1,10-phenanthroline. To avoid the interference from Fe^{3+} , ammonium fluoride was used as a masking agent for Fe^{3+} (Qiang et al., 2003). The concentrations of heavy metals were determined by the inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500 series, USA).

The concentrations of selected antibiotics were determined with liquid chromatography-mass spectrometry (LC-MS) according to our previous work with certain modifications (Ben et al., 2008). The LC system was Alliance Liquid Chromatography 2695 and the MS system was a single-quadrupole mass spectrometer ZQ 4000 (Waters, USA). A Waters Symmetry C18 column (2.1 mm × 150 mm, 5- μ m pore size) was used for separation of antibiotics in combination with a guard column (2.1 mm × 10 mm) for protection purpose. The injection volume of each sample was 20 μ L and the column temperature was maintained at 30 °C. A binary gradient with a total flow rate of 0.2 mL/min was adopted. Mobile phase A and B were 0.2% formic acid aqueous solution (v/v) and acetonitrile, respectively. The gradient program was set as follows: the mobile phase ratio of A:B was 90:10 at 0 min, 60:40 at 10 min, 20:80 at 16 min, 90:10 at 20 min, and then maintained for 15 min for column equilibration. The ratio of mobile phases was changed linearly within each ramp. The six antibiotics could get eluted within 15 min.

The MS system was operated under the positive electrospray ionization (ESI+) mode. The MS parameters were optimized as follows: capillary temperature 120 °C, desolvation temperature 300 °C, capillary voltage 3.5 kV, and desolvation gas flow 300 L/h. The selected ion recording (SIR) mode was chosen to quantify the antibiotics. Cone voltage was optimized individually for each antibiotic to obtain a high sensitivity. For each antibiotic, the protonated molecular ion (i.e.,

$[M+H]^+$) was chosen as a precursor ion for quantification, and one qualifier ion was chosen for confirmation along with the retention time (Table 1). The total-ion SIR chromatogram of the selected antibiotics is shown in Fig. 1.

The most probable number (MPN) method was adopted to evaluate the disinfection efficiency of Fenton's reagent on swine wastewater bacteria (Atlas et al., 1984). The MPN analysis was performed by preparing 10-fold serial dilutions of sample to 10^{-10} in triplicate. The serially diluted tubes were incubated at 37 °C for 3 d. Tubes exhibiting increased turbidity after incubation were considered as positive. The MPN was determined by comparing the distribution pattern of positive tubes with the statistical MPN table.

The acute toxicity of the reaction solution was measured using the 24 h water flea (*Daphnia magna*) test (ISO, 1996). *Daphnia magna* was cultured for more than three generations and fed with green algae (*Scenedesmus obliquus*, about 1×10^5 cell/mL) according to Ren et al. (2009). The toxicity test was performed in duplicate using eight healthy neonates

Fig. 1 – Total-ion SIR chromatogram of six selected antibiotics (1.0 mg/L each) prepared in the SBR effluent. The LC-MS conditions are described in the section of analysis.

(about 24 h) in each glass beaker containing 50 mL of reaction solution. The residual H_2O_2 concentration in reaction solution after Fenton's reagent treatment was allowed to completely decay before the toxicity test was performed. The photoperiod included 16 h light and 8 h darkness and the temperature was $20 \pm 1^\circ\text{C}$. The immobility of *Daphnia magna* was recorded after 24 h and the toxicity was expressed by the immobilization percentage.

2.3. Sequencing batch reactor

An auto-controlled SBR system was utilized to pretreat the raw swine wastewater prior to Fenton oxidation process, particularly aiming at the removal of COD to significantly reduce the DOM which would otherwise compete for $\cdot\text{OH}$. The operation conditions of the SBR system were described as follows: effective reactor volume 9.0 L, hydraulic retention time 10 d, sludge retention time 45 d, mixed liquor suspended solids concentration 6500–7500 mg/L, and ambient temperature $20 \pm 3^\circ\text{C}$. The SBR reactor was operated in five sequences: influent feeding, anoxic phase, aerobic phase, sludge settling and effluent discharge. The lengths of anoxic and aerobic phases were automatically controlled by the computer program on the basis of the process variables respectively monitored with the oxidation-reduction potential, pH or dissolved oxygen probes. For each reaction cycle, the lengths of the anoxic and aerobic phases were approximately 3.5 and 2.5 h, respectively, though the exact values somewhat changed with the real-time conditions. The lengths of influent feeding, sludge settling and effluent discharge were fixed at 5, 55 and 5 min, respectively.

The raw swine wastewater, which was collected from a swine feeding plant located in Tongzhou District of Beijing, mainly consisted of feces and urine. After removing the majority of SS by natural sedimentation, the swine wastewater was pumped into the SBR reactor as influent. The major physico-chemical properties of the swine wastewater after natural sedimentation (i.e., SBR influent) and SBR effluent under steady-state operations are given in Table 2, including pH, COD, SS, TN, $\text{NH}_3\text{-N}$ and TP. The results indicate that the SBR system could effectively remove COD, SS,

TN and $\text{NH}_3\text{-N}$ from the swine wastewater. The concentrations of COD, SS, TN and $\text{NH}_3\text{-N}$ were respectively decreased from 5417 to 145 mg/L, 1380 to 68 mg/L, 1130 to 40 mg/L and 995 to 0.4 mg/L after the SBR treatment with removal efficiencies all above 95%. In contrast, TP was removed less effectively than TN (i.e., 61.8% removal). Table 2 also lists the Maximum Contaminant Level (MCL) for the individual macro-pollutants regulated by the Discharge Standard of Pollutants for Livestock and Poultry Breeding (GB18596-2001) (DSPLPB) in China. It is seen that except TP, all the other macro-pollutants could meet the discharge standard after SBR treatment. However, it should be pointed out that as a group of emerging contaminants, no MCL has been regulated for antibiotics in livestock wastewater up to date. The SBR effluent under steady-state operation conditions was used to prepare the reaction solutions for Fenton's reagent oxidation in this study.

2.4. Experimental procedures

The SBR effluent was filtered through a medium-rate quantitative filter paper (30–50 μm) placed in a Buchner funnel under vacuum to remove the residual SS. All the experiments were conducted in the filtered SBR effluent until otherwise stated. The selected antibiotics (i.e., STZ, SMX, SML, SMN, SDM and TIA) were spiked into the SBR effluent to prepare the reaction solution with an individual concentration of 1.0 mg/L. Compared to the relatively high spiked concentrations, the inward concentrations of studied antibiotics in the swine wastewater could be neglected (Ben et al., 2008). Fenton oxidation experiments were carried out in a 250-mL glass reactor under ambient temperature ($20 \pm 3^\circ\text{C}$) and completely stirring conditions. For each experiment, 200 mL of reaction solution was used. To assess the effect of SS concentration on antibiotics degradation, a desired amount of freeze-dried SS was added into the SBR effluent. The activated sludge in the SBR reactor was directly used to prepare the freeze-dried SS.

The experimental procedures for antibiotics degradation by Fenton's reagent were described as follows: 1) adjust the pH of reaction solution if required; 2) deliver a desired amount of H_2O_2 and Fe^{2+} into the reactor to initiate Fenton's reaction; 3) withdraw 1 mL of sample at pre-selected reaction times, and immediately quench $\cdot\text{OH}$ by adjusting the sample pH to above 11 with 5.0 M NaOH; 4) filter the sample with 0.45- μm syringe filter to remove potential ferric flocs; and 5) analyze the residual concentrations of antibiotics with LC-MS.

The dosing mode of Fenton's reagent could notably affect the degradation of organic compounds (Qiang et al., 2008). Both H_2O_2 and Fe^{2+} compete for $\cdot\text{OH}$ with the second-order rate constants of 2.7×10^7 and $3.2 \times 10^8 (\text{mol/L})^{-1} \text{s}^{-1}$, respectively. If either H_2O_2 or Fe^{2+} is dosed in batch at the beginning of reaction, its initial excessive concentration will stress the competition for $\cdot\text{OH}$. To assess the effect of Fenton's reagent dosing mode on antibiotics degradation, four different dosing modes (i.e., one batch, two pseudo-continuous and one continuous modes) were employed to deliver H_2O_2 and Fe^{2+} into the reactor. Specifically, both H_2O_2 and Fe^{2+} were added at the beginning of reaction in the batch mode; one reagent was singly added at the beginning of reaction and the other was

Table 2 – Major physico-chemical properties of swine wastewater (SBR influent) and SBR effluent under steady-state operation conditions.

Properties	SBR influent ^a	SBR effluent	Removal efficiency (%)	MCL ^b
pH	8.6 ± 0.4	8.5 ± 0.3	–	6.0–9.0
COD (mg/L)	5417	145	97.3	400
SS (mg/L)	1380	68	95.1	200
TN (mg/L)	1130	40	96.5	–
$\text{NH}_3\text{-N}$ (mg/L)	995	0.4	99.9	80
TP ($\text{mg PO}_4^{3-}/\text{L}$)	152	58	61.8	8.0

a Supernatant after natural sedimentation of raw swine wastewater.

b Discharge Standard of Pollutants for Livestock and Poultry Breeding (GB18596-2001) in China.

Fig. 2 – Effect of Fenton's reagent dosing mode on antibiotics degradation: (a) degradation of six antibiotics in the batch dosing mode, (b) comparison of SMX degradation in four different dosing modes, and (c) comparison of TIA degradation in four different dosing modes. Experimental conditions: $C_{0, \text{antibiotic}} = 1.0 \text{ mg/L}$, $[H_2O_2] = 1.37 \text{ mmol/L}$, $[Fe^{2+}] = 0.91 \text{ mmol/L}$, $pH_0 = 5.0$, $COD_0 = 229 \text{ mg/L}$. All the six selected antibiotics were simultaneously degraded by Fenton's reagent in the SBR effluent.

continuously delivered throughout the reaction course in the pseudo-continuous modes; and both reagents were continuously delivered throughout the reaction course in the continuous mode. In addition, the molar ratio of H_2O_2 to Fe^{2+} was optimized to achieve a high removal of antibiotics and meanwhile minimize the dosage of Fenton's reagent to save treatment cost.

3. Results and discussion

3.1. Dosing mode of Fenton's reagent

Four different dosing modes of Fenton's reagent as above mentioned were adopted to examine the competitive effect of H_2O_2 and Fe^{2+} on the degradation efficiency of antibiotics. The total dosages of H_2O_2 and Fe^{2+} were maintained constant at 1.37 and 0.91 mmol/L in all the four dosing modes, respectively, which corresponded to a molar ratio of $[H_2O_2]/[Fe^{2+}] = 1.5:1$. Fig. 2a shows that in the batch dosing mode, the kinetic curves of selected antibiotics were all characterized by a rapid degradation in the initial 2 min and a very slow degradation in the following 8 min. Since both H_2O_2 and Fe^{2+} were dosed in batch, $\cdot OH$ was mostly generated in the initial period of reaction and consumed immediately by antibiotics. Thereafter, the rapid depletion of Fe^{2+} led to the termination of $\cdot OH$ generation. The results also show that TIA was degraded more effectively than all the sulfonamides. Upon the completion of reaction time (i.e., 10 min), TIA and sulfonamides were degraded by 99% and 92–97%, respectively. The five sulfonamides exhibited similar degradation kinetics with their reaction rates towards $\cdot OH$ following a slightly increasing order of $SML < SMN < SMX \approx SDM < STZ$.

The effect of Fenton's reagent dosing mode on antibiotics degradation was specifically exemplified by SMX (Fig. 2b) and TIA (Fig. 2c). Fig. 2b shows that SMX was most rapidly degraded in the batch dosing mode, while most slowly degraded in the continuous dosing mode. The degradation rates of SMX in the two pseudo-continuous dosing modes fell in between. It implies that the reaction rate of SMX towards $\cdot OH$ largely exceeds those of H_2O_2 ($2.7 \times 10^7 \text{ (mol/L)}^{-1} \text{ s}^{-1}$) and Fe^{2+} ($3.2 \times 10^8 \text{ (mol/L)}^{-1} \text{ s}^{-1}$), which overwhelms the competition of H_2O_2 and Fe^{2+} for $\cdot OH$. In fact, Huber et al. (2003) reported that the reaction rate of SMX towards $\cdot OH$ was $5.5 \times 10^9 \text{ (mol/L)}^{-1} \text{ s}^{-1}$ at pH 7.0. The final removal efficiencies of SMX in the four dosing modes were similar, ranging from 89% in the continuous dosing mode to 95% in the batch dosing mode. It is noted that the initial pH of the reaction solution was adjusted to 5.0. The batch addition of acidic Fe^{2+} solution quickly brought down the solution pH to approximately 4.0 (after 1 min) which was more favorable for Fenton's reaction. In contrast, the continuous addition of Fe^{2+} solution gradually decreased the solution pH. The fast reaction rate of SMX toward $\cdot OH$ and the favorable pH condition accounted for a comparatively higher removal efficiency of antibiotics in the batch dosing mode.

Similar results were obtained for TIA, as shown in Fig. 2c. Because TIA could react with $\cdot OH$ more rapidly than sulfonamides as aforementioned, the competitive effect of H_2O_2 and Fe^{2+} was even more counteracted. Therefore, the final removal efficiencies of TIA in the four dosing modes only differed by 3%. Since the batch dosing mode could yield a high removal efficiency of all selected antibiotics as well as greatly shorten the reaction time, it was considered as the optimal dosing mode of Fenton's reagent and adopted for subsequent experiments.

Fig. 3 – Effect of $[\text{H}_2\text{O}_2]/[\text{Fe}^{2+}]$ molar ratio on antibiotics degradation: (a) removal efficiencies of six selected antibiotics, and (b) percent consumption of H_2O_2 and Fe^{2+} . Experimental conditions: batch dosing mode, $C_{0, \text{antibiotic}} = 1.0 \text{ mg/L}$, $[\text{H}_2\text{O}_2] = 1.37 \text{ mmol/L}$, $[\text{Fe}^{2+}] = \text{varying}$, $\text{pH}_0 = 5.0$, $\text{COD}_0 = 220 \text{ mg/L}$, reaction time = 10 min. Experiments were conducted in duplicate. The standard deviation of all data points was below 2%.

3.2. Molar ratio of H_2O_2 to Fe^{2+}

From the viewpoint of practical application, an optimal molar ratio of $[\text{H}_2\text{O}_2]/[\text{Fe}^{2+}]$ will not only yield an effective degradation of antibiotics but also minimize the dosages of H_2O_2 and Fe^{2+} (i.e., leaving the least residuals when the reaction is complete). Experiments were conducted at an initial pH of 5.0 using the batch dosing mode. The dosage of H_2O_2 was maintained constant at 1.37 mmol/L while the dosage of Fe^{2+} was adjusted to obtain the desired molar ratios of 2:1, 1.5:1 and 1:1.

Fig. 3a shows that the removal efficiency of antibiotics slightly increased with the increasing dosage of Fe^{2+} (i.e., the decreasing molar ratio of $[\text{H}_2\text{O}_2]/[\text{Fe}^{2+}]$). For example, the removal efficiency of SML was 85%, 91% and 94% at the molar ratio of 2:1, 1.5:1 and 1:1, respectively. To achieve more than 90% removal of all selected antibiotics, a molar ratio of 1.5:1 was required. Further increasing the dosage of Fe^{2+} could not notably enhance the removal of antibiotics. The residual

concentrations of H_2O_2 and Fe^{2+} were determined to assess their actual consumption during the reaction course. Fig. 3b shows that the percent consumption of H_2O_2 increased from 93% to 99%, while that of Fe^{2+} decreased from 97% to 87% as the molar ratio of $[\text{H}_2\text{O}_2]/[\text{Fe}^{2+}]$ decreased from 2:1 to 1:1. Therefore, taking both antibiotics removal efficiency and Fenton's reagent dosage into account, 1.5:1 was considered as the optimal molar ratio.

3.3. Effect of initial pH

It is well known that Fenton's reagent exhibits its highest efficiency at pH 2.8–3.0 (Zazo et al., 2005). The acidic condition maintains iron stability and lowers the redox potential of the reaction system, thus promoting the most efficient generation of $\cdot\text{OH}$. On the other hand, adjusting the original pH of the SBR effluent (8.5 ± 0.3 , Table 2) to the optimal range (2.8–3.0) will consume a considerable amount of acid. Therefore, it is a tradeoff between reaction efficiency and treatment cost. The effect of initial pH on antibiotics degradation was evaluated at pH values of 3.0, 5.0 and 8.8 using the batch dosing mode. The pH values of 3.0 and 5.0 were adjusted prior to the start of Fenton's reaction, while pH 8.8 was the original value of the SBR effluent.

The results indicate that the final removal efficiency of all selected antibiotics exhibited a slight decrease (by <6% for individual antibiotic) as the initial pH was increased from 3.0 (Fig. 4a) to 5.0 (Fig. 4b), while a dramatic decrease in antibiotics removal was observed as the initial pH was further increased to 8.8 (Fig. 4c). At the initial pH 8.8, only 12–22% of sulfonamides and 41% of TIA were degraded upon the completion of reaction. Fenton's reaction will be significantly inhibited at high pH values (e.g., $\text{pH} > 4$) due to the formation of Fe(II) complexes, precipitation of ferric oxyhydroxides, and decreased oxidation potential of $\cdot\text{OH}$ (Gogate and Pandit, 2004). Fig. 4d shows that the pH of the reaction solution rapidly decreased after the batch addition of Fe^{2+} , then maintained nearly constant throughout the remaining course of reaction. During the first 1 min, the initial pH values of 3.0, 5.0 and 8.8 dropped quickly to 2.9, 4.0 and 7.7, respectively. This pH drop mainly accounted for the comparable removal efficiencies of antibiotics at the initial pH values of 5.0 and 3.0, as aforementioned. As a result, the initial pH 5.0 was considered as the optimal value for antibiotics degradation by Fenton's reagent in the SBR effluent.

3.4. Effect of initial COD and SS concentrations

Water matrix usually has a strong impact on the removal of target pollutants (Bautitz and Nogueira, 2007). Due to the non-selective oxidation nature of $\cdot\text{OH}$, both DOM (expressed by COD) and solid organic particles (expressed by SS) in the SBR effluent may interfere with antibiotics degradation by competing for $\cdot\text{OH}$.

To assess the effect of background COD on antibiotics degradation, experiments were carried out with three different COD concentrations (i.e., 115, 229 and 419 mg/L) in the SBR effluent using the optimal dosage of Fenton's reagent (i.e., $[\text{H}_2\text{O}_2] = 1.37 \text{ mmol/L}$ and $[\text{Fe}^{2+}] = 0.91 \text{ mmol/L}$). The blank experiment was conducted in DI water (i.e., $\text{COD} = 0 \text{ mg/L}$) for comparison purpose. The background COD concentrations were

Fig. 4 – Degradation of antibiotics under various initial pH conditions: (a) pH₀ 3.0, (b) pH₀ 5.0, (c) pH₀ 8.8, and (d) change of pH during the reaction course. Experimental conditions: batch dosing mode, C_{0, antibiotic} = 1.0 mg/L, [H₂O₂] = 1.37 mmol/L, [Fe²⁺] = 0.91 mmol/L, COD₀ = 209 mg/L.

selected according to two facts: 1) the COD concentration of the SBR effluent usually ranged from 120 to 250 mg/L under steady-state conditions, and 2) the DSPLPB regulates a maximum COD concentration of 400 mg/L for the discharge of livestock wastewater. Fig. 5a shows that SMX could be completely degraded during the initial 0.5 min in DI water. A background COD concentration of 115 mg/L had little interference with SMX degradation and 99% removal of SMX was quickly achieved in 1 min. It implies that the reaction rate of SMX towards $\cdot\text{OH}$ is much higher than that of background DOM. As the background COD increased to 229 and 419 mg/L, the final removal efficiency of SMX decreased by 2% and 15%, respectively. It is seen that even the background COD concentration was close to the discharge permit (i.e., 400 mg/L), about 85% of antibiotics could still be removed. Therefore, Fenton's reagent was resistant to the COD variation of the SBR effluent. Moreover, the ferric flocs formed after Fenton's reaction could also contribute to COD removal via coagulation and precipitation. For example, the background COD concentration was reduced from 419 to 255 mg/L after Fenton's reaction, achieving a removal efficiency of about 40%. It should be pointed out that both H₂O₂ and Fe²⁺ can be oxidized by potassium dichromate, thus their residual concentrations will contribute to the background COD. Their COD equivalences are calculated to be 0.471 mg O₂/mg H₂O₂ and 0.143 mg O₂/mg Fe²⁺. The contribution of the residual H₂O₂ and Fe²⁺ to the COD concentration was corrected in this work.

The effect of background SS on antibiotics degradation was examined with two different SS concentrations (i.e., 100 and 250 mg/L) in the SBR effluent. The blank experiment was conducted in the filtered SBR effluent (SS = 0 mg/L). A certain amount of freeze-dried SS was added into the filtered SBR effluent to obtain desired SS concentrations. The SBR effluent, after addition of the freeze-dried SS, was stirred for about 1 h and the soluble chemical oxygen demand (SCOD) concentration was analyzed. The SCOD concentrations were 205, 216 and 240 mg/L for the prepared effluents with 0, 100 and 250 mg/L SS, respectively. It is seen that the addition of different amounts of SS only slightly changed the SCOD concentration of the SBR effluent, thus maintaining a similar influence of the background COD. In addition, our supplementary experiments showed that the adsorption of antibiotics on SS was insignificant. Fig. 5b shows that the background SS concentration had insignificant impact on antibiotics degradation in the studied range from 0 to 250 mg/L. The final removal efficiency of SMX only decreased by 4% and 7% at 100 and 250 mg/L SS, respectively, in comparison to that in the absence of SS. It implies that the reaction rate of SMX towards $\cdot\text{OH}$ largely exceeds those of organic materials on SS surface. Therefore, Fenton's reagent was also resistant to the SS variation of the SBR effluent.

3.5. Secondary benefits achieved by Fenton's reagent

Besides degrading antibiotics, Fenton's reagent may also remove TOC, heavy metals and TP as well as inactivate bacteria and reduce wastewater toxicity due to the dual functions of ferric adsorption/co-precipitation and $\cdot\text{OH}$ oxidation. Experiments were conducted in the SBR effluent with a background COD concentration of 205 mg/L at an initial

Fig. 5 – Effect of SBR effluent quality on antibiotics degradation exemplified by SMX: (a) effect of initial COD concentration, and (b) effect of initial SS concentration. Experimental conditions: batch dosing mode, $C_{0, \text{antibiotic}} = 1.0 \text{ mg/L}$, $[\text{H}_2\text{O}_2] = 1.37 \text{ mmol/L}$, $[\text{Fe}^{2+}] = 0.91 \text{ mmol/L}$, $\text{pH}_0 = 5.0$. All the six selected antibiotics were simultaneously degraded by Fenton's reagent in the SBR effluent.

pH of 5.0 using the batch dosing mode. Two dosage levels of Fenton's reagent were evaluated including the optimal dosage (i.e., $[\text{H}_2\text{O}_2] = 1.37 \text{ mmol/L}$ and $[\text{Fe}^{2+}] = 0.91 \text{ mmol/L}$) which was denoted as the low dosage (LD), and five times the optimal dosage which was denoted as the high dosage (HD) for comparison purpose. After Fenton's reaction was complete, the solution pH was adjusted from approximately 3.7 (Fig. 4d) to 6.0. The DSPLPB regulates a pH range of 6.0–9.0 for the discharge of livestock wastewater. Thereafter, the generated ferric flocs were allowed to settle statically for about 20 min and the supernatant was withdrawn for analysis of TOC, heavy metals, TP, MPN and acute toxicity. The results are summarized in Fig. 6.

3.5.1. TOC removal

The removal of TOC partially reflects the mineralization degree of organic compounds. Fig. 6a shows that TOC was removed by about 40% and 60% in the SBR effluent with an initial TOC concentration of 67 mg/L at LD and HD, respectively. In contrast, in the DI water sample consisting of the six selected

antibiotics (1.0 mg/L each), TOC was only removed by 22% and 45% at LD and HD, respectively. The results indicate that the background organic materials, which contributed to most of TOC in the SBR effluent, were more easily removed than antibiotics by Fenton's reagent. Compared to the nearly complete removal of antibiotics under similar experimental conditions (e.g., Fig. 2a), the lower removal efficiency of TOC implies that most of antibiotics were degraded into organic byproducts instead of being mineralized to CO_2 and H_2O . Further study is needed to identify the byproducts, particularly focusing on the functional structures which exert anti-bacteria activity.

3.5.2. Heavy metal and TP removal

Heavy metals are frequently applied as feed additives to promote the growth rate and improve the meat color of livestock. As a consequence, swine wastewater is also an important pollution source of heavy metals. ICP-MS analysis showed that the concentrations of As, Cu and Pb were 330.6, 101.5 and 1.3 $\mu\text{g/L}$ in the SBR effluent, respectively. Fig. 6b shows that Fenton's reagent could remove 78% and 89% of As, 36% and 78% of Cu, and 18% and 25% of Pb at LD and HD, respectively. Increasing Fenton's reagent dosage from LD to HD had relatively low impact on the removal of As and Pb, while significantly enhanced the removal of Cu. Pb was most difficult to remove probably due to its lowest concentration. The removal of heavy metals was ascribed to adsorption and co-precipitation by ferric flocs and/or precipitation of heavy metal hydroxides. Although the DSPLPB does not regulate heavy metals, the removal of heavy metals helps to protect the receiving surface water. For example, the Environmental Quality Standards for Surface Water (GB3838-2002) in China has set an MCL of 0.05, 1.00 and 0.05 mg/L for As, Cu and Pb in Class-III (middle level) surface water, respectively.

As mentioned above, the SBR reactor could only achieve 61.8% removal of TP. Since Fenton's reagent was effective in removing As through adsorption and co-precipitation, it is analogously expected that TP may get effectively removed through the similar mechanism. Fig. 6b shows that at the LD of Fenton's reagent, TP concentration was remarkably reduced from 60.5 to 6.9 mg/L (as PO_4^{3-}), achieving a removal efficiency of 89%. The final concentration of TP (6.9 mg/L) was lower than the MCL regulated by DSPLPB (8.0 mg/L, Table 2). Moreover, TP could be completely removed at the HD of Fenton's reagent. It is seen that although the SBR reactor only achieved a moderate removal of TP, the subsequent treatment with Fenton's reagent could easily reduce its concentration to below MCL.

3.5.3. Bacteria inactivation and toxicity reduction

Fenton's reagent can also inactivate bacteria in the SBR effluent, which helps to prevent the release of antibiotic resistant bacteria from swine wastewater into the surrounding environment. For simplicity, MPN analysis that enumerates total bacteria populations was employed to evaluate the disinfection efficiency instead of appraising antibiotic resistant bacteria. Fig. 6c shows that a disinfection efficiency of 2.4 log and 4.4 log was achieved at LD and HD, respectively. Increasing Fenton's reagent dosage resulted in a significant increase in disinfection efficiency. It is seen that Fenton's reagent is also quite effective in inactivating bacteria, which is somewhat comparable to ozonation and chlorination (Macauley et al., 2006; Qiang et al., 2006).

Fig. 6 – Secondary benefits achieved by Fenton's reagent: (a) TOC removal, (b) heavy metal and TP removal, (c) bacteria disinfection, and (d) toxicity reduction. Experimental conditions: batch dosing mode, $C_{o, \text{antibiotic}} = 1.0 \text{ mg/L}$, $\text{pH}_0 = 5.0$, $\text{COD}_0 = 205 \text{ mg/L}$, reaction time = 10 min. The initial concentrations of heavy metals and TP in the SBR effluent were as follows: $[\text{As}] = 330.6 \text{ } \mu\text{g/L}$, $[\text{Cu}] = 101.5 \text{ } \mu\text{g/L}$, $[\text{Pb}] = 1.3 \text{ } \mu\text{g/L}$, $[\text{TP}] = 60.5 \text{ mg PO}_4^{3-}/\text{L}$. The error bars represent the standard deviation of duplicate experiments.

Because it was difficult to identify the degradation byproducts of antibiotics in the complex matrix of SBR effluent which also contained a variety of background organic substances, the acute toxicity of reaction solution was measured instead. Fig. 6d shows that the healthy neonates of *Daphnia magna* were immobilized by 69% in the SBR effluent after culturing for 24 h. The immobilization percentage decreased to 44% at the LD of Fenton's reagent, and further increasing Fenton's reagent dosage to HD had insignificant impact on toxicity reduction. This result indicates that the toxicity of swine wastewater could be reduced by approximately 25% after Fenton oxidation process.

3.6. Process applicability

At the optimal dosage of Fenton's reagent (i.e., $[\text{H}_2\text{O}_2] = 1.37 \text{ mmol/L}$, $[\text{Fe}^{2+}] = 0.91 \text{ mmol/L}$), the total cost of chemicals including the Fenton's reagent (i.e., H_2O_2 and FeSO_4) and the acid/base for pH adjustment (i.e., H_2SO_4 and NaOH) was estimated to be CNY $0.67/\text{m}^3$ wastewater treated (equal to USD $0.10/\text{m}^3$). At this dosage, Fenton's reagent could not only effectively degrade antibiotics, but also achieve a number of secondary benefits such as removal of TOC, heavy metals and TP, inactivation of bacteria, and reduction of wastewater toxicity. It is seen that Fenton's reagent is quite cost-effective in practice. Of the total chemical cost, Fenton's reagent only occupied 30% while the acid/base occupied 70% due to a quite strong buffering capacity of the swine wastewater. If the dosage of Fenton's reagent was increased by five times, the total chemical

cost only increased to about CNY $1.47/\text{m}^3$ wastewater treated (equal to USD $0.22/\text{m}^3$) because the consumption of acid/base retained similar. The practical dosage of Fenton's reagent can be readily adjusted according to the real concentration of antibiotics in swine wastewater.

The DSPLPB recommends that the treated swine wastewater that meets the discharge standard can be used for agricultural irrigation. Since the effluent quality largely surpasses the discharge standard after comprehensive treatment with the integrated process of SBR and Fenton's reagent, it may also be recycled to flush swine barns to reduce water consumption.

The solid waste produced, which mainly consisted of ferric hydroxide, was about $97 \text{ g}/\text{m}^3$ wastewater treated (dry weight) at the optimal dosage of Fenton's reagent. It was a relatively small amount. The toxicity of the solid waste was mainly ascribed to the heavy metals it contained. However, this toxicity is not problematic if the solid waste gets appropriately disposed. In general, the solid waste after drying can make low-grade bricks and road stabilizers, or go landfill.

4. Conclusions

The removal of antibiotics by Fenton's reagent in the SBR pretreated swine wastewater was investigated in this work. The SBR pretreatment could remove more than 95% of COD, SS, TN and $\text{NH}_3\text{-N}$ from the swine wastewater, thus providing

favorable conditions for Fenton oxidation process. The optimal operation conditions for Fenton's reagent and the effects of SBR effluent quality parameters were systematically assessed to achieve both effective removal of antibiotics and economical dosage of chemicals. Based on the experimental results, the following conclusions are drawn:

- Fenton's reagent could effectively remove all the selected antibiotics, including five sulfonamides and one macrolide at an initial concentration of 1.0 mg/L, from the SBR pretreated swine wastewater. From the viewpoint of practical application, the optimal operation conditions for Fenton's reagent were determined as follows: batch dosing mode, 1.5:1 molar ratio of $[H_2O_2]/[Fe^{2+}]$ ($[H_2O_2] = 1.37$ mmol/L, $[Fe^{2+}] = 0.91$ mmol/L), and initial pH 5.0.
- Due to the dual functions of ferric adsorption/co-precipitation and $\cdot OH$ oxidation, Fenton's reagent could also help to remove TOC, heavy metals and TP as well as disinfect bacteria and reduce toxicity of the SBR effluent. At the optimal dosage for antibiotics degradation as aforementioned, Fenton's reagent could not only remove about 40% TOC, 78% As, 36% Cu, 18% Pb and 89% TP, but also achieve 2.4 log of bacteria disinfection and 25% of toxicity reduction. In comparison, increasing the dosage of Fenton's reagent by five times could significantly enhance the removal of TOC and Cu as well as the disinfection of bacteria, but had relatively insignificant impact on the removal of As, Pb and TP as well as the reduction of wastewater toxicity.
- This work demonstrates that the integrated process, which combined SBR with Fenton's reagent, was feasible for the comprehensive treatment of swine wastewater. This integrated process could effectively remove both macro-pollutants (i.e., COD, SS, TN, NH_3-N , TP and bacteria) and micro-pollutants (i.e., antibiotics and heavy metals) from swine wastewater, thus greatly reducing its environmental risks.

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