Study on Pollution Situation of Wastewater from Piggery Farms and Treatment Feasibility of SMBR and IASBR Processes in Jiaxing City

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Li LI

Abstract

Discharge of chemical oxygen demand (COD), nitrogen, phosphorus, and exogenous chemicals such as heavy metals and antibiotics from livestock industries has caused severe water pollution in China. It is in an urgent need to understand the pollution profiles of the piggery wastewater and develop efficient treatment technologies. Since application of anaerobic digestion process for wastewater treatment is a national regulation and requirement for large-scale piggery farms, the piggery wastewater used in the study in fact is anaerobically digested piggery wastewater (ADPW).

With Jiaxing, an important pig breeding base in the southeast of China, as the research object, this study investigated the pollution characteristics of ADPW from ten large-scale piggery farms, the influence of ADPW on the surrounding water environment, and the long-term operational performance of a submerged membrane bioreactor (SMBR) and three intermittently aerated sequencing batch reactors (IASBRs) for the treatment of ADPW.

The water quality of ADPW was found to greatly vary in piggery farms as well as in seasons. Pollution levels of ADPW tended to be the lowest in summer while the highest in spring. COD, total nitrogen (TN), ammonia nitrogen (NH_4-N) and total phosphorus (TP) in spring varied in a range of 1,008 ~ 18,479 mg/L, 205 ~ 2,228 mg/L, 119 ~ 1,936 mg/L and 32.6 ~ 306 mg/L, respectively, with their average values four times higher than those in summer. Six heavy metals (Cu, Zn, Pb, Cd, Ni and Cr) and ten antibiotics (including three tetracyclines, two sulfonamides, three macrolides and two quinolones) were all detectable in the studied ADPW. Cu and Zn accounted for 97 ± 3% of the total metal concentration, and their concentrations in ADPW always exceeded the discharge limits of the tentative discharge standard in China. The determined total concentration of the ten antibiotics ranged between 10,140 to 1,090,250 ng/L, by far exceeding the antibiotics limit of 10 ng/L in water environment specified by the European Union (EU).

Investigation on surface water revealed the total concentration of the ten antibiotics in urban rivers ranged from 20.1 to 61.2 ng/L. The highest proportion was taken by tetracyclines, accounting for 39 ~ 95%, with an concentration up to 44.0 ng/L. Quinolones shared the second largest proportion with a total concentration up to 21.6 ng/L. Concentrations of sulfonamides and macrolides were low, respectively below 2.7 ng/L and 6.3 ng/L. Antibiotics concentrations in rural rivers were much higher than those in urban rivers. The highest total concentration of the ten antibiotics in rural rivers was up to 471 ng/L, 60% of which was attributed to tetracyclines, with the highest concentration of 253 ng/L. Sulfonamides shared 20% of the total concentration with the highest concentration of 165 ng/L. The highest concentration of macrolides and quinolones was 14.6 ng/L and 14.5 ng/L, respectively.

Removals of COD, NH_4-N, heavy metals and antibiotics were studied in the SMBR when hydraulic retention time (HRT) was gradually shortened from 12 d to 2.7 d, and the volumetric loading rates were increased from 0.4 ± 0.1 kg-COD/m^3 d and 0.13 ± 0.04 kg-NH_4-N/m^3 d to 2.8 ± 0.6 kg-COD/m^3 d and 0.49 ± 0.07 kg-NH_4-N/m^3 d. Effluent concentrations of COD, Cu and Zn remained low and stable at all loadings. The effluent NH_4-N concentrations remained below 10 mg/L at volumetric loadings of 0.33 ± 0.06 kg-NH_4-N/m^3 d, and rose to 403 mg/L at 0.49 ± 0.07 kg-NH_4-N/m^3 d. No significant difference was observed among the removals of NH_4-N and COD at different HRTs, but the removal efficiency of tetracycline antibiotics significantly decreased with the decrease in HRT. It suggests that the volumetric loading of NH_4-N could be of the control factor when applying the SMBR for the removal of NH_4-N and antibiotics.

IASBR is a novel technology that appropriate for the treatment of wastewater with high TN concentration but low COD/TN ratio. It was found that the IASBR was effective to remove antibiotics for more than 84%. And a non-aeration session of no shorter than 50 min and an aeration session of 50 ~ 120 min could be more feasible for ADPW treatment. When started up in winter at temperature lower than 18 ℃ and NH_4-N loading rate of 0.15 ~ 0.25 kg-NH_4-N/m^3 d, the reactor could remove 60 ~ 88% of TN.

In conclusion, ADPW brings high concentrations of not only COD, TN and TP, but also antibiotics and heavy metals, and it has caused serious deterioration in the surface water quality in Jiaxing City. ADPW is a kind of wastewater with low COD/TN ratio but high concentrations of TN, TP and antibiotics, implying its difficulty in meeting the discharge standards after biological treatment process at high construction and operation costs. The SMBR can retain high sludge concentration and nitrifying bacteria in the reactor, but it has little effect on TN removal, due to lack of anoxic process thus leading to highly accumulated nitrite (NO_2-N) and nitrate (NO_3-N) in the reactor; The IASBR can resist shock loadings and low temperatures, but it is sensitive to operational upsets and lack of control experience. Therefore, enhancement of the biological treatment technologies based on efficiency and economics and its combination with physicochemical treatment processes will be more promising alternative solutions which need future research.

Key words: anaerobically digested piggery wastewater (ADPW); pollution status; antibiotics; submerged membrane bioreactor (SMBR); intermittently aerated sequencing batch reactor (IASBR)

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Abbreviations

ADPW: anaerobically digested piggery wastewater

AOB: ammonia oxidizing bacteria

AUR: ammonia oxidation rate

COD: chemical oxygen demand

CIP: ciprofloxacin

CTC: chlortetracycline

DB: denitrifying bacteria

DO: dissolved oxygen

ENR: enrofloxacin

EU: the European Union

HRT: hydraulic retention time

IASBR: intermittently aerated sequencing batch reactor

IDL: instrument detection limit

IQL: instrument quantification limit

LC: liquid chromatography

LOD: limit of detection

LOQ: limit of quantification

MBR: membrane bioreactor

MLSS: higher mixed liquor suspended solids

MLVSS: mixed liquor volatile suspended solids

MS: mass spectrometer

NOB: nitrite oxidizing bacteria

NOR: norfloxacin

NUR: nitrite oxidation rate

ORP: oxidation reduction potential

OTC: oxytetracycline

PAOs: polyphosphate accumulating organisms

RTM: roxithromycin

SAUR: specific ammonia oxidation rate

SBR: sequencing batch reactor

SMBR: submerged membrane bioreactor

SMD: sulfadimidine

SMX: sulfamethoxazole

S/N: signal-to-noise

SNUR: specific nitrite oxidation rate

SV: sludge settlement ratio

SVI: sludge volume index

SPE: solid-phase extraction

SRT: sludge retention time

SS: suspended solids

TC: tetracycline

TN: total nitrogen

TP: total phosphorus

TYL: tylosin

Chapter 1 Introduction

1.1. ADPW and its impact on water environment

1.1.1. Pollution of livestock and poultry industry in China

Recently, livestock and poultry industry has developed rapidly in China, which improves the efficiency of management and decreases the cost of production, and also brings a high pressure on the ecological environment. The pig inventory was about 466 million in 2012 in China, with annual production of about 3.36 billion tons of piggery wastewater. According to the first general survey on nationwide pollution sources in China, 2010, livestock and poultry industry accounts for 41.9% of the total COD and 41.5% of total NH_4-N discharged.

It was reported that the pollution from livestock and poultry industry was one of the main non-point sources in China. Moreover, wastewater discharges without proper treatment will accelerate the eutrophication of surface waters that is also difficult for ecological remediation and thus results in the leaching of the exotic and harmful substances into groundwater (Zhou et al., 2009). So the control of livestock pollution has been listed in the whole water pollution control framework in China.

1.1.2. Water quality characteristics and control strategy of ADPW pollution

(1) Source of ADPW and pollution control

Based on the related regulations and requirements of national policies and standards, anaerobic digesters should be installed in large-scale piggery farms to treat and reutilize the waste and wastewater discharged. The anaerobic digestion process can produce renewable and combustible biogas (mainly CH_4) and reduce the pollution load when treating organic wastes like straw and swine waste or high strength organic wastewater. However, the effluent from the anaerobic digester, *i.e.* anaerobically digested piggery wastewater (ADPW) still contains lots of organic and inorganic substances, which need further treatment and recirculation. Jiaxing City is an important pig breeding base in the Yangtze River Delta region. The city supplied 2.8 million of living pigs in 2012, and discharged more than 3 million tons of ADPW. To find an appropriate treatment process and proper management of ADPW is the target of this study.

(2) Characteristics of ADPW

In the process of anaerobic fermentation to produce biogas, most of the fermented liquid residues are turned into ADPW. Biogas fermentation can not only control this non-point source pollution to a large extent, but also provide clean energy for the nearby residents to reduce the consumption of non-renewable energy such as coal, which may induce air pollution by carbon dioxide (Zhao, 1998). On the other hand, the blooming construction of biogas digesters increases the release of ADPW with low COD/TN ratio, which can be used as fertilizer due to lots of nutrients are still remained in it. Serious eutrophication is inevitable if ADPW is directly discharged into water without proper treatment, especially with its high concentrations of antibiotics (Song, 2011; Sui et al., 2011).

1.1.3. Difficulties in ADPW management and pollution control

(1) New discharge standard

A new Discharge Standard of Water Pollutants for Livestock and Poultry Breeding (draft) has been promulgated now and this new standard will replace the old one (GB18596-2001). The new standard is much more strict on effluent water quality than the old one (Table 1-1), and the existing treatment processes cannot meet the new standard.

(2) Present treatment technologies of ADPW

There are two major technologies for ADPW treatment now, seed soaking irrigation and biological treatment process. Seed soaking irrigation is simple, practical, quick and low cost, but the discharge of ADPW increases with the construction of large-scale livestock farm so it cannot be utilized completely because of the limited land resources. Traditional biological treatment technologies also have some disadvantages, such as low denitrification efficiency in both A/O and A^2O process, but the increase of reflux ratio would carry many aerobes and oxygen to the anaerobic pond resulting in decreased efficiency of denitrification. So, it is necessary to develop a new and efficient treatment process.

1.2. Antibiotics, a new pollution brought by ADPW

Antibiotics, the chemicals frequently used in daily life, are also applied in livestock breeding. Due to their effect on the generation of drug-resistant bacteria, the potential effect of antibiotics discharged into the environment has attracted more and more attentions recently.

1.2.1. Antibiotics types and their physical and chemical properties

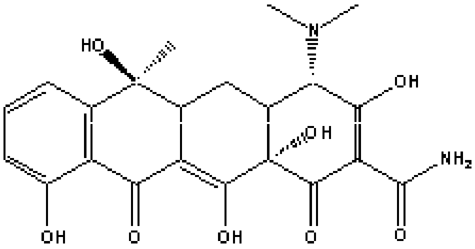
Nowadays, the widely used antibiotics can be classified according to their chemical structures into tetracyclines, macrolides, sulfonamides, β-lactam, quinolones, glycopeptides, lincosamides, aminoglycosides and others.

The following four classes of ten antibiotics are widely used in Jiaxing city.

(1) Tetracyclines

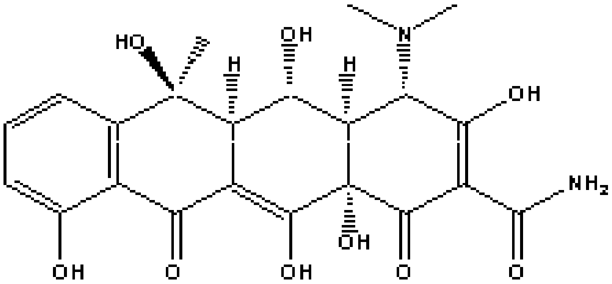
a. Tetracycline: $C_{22}H_{24}N_2O_8$; abbreviation: TC.

TC is a yellow crystal that can be decomposed at 170 ~ 175℃. It is slightly soluble in water while soluble in ethanol and acetone. TC is stable in air, but easy to absorb moisture. It’s easy to discolor when under strong sunlight, and unstable in acidic or alkaline conditions, which leads to activity decrease or forming inactivated compounds.



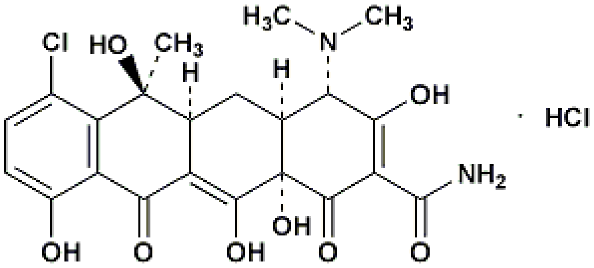
b. Oxytetracycline: $C_{22}H_{24}N_2O_9$; abbreviation: OTC

OTC is a faint yellow or yellow crystalline powder that can be decomposed at 181 ~ 182℃. It's slightly soluble in ethanol, and imperceptibly soluble in water. OTC is stable in air, and its color will darken when exposure in sunlight, and be damaged in aqueous alkali.



c. Chlortetracycline: $C_{22}H_{23}ClN_2O_8{\bullet}HCl$; abbreviation: CTC

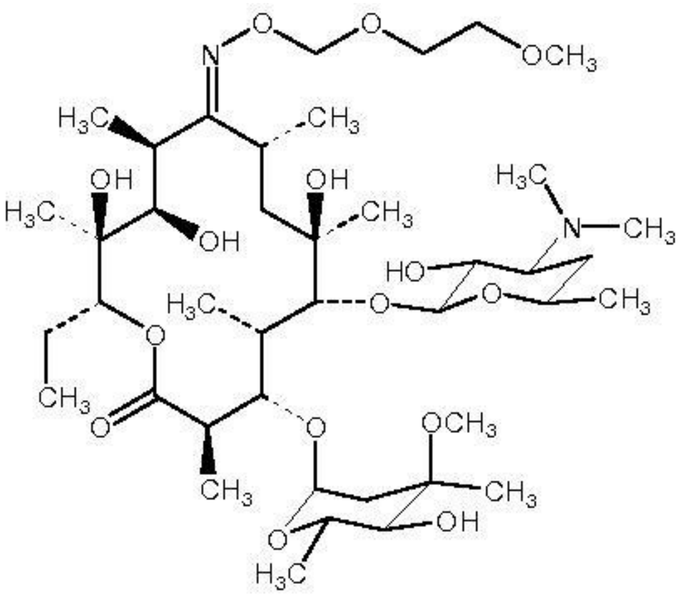
CTC is a golden yellow or yellow crystal that can be decomposed above 210℃. It's slightly soluble in ethanol and water, and almost insoluble in acetibe, ethyl ether and chloroform.



(2) Macrolides

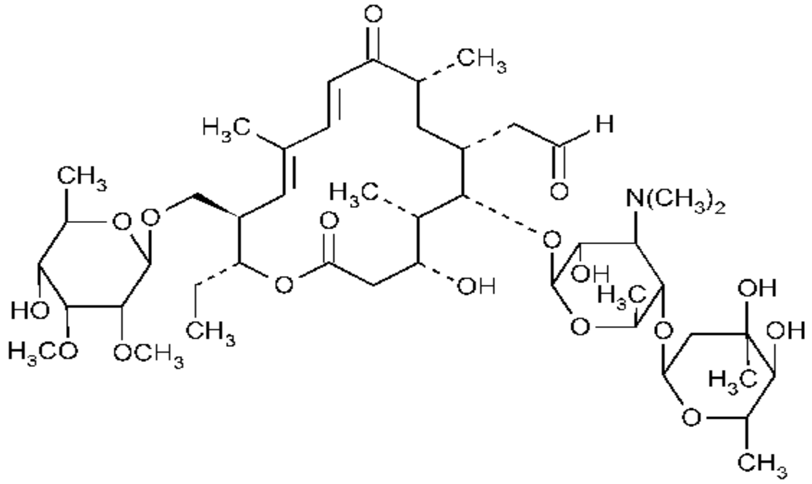
a. Roxithromycin: $C_{41}H_{74}N_2O_{15}$; abbreviation: RTM

RTM is a white crystal, and can be decomposed at 111 ~ 118 ℃. It's soluble in water and acetone, but more difficult dissolve in methanol and diethyl ether, and almost insoluble in water.



b.Tylosin: $C_{46}H_{77}NO_{17}$; abbreviation: TYL

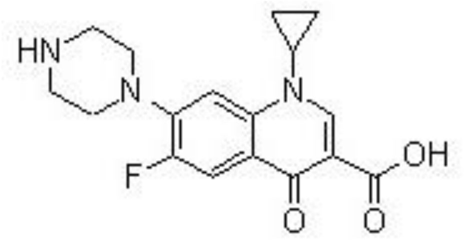
TYL is a white tabular crystal that can be decomposed at 18 ~ 132℃. It's soluble in water, slightly soluble in ethanol, and these solutions are stable at pH 4 ~ 9.



(3) Quinolones

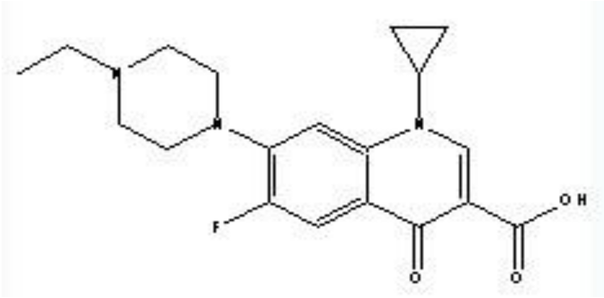
a. Ciprofloxacin: $C_{17}H_{18}FN_3O_3$; abbreviation: CIP

Ciprofloxacin is the third generation synthetic quinolone antibacterial agents. They not only have a broad spectrum of antimicrobial activity, but also effective, with effectiveness of 2 to 4 times higher than norfloxacin and enoxacin. This kind of antibiotics is effective on enteric bacilli, pseudomonas aeruginosa, haemophilus influenza, gonococcus, streptococcus, legionella and staphylococcus aureus.



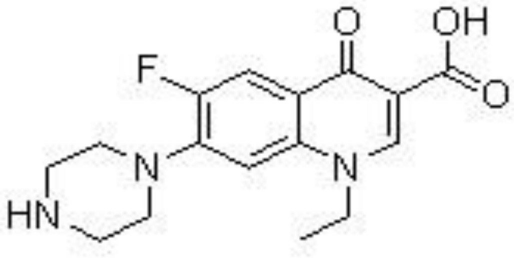
b. Enrofloxacin: $C_{19}H_{22}FN_3O_3$; abbreviation: ENR

ENR is a yellowish or pale yellow crystalline powder that can be decomposed at 221 ~ 226℃. It’s very slightly soluble in water and ethanol, but soluble in acetic acid, hydrochloric acid and sodium hydroxide.



c. Norfloxacin: $C_{16}H_{18}FN_3O_3$; abbreviation: NOR

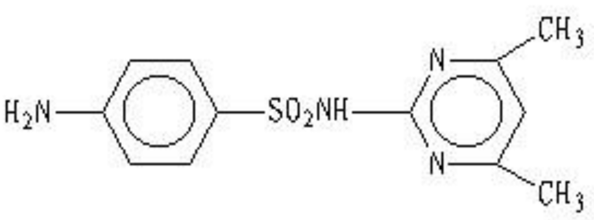
NOR is a white or light yellow powder that can be decomposed at 218 ~ 224℃. It’s easy to absorb moisture in air and to discolor gradually when in the sunlight. NOR is soluble in acetic acid, hydrochloric acid and sodium hydroxide, but slightly soluble in dimethylformamide while very slightly soluble in water and ethanol.



(4) Sulfonamides

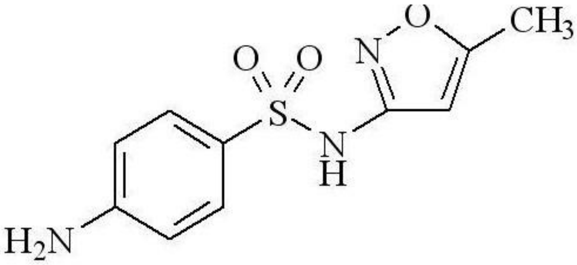
a. Sulfadimidine: $C_{12}H_{14}N_4O_2S$；abbreviation: SMD

SMD is a white or light yellow powder that can be decomposed at 197 ~ 200℃. It will discolor gradually when in the sunlight. SMD is soluble in hot ethanol, dilute acid solution and dilute alkali solution, but almost insoluble in water and diethyl ether.



b. Sulfamethoxazole: $C_{10}H_{11}N_3O_3S$；abbreviation: SMX

SMX is a white crystalline powder that can be decomposed at 168 ~ 172℃. It's soluble in diluted hydrochloric acid, sodium hydroxide solution and ammonia water, but almost insoluble in water.



1.2.2. Sources and residues of antibiotics in the environment

(1) Source of antibiotics

Antibiotics residues in environment mainly come from the antibiotics wastewater produced from antibiotics manufactories and the remaining parts after being medically used by people and animals. Because of the low volatility of antibiotics, their main migration path in environment is through water bodies and food chains.

The antibiotics wastewater from antibiotic pharmacy is biohazardous containing recalcitrant substances and highly concentrated organic matters. It was reported 20 ~ 30% of world wide antibiotics wastewater containing more than 70 kinds of antibiotics was produced by about 300 Chinese enterprises (Liu et al., 2008). The antibiotics are hard to degrade and the amount of antibiotics wastewater is huge, causing ecological environmental pollution (Amin et al., 2006; Li et al., 2008).

Antibiotics are commonly used and also abused in the medical industry. It has been reported that a variety of high concentrations of antibiotics can often be detected in hospital wastewaters. Brown *et al*. (2006) found ciprofloxacin, ofloxacin, trimethoprim, lincomycin, penicillin G, sulfamethoxazole and other antibiotics in the wastewater from a hospital, with the relatively high concentration (up to 35,500 ng/L) of ofloxacin. Lindberg *et al*. (2005) detected various antibiotics including two kind of penicillin, three kinds of fluoroquinolone, trimethoprim, sulfamethoxazole and doxycycline in a hospital wastewater treatment plant in Sweden. It was reported that more than 90% of antibiotics in human body went into the environment through feces and urine (Kümmerer et al., 2000), and then entered the sewage system, leaked into the groundwater directly or discharge after simple treatment, causing surface water pollution ultimately. In addition, pollution of large doses of discarded overdue antibiotics to the environment is significant.

Livestock, poultry and aquaculture industry are also important sources of antibiotics pollution, accounting for about half of the total antibiotics usage. Antibiotics are widely used in the prevention and treatment of animal diseases, or added into the feed to promote the growth of animals in many countries. Abuse of antibiotics in aquaculture may induce resistance genes in animals (Luo and Zhou, 2008), and most of these antibiotics that cannot be absorbed were discarded directly into the environment. Antibiotics in human and animal manure could transfer through food chain, especially those degradation-resistant and easy-adsorption antibiotics used as fertilizer, such as Sulfa and Tetracycline class, may cause ecological risk (Nygaard et al., 1992).

(2) Antibiotics residues in the environment

There are many reports about antibiotic residues in water environment recently. Trace quantities of antibiotics exist in the environment and water body are about tens to hundreds of ng/L (Tuan and Munekage, 2004; Cha et al., 2005). Sachera *et al*. (2001) detected more than 60 kinds of drugs in 108 underground water samples in German, including two commonly used antibiotics, erythrocin and sulfamethoxazole, with the highest concentration of 410 ng/L, by far exceeding the antibiotics limit of 10 ng/L in water environment specified by EU (Chen et al., 2010). Xu *et al*. (2006) analyzed 9 kinds of typical antibiotics in Hong Kong's Victoria Harbour and the Pearl River water of Guangzhou, The results showed that the concentration of norfloxacin and ofloxacin was between 53 ~ 108 ng/L and 117 ~ 251 ng/L, respectively, and the concentration of erythromycin and luo erythromycin was between 13 ~ 423 ng/L and 0 ~ 105 ng/L. Ye *et al*. (2007) investigated 9 kinds of typical antibiotics in the Pearl River Delta, and the results showed that the most serious pollution was erythromycin and sulfamethoxazole, with concentration between 779 ~ 1,340 ng/L and 517 ~ 880 ng/L, respectively. The concentration of luo erythromycin was between 184 ~ 206 ng/L, and the highest concentrations of sulfadiazine and sulfadimidine were 292 ng/L and 469 ng/L, respectively. The pollution of quinolones including levofloxacin and norfloxacin was not very serious: levofloxacin was between 16 ~ 110 ng/L, and the highest concentration of norfloxacin was 44 ng/L. erythromycin and sulfamethoxazole were also the main residual in Shenzhen Bay, 281 ng/L and 248 ng/L respectively, and other antibiotics were about dozens of ng/l in the water. Ye and Weinberg (2007) investigated the antibiotics residues in tap water which has disinfected by chlorine disinfection: 6 kinds of antibiotics were detectable and the concentrations of sulfamethoxazoles, macrolides and quinolones were 3.0 ~ 3.4 ng/L, 1.4 ~ 4.9 ng/L and 1.2 ~ 4.0 ng/L, respectively.

Pollution caused by abuse of veterinary antibiotics in the livestock breeding is more and more serious, and reports about antibiotic residues in the livestock excrement increased in recent years. Hamscher *et al*. (2002) reported tetracycline and aureomycin in liquid manure were 4.0 mg/kg and 0.1 mg/kg, respectively. Zhang *et al*. (2005) used high performance liquid chromatography (HPLC) to detect antibiotics in typical livestock and poultry dung in seven provinces of China, and the analysis results showed the average concentration of oxytetracycline, tetracycline, aureomycin was 9 mg/kg, 5.2 mg/kg, and 3.6 mg/kg, topped to 134.8 mg/kg, 78.6 mg/kg and 121.8 mg/kg. Zhang *et al*. (2008) collected 93 feces specimen from large-scale livestock and poultry farms in the north of Zhejiang Province, China. The results showed that tetracycline, oxytetracycline and aureomycin residues in livestock and poultry manure were below the detection limit to 16.8 mg/kg, 29.6 mg/kg and 11.6 mg/kg, totally 1.6 mg/kg, 3.1 mg/kg and 1.8 mg/kg on average. Liu *et al*. (2008) collected livestock excrement from 181 intensive livestock and poultry farms in Jiangsu Province, then analyzed the samples by HPLC, and the detection rate of oxytetracycline, aureomycin, methacycline, doxycycline in feces samples was 16.6%, 38.1%, 18.8%, 17.1%, respectively.

1.2.3. Potential hazards of antibiotic residues in the environment

The application of antibiotics generates the drug resistance to the bacterium, becoming one of the largest potential risks of antibiotics. Studies have shown that bacteria existing in the environment may be a potential source of antibiotic resistance in the food chain (Kennedy et al., 1998; Aga et al., 2005). Although concentrations of most antibiotics in the environment are less than 1 μg/L, but they can create favorable conditions for the growth of bacterium inducing drug resistant, due to the stable coexistence of a variety of antibiotics (Cherlet et al., 2002).

Antibiotics inhibit the growth of certain bacteria, kill the sensitive strains in water and soil, and then leave the resistant strains as the dominant bacteria. So the existence of the low concentration of antibiotics for a long time influence the microbial community to a certain degree, and destroy the balance of the ecosystem (Halling-Srensen et al., 2002). A previous study found that 1 mg/kg of tetracycline in the soil could significantly inhibit soil dehydrogenase and phosphatase activity (Boleas et al., 2005). So the antibiotics have far-reaching influence on microbial community structure and function.

Long-term intake of low doses of antibiotics by livestock and poultry and aquatic animals, will generate their resistance to antibiotics. And the animal products such as meat, milk and eggs have antibiotic residues due to the accumulation of antibiotics in animals (Wang et al., 2006). Some commonly used drugs bring specific toxicity to human health. For example, penicillin, streptomycin and sulfa drugs are easy to make the person having allergy and abnormal reaction; chloramphenicol will cause disease of regeneration barrier and hemolytic anemia; tetracycline cause photosensitivity and gastrointestinal reaction; olaquindox is gene mutagen, etc. Long-term intakes of trace antibiotics from drinking water will affect the immune system, and cause seriously interfere to human physiological function.

1.2.4. Treatment technologies for antibiotics removal

Biochemical technologies such as the biological treatment (Jiang et al., 2008), advanced oxidation (Carballa et al., 2004; Huber et al., 2005), and membrane filtration (Li et al., 2004; Kosutic et al., 2007) are the most commonly used methods for removal of antibiotics from wastewater. The traditional biological method was found inefficient, which could remove little sulfonamides and only approximately 60% of other antibiotics (Ternes et al., 2004; Karthikeyan et al., 2006). The advanced oxidation method was reported to achieve an antibiotics removal of 30 ~ 90%, but the method is limited for wide application to farms owing to its high initial investment as well as high running cost. Membrane filtration can separate antibiotics from effluent by efficient interception, but the retained antibiotics are still remained in the concentrated liquid, which needs further countermeasures to remove. Further study is required to develop efficient but low cost technology for the removal of antibiotics.

1.3. Biological treatment technologies of ADPW

Aerobic biological treatment has been widely applied for removal of organic pollutants and NH_4-N from ADPW. The most commonly used technologies are conventional activated sludge process, contact oxidation process and sequencing batch reactor (SBR) process (Yamamoto et al., 2006; Dosta et al., 2008; Chen et al., 2010). Using static precipitation for solid-liquid separation, the sludge concentration in the reactor is unable to reach a high concentration. As a result, these processes suffer from a low volumetric loading rate when they are used to treat ADPW, leading to a large reactor volume, weak to shock loading, and significant fluctuation in effluent quality impacted by the high concentrations of pollutants in the influent.

1.3.1. Membrane bioreactor (MBR)

Membrane bioreactor (MBR) is the combination of a membrane process like microfiltration or ultrafiltration with a suspended growth bioreactor, and is widely used for municipal and industrial wastewater treatment now.

(1) Advantages of MBR

The advantages of MBRs over conventional processes include small footprint, easy retrofit and upgrade of old wastewater treatment plants. It is possible to operate MBR processes at higher mixed liquor suspended solids (MLSS) concentrations compared to conventional sedimentation/separation systems, thus the reactor volume could be reduced to achieve the same loading rate.

Recently, the MBR has become an established process option to treat wastewaters because of technical innovation and significant membrane cost reduction. As a result, the MBR process has become an attractive option for the treatment and reuse of industrial and municipal wastewaters, as their the treatment plants number and their treatment capacity constantly increase. The current MBR market has been estimated to value around US$216 million in 2006 and to rise to US$363 million by 2010 (Atkinson, 2006).

(2) Disadvantages of MBR

The MBR filtration performance inevitably decreases with filtration time. This is due to the deposition of soluble and particulate materials onto and into the membrane, attributable to the interactions between activated sludge components and the membrane. This major drawback and process limitation has been investigated early, and remains one of the most challenging issues facing further technical development (Cui et al., 2003; Kraume et al., 2005).

Membrane fouling is the most serious problem affecting system performance with other membrane separation processes, which leads to a significant increase in hydraulic resistance. Therefore, alternatively frequent membrane cleaning and more frequently membrane replacement are required, increasing the operating costs significantly. Membrane fouling results from interaction between the membrane material and the components of the activated sludge liquor, which include biological flocs formed by a large range of living or dead microorganisms along with soluble and colloidal compounds. Figure 1-1 shows the main factors influencing membrane fouling.

(3) Application feasibility of MBR

Submerged membrane bioreactor (SMBR) is feasible for the treatment of ADPW. The SMBR can retain high sludge concentration, which is beneficial for improving volumetric loading rate, saving land occupation and maintaining a stable and excellent effluent quality; the membrane filtration can retain all nitrifying bacteria in the reactor, thus greatly increasing the nitrification efficiency; and it is easy to automation, thus convenient for operation in rural areas where is short of hands and technologies.

But the SMBR has little effect on TN removal due to its lack of anoxic process, leading to highly accumulated NO_2-N and NO_3-N. An improved technology of MBR is required for a better performance on ADPW treatment.

1.3.2. Intermittently aerated sequencing batch reactor (IASBR)

(1) Brief introduction to IASBR

A conventional sequencing batch reactor (SBR) operation cycle including five stages: inflow, reaction, sedimentation, outflow and idle. Its denitrification principle is based on the traditional biological nitrogen removal: (1) in the aerobic nitrification stage, NH_4-N is oxidized to NO_2-N by autotrophic ammonia bacteria, and then to NO_3-N; (2) in the anoxic denitrification stage, NO_3-N is reduced to nitrogen by heterotrophic denitrifying bacteria. Conventional biological denitrification process requires enough organic carbon (COD/TN > 5) to meet the need of heterotrophic denitrifying bacteria in the wastewater, so the traditional SBR denitrification technology is not suitable for low COD/TN ratio wastewater, such as ADPW.

Intermittently aerated sequencing batch reactor (IASBR) is an improved technology based on SBR, which innovatively implement multiple alternations between aerobic conditions and anaerobic conditions in a SBR run cycle through self-control procedures. Due to some special efficient microbial community structure generated by controlling operate mode, nitrogen and phosphorus removal from the wastewater at high efficiency could be achieved in the same reactor at the same time (Li et al., 2008). After changing the operation mode, IASBR reactor is fundamentally different from SBR mainly in the following two aspects possibly because of the special microbial population structure: (1) anaerobic ammonium oxidation bacteria (Anammox) was found in the activated sludge of the IASBR (Li et al., 2008), in which Anammox bacteria was first discovered to exist in the aerobic reactor, and the bacteria can convert NH_4-N and NO_2-N into N_2 under anaerobic conditions (Figure 1-2). the oxygen consumption can be saved due to no need of conversion of NO_2-N into NO_3-N, and organic carbon source can also be saved in the heterotrophic denitrification process with the Anammox reaction; (2) IASBR operation mode can cause the accumulation of ammonia-oxidizing bacteria and inhibition of nitrite-oxidizing bacteria, resulting in a short nitrification - denitrification stage in the wastewater treatment system (Healy et al., 2008). That is, in the aerobic nitrification stage, NH_4-N in the wastewater is oxidized to NO_2-N by ammonia-oxidizing bacteria, and then the NO_2-N is reduced to N_2 in the anoxic denitrification stage by heterotrophic denitrifying bacteria (Figure 1-2). And the use of short nitrification - denitrification stage can save 25% of oxygen demand in the nitrification stage and 40% reduction of organic carbon needed in the denitrification stage. Because of these two distinct features, IASBR technology has potential for removing nitrogen efficiently with reduced energy consumption in the treatment of low COD/TN ratio wastewater.

(2) Advantages of IASBR

Compared with other wastewater treatment processes, IASBR technology has the following advantages: 1) With the integrated device and simple structure, it can save 20% or more infrastructure investment than conventional processes; 2) It is economical and efficient for nitrogen removal, and it can achieve long-term stable short-cut nitrification, so a new nitrification technique was developed with this feature (Li et al., 2011); 3) An IASBR can also remove phosphorus efficiently in the wastewater with very low organic carbon; 4) IASBR is convenient in operation management, and has a wide range of application from small- to medium-scale wastewater treatment systems.

(3) Application feasibility of IASBR

IASBR technology is a new technology proposed by an Irish scientist recently. Long-term lab and pilot tests have shown that IASBR was effective to treat piggery wastewater, which contains hundreds of mg/L of TN and the COD/TN ratio was only about 3. In addition, the IASBR was found to resist shock loadings and low temperatures. Despite of the violent fluctuations of piggery wastewater quality with season, the effluent of IASBR remained stable and qualified. Moreover, efficient removal of total nitrogen and phosphorus could be achieved at low temperature of about 11℃, which is almost impossible for any other bioreactors up to date.

1.4. Research objectives

On the basis of investigation on pollution situation and the characteristics of wastewater quality of the typical livestock and poultry breeding sites in Jiaxing City, China, this study focused on the effect and suitable operation parameters of two kinds of wastewater treatment technologies, SMBR and IASBR, which will provide data and technical support for pollution control of ADPW.

1.5. Originality and structure

1.5.1. The main originality of this research

(1) This study gave an overall and systematic survey on the veterinary antibiotics pollution status in the river water and ADPW in Jiaxing, a city as a nutshell of Yangtze River Delta region that is densely populated and at the same time rapidly developed in piggery industries. Up to now little information could be found in the literature. The data of antibiotics, together with the data of conventional water quality indices, obtained in this study are useful for further risk management and pollution control of ADPW.

(2) Few study focused on the influence of bioreactor operational parameters on the removal performance of antibiotics from piggery wastewater. This study found that antibiotics removal in a SMBR was greatly influenced by HRT. A high removal of tetracycline antibiotics could only be achieved at HRT much longer than what was required for removal of NH_4-N and COD.

(3) Cooperated with Irish scientists, a novel technology was developed through this study, which fitted the treatment of wastewater of high TN concentration and low COD/TN ratio. It was found that the IASBR was effective to remove antibiotics and TN, and might be resistant to low temperatures.

1.5.2. Technology roadmap

First of all, pollution status analysis of groundwater quality in Jiaxing city is very important as it is seriously deteriorated by the ADPW, and extensive use of veterinary antibiotics in pig breeding industry has aggravated the ecosystem risk. 4 classes of 10 veterinary antibiotics are found to be commonly used in the pig farms through investigation, and a method to analyze these antibiotics simultaneously was established by solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC/MS/MS). Water quality of ADPW from ten large-scale pig farms in Jiaxing was investigated in this study, including conventional pollutants, heavy metals and antibiotics (Chapter 2). Then the pollution situation of the 10 antibiotics in 10 typical rural river sections and 21 urban river sections was investigated and compared (Chapter 3).

Next, an SMBR (Chapter 4) and three IASBRs (Chapter 5) were used respectively to treat ADPW. Removal performance of not only conventional water quality indexes but also heavy metals and antibiotics was studied. The whole structure of this thesis is illustrated in Figure 1-3.

Table 1-1 Comparison between the old and new discharge standards of water pollutants for livestock and poultry breeding

Water quality indices	Current standard	Tentative discharge standard (2011)	
		2-year transition period of the old field	After the 2-year transition period
pH	-	6-9	6-9
COD (mg/L)	400	150	100
BOD (mg/L)	150	40	30
SS (mg/L)	200	150	70
NH_4-N (mg/L)	80	40	25
TN (mg/L)	-	70	40
TP (mg/L)	8.0	5.0	3.0
Fecal coliform (/100 ml)	1000	1000	400
Ova of roundworm (/L)	2.0	2.0	1.0
Cu (mg/L)	-	1.0	0.5
Zn (mg/L)	-	2.0	1.5

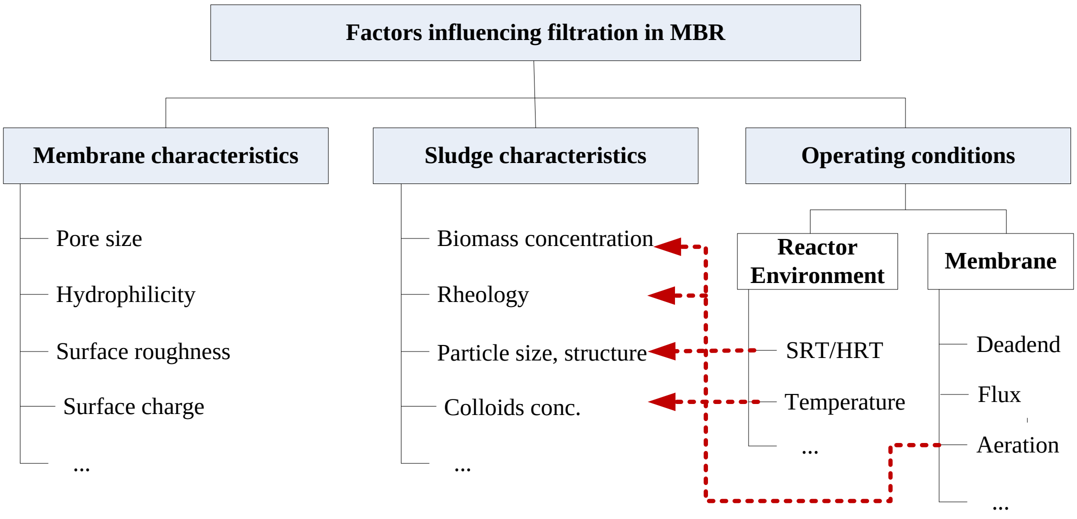


Figure 1-1 Factors influencing fouling (interactions are expressed in dotted line)

HRT: hydraulic retention time

SRT: sludge retention time

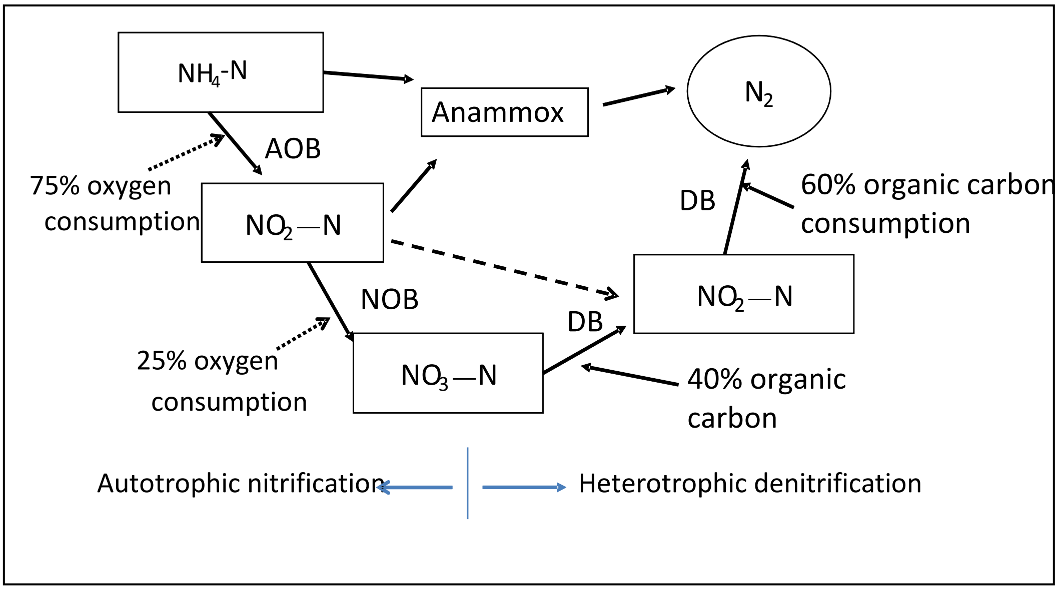


Figure 1-2 Mechanisms involved in the biological denitrification

AOB: Ammonia oxidizing bacteria

NOB: nitrite oxidizing bacteria

DB: denitrifying bacteria

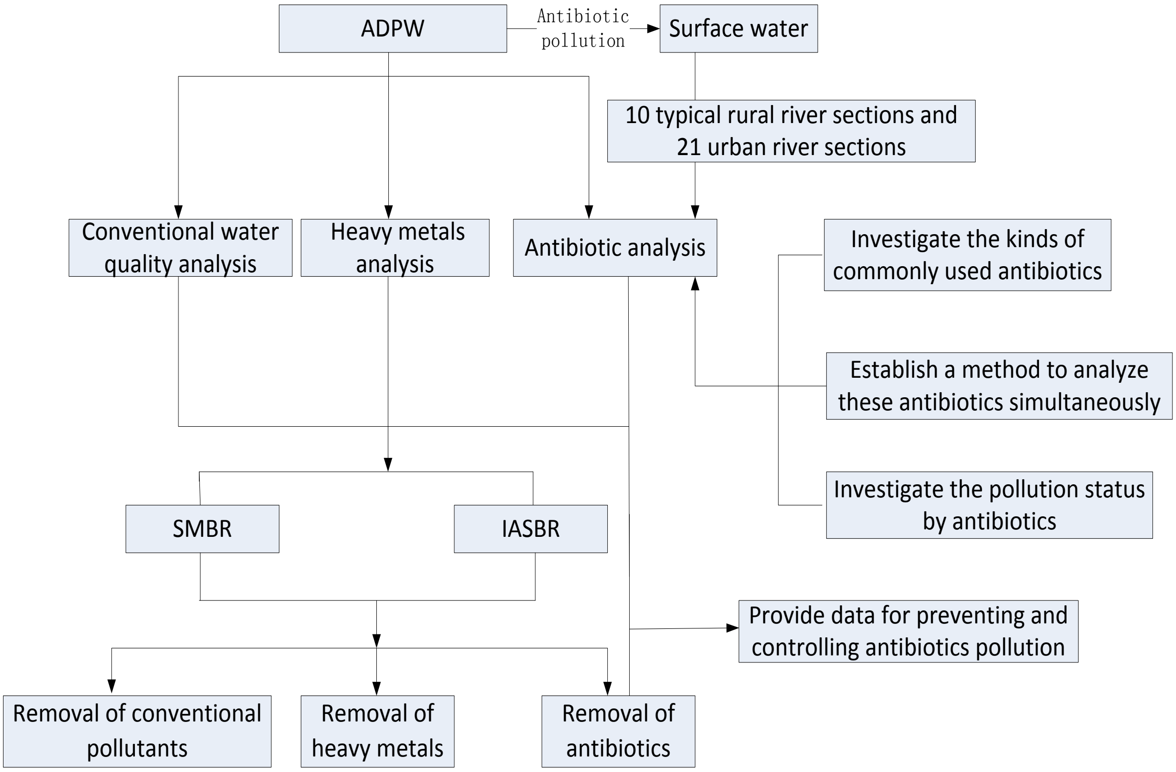


Figure 1-3 Research route and framework of this thesis

Chapter 2 Investigation on water quality of ADPW in Jiaxing

2.1. Introduction

Water pollution caused by COD, nitrogen and phosphorus from livestock industries has attracted extensive public attention in China (Xu et al., 2004; Shi et al., 2011). COD and NH_4-N have been listed in the total discharge reduction plan of the national 12th Five Year Plan made by the Ministry of Environmental Protection, which requires 8% of COD reduction and 10% of NH_4-N reduction, respectively in the effluent discharge from large-scale livestock farms during 2011 ~ 2015. At the same time, exogenous chemicals such as heavy metals and antibiotics have been widely employed in livestock industries as feed addictives or therapeutic drugs in order to promote growth or prevent disease (Jiang et al., 2010). Only a small fraction of these ingested chemicals could be utilized by the livestock while more than 85% left are excreted and finally enter the environment. Environmental pollution and ecosystem risk caused by these exogenous chemicals has aroused extensive concern in the academic world (Costanzo et al., 2005; Binh et al., 2008; Jiang et al., 2008; Kumarasamy et al., 2010; D'Costa et al., 2011). In the tentative discharge standard, the discharge limit of Zn and Cu is proposed to supplement, clearly indicating Chinese government is paying a close attention to the exogenous chemicals pollution from livestock industries.

Possibly due to the complex and costive of the analysis, little dada have been released up to date on the pollution status of heavy metals and antibiotics in ADPW. A comprehensive data of exogenous pollutants together with conventional pollution indicators, seasonal changes and variation trends among different farms, are of especially valuable for the safe treatment of ADPW. However, the data are scarce.

Jiaxing City is an important pig breeding base in the Yangtze River Delta region. The city supplied 2.8 million of living pigs in 2012, and discharged more than 3 million tons of ADPW. Taking the city as a case, the study investigated the seasonal change in the wastewater quality from ten large-scale pig farms. ADPW was analyzed for not only the conventional water quality indicators, but also the concentrations of six heavy metals and ten typical veterinary antibiotics. This chapter aimed to provide basic data on the wastewater quality, which might be helpful for effective treatment and safe management of ADPW.

2.2. Materials and methods

2.2.1. Sampling

The Southlake district supplies 1/4 of the pigs in Jiaxing city with totally 17 large-scale pig farms in it. Water samples were collected from ten pig farms, accounting for 60% of the total farms in the district, and thus the obtained data could be representative for the city.

ADPW was sampled from the ten farms on the same day, four times over a year: autumn (October 30, 2012), winter (December 26, 2012), spring (April 15, 2013) and summer (August 5, 2013). 500 ml of ADPW was sampled, preserved in a sample box with ice, and then brought back to the lab for immediate analysis or immediate pretreatment. Those samples, not to be analyzed on the sampling day, were stored at 4℃ and the subsequent analysis would be carried out within three days.

2.2.2. Analytical methods

COD, total nitrogen (TN), ammonia nitrogen (NH_4-N), and total phosphorus (TP) were analyzed according to the national standard methods (SEPA, 2002). The soluble indicators were determined with the supernatant of samples after centrifuged at 3000 rpm for 8min.

Heavy metals of Cu, Zn, Cr, Ni, Cd and Pb were analyzed by flame atomic absorption spectrometry (240AA Duo, Agilent Technologies Co., Ltd) after microwave digestion (MDS-10, Shanghai Sineo Microwave Chemistry Technology Co., Ltd).

Antibiotics were analyzed with an internal standard method (Luo et al., 2011). Liquid chromatography (LC, Waters e2695) coupled to a triple quadrupole-linear mass spectrometer (MS, waters TQ Detector) (Waters Science and Technology Co., Ltd., USA); Twelve hole solid-phase extraction device (Supelco Co., Ltd., USA); Nitrogen purging instrument (HSC-12-a, Hengao Science and Technology Development Co., Ltd., Tianjin); Oasis HLB solid-phase extraction column (3 mL/60 mg, Waters Science and Technology Co., Ltd., USA); Circulating water vacuum pump (SHZ-III, Yarong Biochemical Instrument Factory, Shanghai); Glass fiber membrane (0.7μm GF/F, GE Healthcare, Ltd, UK.); PTFE membrane (0.45 μm, Anpu Co., Ltd. Shanghai). The standards of tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), norfloxacin (NOR), enrofloxacin (ENR), ciprofloxacin (CIP), tylosin (TYL), and roxithromycin (RTM) were purchased from Dr. Ehrenstorfer GmbH Company, German. The standards of sulfamethoxazole (SMX) and sulfadimidine (SMD) were purchased from the Pharmaceutical and Biological Products Research Institute, China. Methanol, acetonitrile and formic acid were chromatographically pure. EDTA and HCl were analytically pure. All the water used in this study was Milli-Q water.

2.3. Results and discussion

2.3.1. Conventional pollution indicators

Concentrations of total COD, TN, NH_4-N and TP of ADPW from the ten farms are shown in Figure 2-1. A great variation was observed in the water quality among the four seasons. In most farms, pollution levels of ADPW tended to the lowest in summer while the highest in spring.

In spring, the COD concentration of ADPW was 6,596 ± 5,342 mg/L in the ten farms. The highest COD of 18,479 mg/L was observed in farm GQ, and the lowest COD of 1,008 mg/L was observed in farm FX, with the difference in COD between farms being as large as 18 times. COD of below 2,000 mg/L and 2,000 ~ 6,000 mg/L was respectively observed in 3 farms, and COD of over 6,000 mg/L was observed in 4 farms. The TN concentration was 1,322 ± 621 mg/L with the maximum value of 2,228 mg/L, about 11 times of the minimum value of 205 mg/L. The concentration of NH_4-N varied from 119 mg/L to 1,936 mg/L (mean value ± standard deviation being 1,150 ± 563 mg/L), accounting for 84 ± 11% of the TN. Seven pig farms had concentrations of TN and NH_4-N over 1,000 mg/L, while only three had TN and NH_4-N concentrations of below 1,000 mg/L. The TP concentration fluctuated between 32.6 mg/L and 306 mg/L (mean value ± standard deviation being 121 ± 83 mg/L), and TP of below 60 mg/L was observed in three farms, 60 ~ 150 mg/L in three farms, and over 150 mg/L in four farms.

By comparison, the pollution level in summer sharply decreased, although a violent difference was still observed among the farms. The COD concentration of ADPW was 1,510 ± 1,163 mg/L, the average value of the ten farms less than one quarter of the value in spring. Eight farms had COD of below 2,000 mg/L, and only two had COD of 2,000 ~ 6,000 mg/L. No farms had COD of over 6,000 mg/L. The concentrations of TN and NH_4-N were as low as 728 ± 422 mg/L and 384 ± 269 mg/L, respectively, and nine pig farms had concentrations of TN and NH_4-N below 1000 mg/L. The TP concentration was also half decreased. The maximum and minimum concentrations of TP were 87.5 mg/L and 30 mg/L, respectively, with mean value ± standard deviation being 57 ± 20 mg/L. TP of below 60 mg/L was observed in five farms, while 60 ~ 87.5 mg/L in other five farms.

COD of ADPW sharply decreased in summer, as might be explained by the following two reasons. (1) The temperature in the biogas digester increases to higher than 40℃ in summer while decreases to below 18℃ in winter. High temperature would lead to a more complete fermentation process and thereby an efficient organic removal in summer, as reported in literature (Zhao, 2012). (2) Double or triple times volume of water is used for drinking and washing in pig-breeding industries in summer, which may lead to dilution of the ADPW. The average concentrations of TN and TP also decreased in summer, but not as sharply as COD. This decrease might principally be attributed to the dilution effect by the increasing water consumption in summer. Fermentation process presented almost no removal of TN and TP, although it showed excellent degradation capacity of organic matters (Xu et al., 2004).

ADPW is well known for its unbalanced nutrition, especially its lower COD/TN ratio resulted from no apparent removal of nitrogen content while efficient biogasification from carboneous organic substances in the anaerobic digester, thereby the wastewater is difficult to be further efficiently biodegraded and utilized by microorganisms. The COD/TN ratios of the study were 4.6 ± 2.4 in spring, with 60% farms below 5.0 and the lowest two farms being 1.5 and 1.8. In summer, the COD/TN ratio decreased to 2.7 ± 2.6 owing to an abrupt decrease in COD. Eight out of ten farms had COD/TN ratios below 3.0 and the lowest was 0.7, respectively. Generally, a COD/TN ratio of 8 ~ 10 is required for efficient biological nitrogen removal (Wu et al., 2003). The low COD/TN ratios for the ADPW in this study suggest that it should be difficult to meet the requirement of TN discharge limit of 40 mg/L specified in the new tentative discharge standard.

Similarly, the water quality of ADPW from the ten farms varied greatly. Farms GQ and XX showed COD, TN, TP and NH_4-N concentrations significantly higher than the other eight farms. This difference was probably contributed by the difference in water consumption, as well as the breeding management, manure removal method (water soak dung, flush or dry dung) and frequency in different pig farms. Moreover, the operation efficiency of the biogas digester in each farm also exerted influence on this difference to a large extent.

Previous studies (Yang et al., 2002; Chen et al., 2010; Jiang et al., 2010; Xun et al., 2010; Zhao, 2012) showed that the concentrations of COD and TP in digested piggery wastewaters of Shanghai, Jiangsu and Hunan provinces were respectively 960 ~ 2,800 mg/L and 20 ~ 50 mg/L, agreeing with the results of this study. However, the TN concentration of ADPW from the ten farms in Jiaxing was about 1,099 ±275 mg/L, and most farms discharged even higher concentrations of TN than the reported high values of 300 ~ 900 mg/L in Shanghai and Jiangsu province, although these farms are located in the same Yangtze River Delta Region. Such pollution characteristics suggest that the biological treatment, especially nitrification and nitrogen removal of ADPW would be of more difficulty in Jiaxing city. Tremendous efforts should be made for developing efficient nitrogen removal technologies such as short-cut nitrification and denitrification (Li et al., 2011), anaerobic ammonium oxidation (Wang et al., 2009) and so on.

The soluble components of COD, TN and TP accounted for 30 ~ 97%, 50 ~ 97% and 30 ~ 96% of the total concentration, respectively. In other words, COD, TN and TP brought by suspended solids accounted for 3 ~ 70%, 3 ~ 50% and 4 ~ 70%, respectively. Considering that the water quality was greatly influenced by suspended solids, it is necessarily to remove the suspended solids as much as possible by enhancing the primary treatment. A reduction in pollutant load on the subsequent biological treatment would benefit for a better effluent quality, as well as a great reduction in the running cost.

2.3.2. Heavy metal

Seasonal change in the six heavy metals in ADPW was shown in Table 2-1. Cu and Zn were ranked as the top two dominant metals, amounting to 97 ± 3% of the total concentration for all ADPW except those sampled from the four farms (HF, FX, JH and YW) in autumn. Pb was ranked as the third, Cd, Ni and Cr were also detectable in most cases, but their concentrations were much lower.

ADPW in spring demonstrated the highest metal concentrations, with six metals detectable from all of the ten farms. Concentrations of Cu and Zn were respectively in a range of 0.82 ~ 8.8 mg/L (mean value ± standard deviation of 3.7 ± 2.7 mg/L) and 1.4 ~ 39.8 mg/L (mean value ± standard deviation of 14.8 ± 13.0 mg/L). All ADPW from the ten farms can’t meet the discharge limits of Cu (0.5 mg/L) and Zn (1.5 mg/L) according to the tentative discharge standard. Pb was ranked the third, with a concentration of 0.15 ~ 0.35 mg/L. Ni and Cr were at similar concentration levels, respectively 0.07 ~ 0.15 mg/L and 0.04 ~ 0.21mg/L. Cd was in the least concentration of 0.01 ~ 0.03 mg/L.

All the six metals were also detectable in the ADPW from most farms in autumn. Concentrations of Cu and Zn varied in a range of 0.24 ~ 3.6 mg/L and 0.32 ~ 13.0 mg/L, respectively. ADPW from eight farms exceeded the discharge limits of Cu (0.5 mg/L) and Zn (1.5 mg/L) based on the tentative discharge standard. Pb was detected in much higher concentrations in autumn than spring in 60% of the samples while undetectable in the other 40% samples. The average concentrations of Cd, Ni and Cr were respectively 0.01 mg/L, 0.04 mg/L, 0.05 mg/L, much lower than corresponding values in spring.

Summer and winter are the two seasons in which the least species of metals were detectable. Cu and Zn in the two seasons were in similar concentration levels, with a mean value lower than 40% of that in spring. According the new discharge standard, two farms could meet the standard of Cu and Zn in summer while four farms could in winter. Pb and Cd were undetectable from all samples in summer. Ni and Cr were undetectable in most of the samples in winter. The detected concentrations of Pb and Cd in winter and concentrations of Ni and Cr in summer were also much lower than that in spring.

Heavy metals in ADPW were principally attributed to the additives in the feed, which is widely used in piggery farming industries for improving the animal growth rate. All of the farms were found to produce ADPW containing Cu and Zn higher than the new discharge standards. Moreover, Pb, Cd, Ni and Cr were detectable in all ADPW from the ten farms. Such wastewater would deteriorate water quality and thereby threaten the aqua life safety if discharged into the water body without proper treatment (Wang, 2010). The heavy metal pollution problem, therefore, should be paid close attention during resource utilization or treatment of swine waste and wastewater.

Biological treatment is one of the main methods for dealing with ADPW pollution. Studies showed that nitrifying activity of the activated sludge would be irreversibly inhibited by copper and zinc at a concentration of below 10 mg/L in the influent, although little influence was observed on the organic removal, the accumulation of metals would also result in a decreased sludge settling property and an increased effluent turbidity (Xie, 2002). Specific consideration should be showed to heavy metal accumulation and its effect on microbial activity when designing a treatment system for ADPW. Metals should be removed as much as possible with physicochemical pretreatment methods before entering a biological treatment process (Guo et al., 2011; Tang et al., 2011).

2.3.3. Antibiotics analysis

Seasonal change in concentrations of the ten antibiotics was shown in Table 2-2. The total concentration of the ten antibiotics amounted to 45,000 ~ 1,090,250 ng/L (with mean value of 367,625 ng/L) in spring, 10,140 ~ 350,882 ng/L (with mean value of 98,514 ng/L) in summer, and 27,984 ~ 716,561 ng/L (with mean value of 187,421 ng/L) in winter. Samples in spring were determined having the highest antibiotics concentration, with averaged value equivalent to 3.7 times of that in summer and 2.0 times of that in winter.

The ten antibiotics were detectable from each farm in all seasons, indicating that these antibiotics were commonly used in piggery farming industries in the whole year. However, the concentrations greatly differed among the farms. The maximum value of the total concentration was respectively 24 times, 35 times and 25 times of the minimum value in spring, autumn and winter. Farm KH was always ranked the top by the total concentration of the ten antibiotics, while farm FX was ranked the tenth in two of the three seasons. The large difference among farms suggested that antibiotics may be overused in some farms, considering that the farms were in the same district and the disease prevention requirement should be similar to each other.

Tetracyclines (including TC, OTC and CTC) were always dominant in the ten antibiotics of ADPW from the farms, averagely accounting for 91±11% of the total antibiotics concentration. The three tetracyclines were detectable in all the ADPW from the ten farms at all seasons. Moreover, their detectable concentrations were high, totally up to 39,800 ~ 1,063,900 ng/L in spring, 8,150 ~ 344,880 ng/L in autumn, and 26,420 ~ 713,070 ng/L in winter. OTC was of the major among the three tetracyclines, accounting for 75 ± 22%. TC and CTC were of the minor ones, but still with concentrations of from hundreds to thousands of ng/L.

The total concentrations of two sulfa antibiotics, SMD and SMX, varied from ND to 59,700 ng/L in spring, 8 ~ 3,501 ng/L in summer and 8 ~ 1,662 ng/L in winter. The seasonal average concentration of the ten farms increased tens times higher in spring compared to the other two seasons. The gap between farms was as large as hundreds to thousands times.

The total concentrations of three macrolides (including ENR, CIP and NOR) varied from 750 to 12,900 ng/L in spring, 290 ~ 7,980 ng/L in summer and 250 ~ 12,140 ng/L in winter. The highest average concentration was found in spring, but the gap between the seasons and the gap between the farms was not as big as those of sulfa antibiotics.

TYL was detectable from almost all farms in every season. The concentration was thousands of ng/L in spring, tens to hundreds of ng/L in summer and winter. RTM was only detectable in one farm in winter, seven farms in autumn and all farms in winter, and the concentration was mostly tens to hundreds of ng/L.

The discharge limit of antibiotics has not been established yet in China. However, this study revealed that antibiotics pollution in ADPW was very severe for all farms. The determined total concentration of the ten antibiotics varied from 10,140 to 1,090,250 ng/L, by far exceeding the antibiotics limit of 10 ng/L in water environment specified by EU (Chen et al., 2010). Long-term exposure to antibiotics would induce resistance gene in flora and fauna. Such resistance gene would then be possibly transferred to nonresistant bacteria in soil, farmland and groundwater so that the number of resistant bacteria will be increased and finally spread to crops and organisms. As a consequence, the antibiotic efficacy is too much reduced to cure diseases. Therefore, much attention should be paid to the antibiotics pollution in ADPW. Besides of policy and guide for rational use of antibiotics, pollution status, efficient removal technologies and ecosystem risk assessment are also required in the future study.

2.4. Summary

Water quality of digested piggery wastewater greatly varied in different piggery farms as well as in different seasons. The above difference and change in the ADPW quality should be taken into full consideration when designing a treatment process. Enhanced primary treatment is required to prevent suspended solids and metals from entering the biological treatment process. Efficient but low cost technologies are required for advanced removal of antibiotics.

Exogenous chemical pollution in ADPW was very severe for all farms. Six metals and ten antibiotics were all detected in ADPW from the ten farms. Cu and Zn were the absolutely dominant metals, with concentrations (0.82 ~8.8 mg/L and 1.4 ~ 39.8 mg/L) in ADPW always exceeding the discharge limits of Cu (0.5 mg/L) and Zn (1.5 mg/L) according to the tentative discharge standard. The determined total concentration of the ten antibiotics was 10,140 ~ 1,090,250 ng/L (maximum), by far exceeding the antibiotics limit of 10 ng/L in water environment specified by EU. Such wastewater would deteriorate water quality and thereby threaten the aqua life safety if being discharged into the water body without proper treatment. Exogenous chemical pollution by metals and antibiotics, therefore, should be paid high attention during resource utilization or treatment of ADPW.

Table 2-1 Seasonal change in heavy metal concentrations of ADPW from ten piggery farms

		Heavy metals (mg/L)					
Season	Farm	Cu	Zn	Pb	Cd	Ni	Cr
Spring	HF	5.5	19.2	0.20	0.02	0.09	0.17
	FX	1.6	5.9	0.30	0.02	0.07	0.07
	JH	7.3	39.8	0.20	0.03	0.15	0.17
	YW	1.7	3.9	0.15	0.01	0.11	0.19
	GQ	8.8	32.3	0.35	0.02	0.39	0.19
	XX	1.7	21.8	0.25	0.03	0.09	0.06
	JF	4.0	10.8	0.25	0.03	0.08	0.14
	KH	4.1	7.0	0.30	0.02	0.09	0.08
	SZ	0.82	1.4	0.20	0.02	0.09	0.04
	DH	1.8	5.6	0.20	0.02	0.07	0.21
Summer	HF	8.6	30.3	ND	ND	0.15	0.18/
	FX	0.17	2.1	ND	ND	ND	ND
	JH	0.70	3.9	ND	ND	0.04	0.02
	YW	0.32	0.75	ND	ND	0.01	ND
	GQ	1.1	10.8	ND	ND	0.03	0.01
	XX	0.70	2.7	ND	ND	0.02	ND
	JF	0.49	2.0	ND	ND	0.01	ND
	KH	0.39	1.9	ND	ND	0.06	ND
	SZ	0.23	0.45	ND	ND	0	ND
	DH	0.88	3.5	ND	ND	0.08	0.08

Autumn	HF	0.53	1.8	0.70	0.01	0.03	0.06
	FX	0.66	1.3	0.70	0.01	0.01	ND
	JH	0.59	2.3	0.70	0.01	0.03	0.08
	YW	0.30	0.42	0.70	0.01	0.04	0.12
	GQ	3.6	13.0	1.4	0.01	0.10	ND
	XX	2.1	9.6	1.4	0.01	0.10	0.03
	JF	1.4	3.0	ND	0.02	0.04	0.02
	KH	1.5	7.0	ND	0.02	0.02	0.06
	SZ	2.5	2.9	ND	0.01	0	0.04
	DH	0.24	0.32	ND	0.01	0	0.02
Winter	HF	0.14	1.7	0.01	0.03	ND	ND
	FX	0.12	0.8	0.01	0.02	ND	ND
	JH	1.3	9.5	0.03	0.02	ND	ND
	YW	0.35	1.4	0.03	0.03	ND	ND
	GQ	4.8	23.1	0.14	0.02	ND	0.12
	XX	2.0	11.4	0.09	0.02	ND	ND
	JF	0.38	1.2	ND	0.01	ND	ND
	KH	1.3	6.8	0.04	0.02	ND	ND
	SZ	0.61	1.2	0.03	0.02	ND	ND
	DH	0.06	0.44	ND	ND	ND	ND
Maximum		8.8	39.8	1.40	0.04	0.39	0.21
Minimum		0.06	0.32	ND	ND	ND	ND
Average		1.88	7.63	0.21	0.01	0.05	0.05
STDEV		2.27	9.64	0.35	0.01	0.07	0.07

ND: not detectable.

Table 2-2 Seasonal change in antibiotics concentrations of ADPW from ten piggery farms

Season	Farms	Antibiotics (ng/L)									
		TC	OTC	CTC	SMD	SMX	ENR	CIP	NOR	TYL	RTM
Spring	HF	750	69600	2950	50	50	1050	1300	2300	21950	ND
	FX	2300	16000	21500	2900	ND	400	650	850	400	ND
	JH	5750	993500	31300	ND	ND	200	1200	650	700	ND
	YW	8600	17550	75050	ND	2300	100	650	ND	ND	3400
	GQ	6650	182300	34500	3800	ND	1600	1100	800	1100	ND
	XX	43000	88500	227500	30250	ND	150	4600	800	1700	ND
	JF	3650	44750	17750	2850	56850	2850	1950	950	6100	ND
	KH	18050	958500	87350	19200	ND	1200	1500	1000	3450	ND
	SZ	9500	36800	41100	800	ND	1500	1100	1050	6650	ND
	DH	13300	287000	64200	13800	200	3900	4600	4400	44100	ND
	Average	**11155**	**269450**	**60320**	**7365**	**5940**	**1295**	**1865**	**1280**	**8615**	**340**
Autumn	HF	400	9780	530	8	ND	220	410	600	160	150
	FX	820	51090	7010	370	20	460	290	570	30	30
	JH	1440	40430	3070	3470	31	1980	5920	80	20	260
	YW	670	14790	2640	5	1610	90	280	160	10	500
	GQ	1740	17700	52800	1590	ND	190	1440	1630	40	40

	XX	1270	4580	2300	330	ND	ND	860	440	360	ND
	JF	3020	68480	18580	90	1170	1390	1530	330	110	ND
	KH	620	331890	12370	460	80	180	2090	1970	1220	2
	SZ	8980	164260	53770	30	3	1310	860	80	790	ND
	DH	400	69770	1280	11	1	50	200	40	3	5
	Average	**1936**	**77277**	**15435**	**636**	**292**	**587**	**1388**	**590**	**274**	**99**
Winter	HF	620	92790	6430	20	2	230	700	400	80	20
	FX	670	21200	4550	390	3	350	570	230	12	9
	JH	810	39490	3800	1660	2	470	1500	60	560	7
	YW	1840	21560	12190	50	10	200	440	80	70	30
	GQ	14170	469060	93670	120	7	5880	5910	350	380	6860
	XX	2000	187930	13810	190	20	309	1930	300	300	320
	JF	890	24320	3240	50	600	680	1770	160	250	9
	KH	5830	671900	35340	200	1	1290	1810	80	100	10
	SZ	3550	46540	20970	80	2	580	680	210	120	10
	DH	300	33590	1040	8	0	30	180	40	120	6
	Average	**3068**	**160838**	**19504**	**277**	**64.7**	**1002**	**1549**	**191**	**199**	**728**

ND: not detectable.

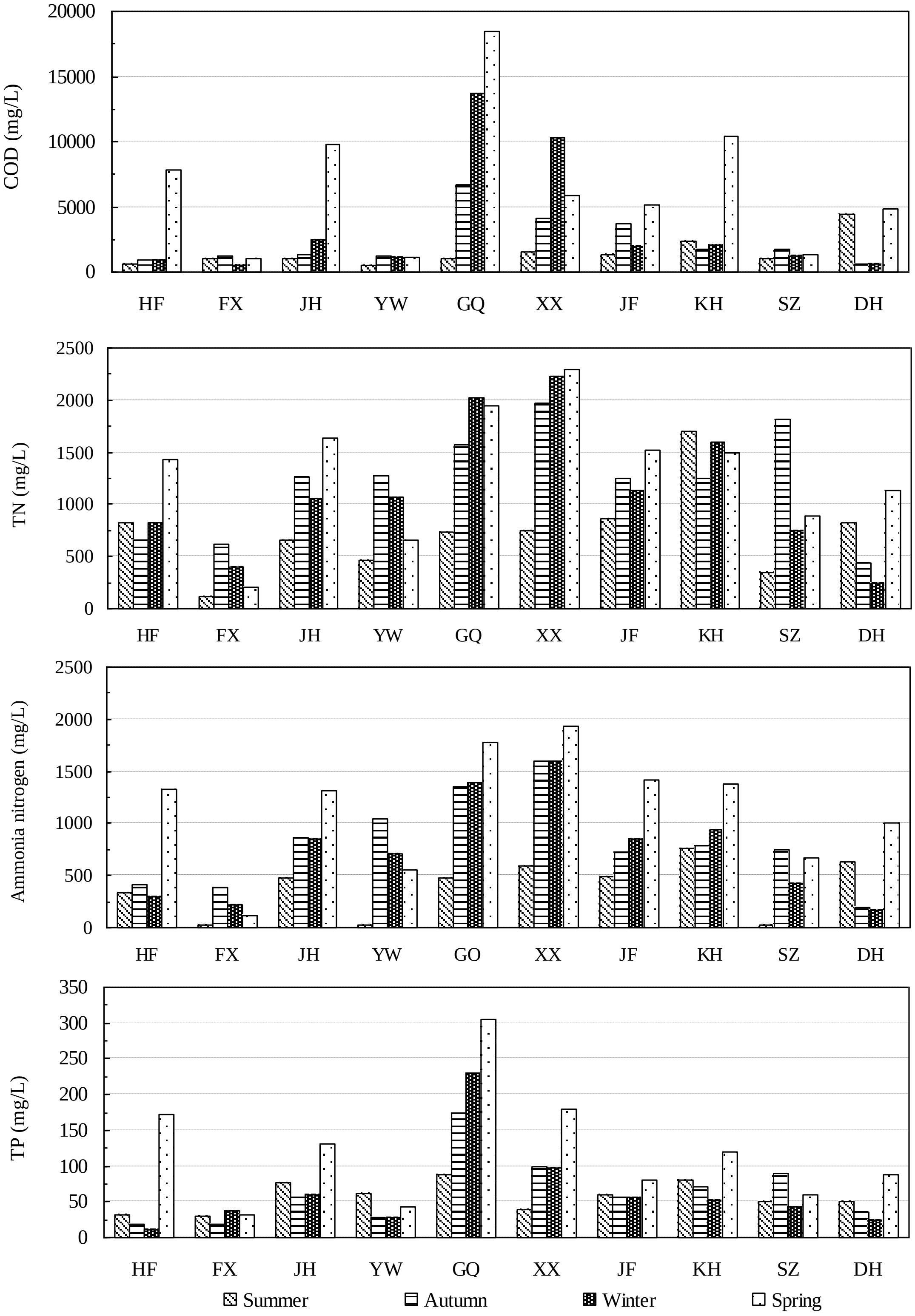


Figure 2-1 Seasonal change in COD, TN, NH_4-N and TP in the ten large-scale farms

Chapter 3 Investigation on antibiotics pollution in the rivers of Jiaxing

3.1. Introduction

Antibiotics are widely used for prevention and treatment of animal diseases. There are about 8000 tons of antibiotics used as feed additives in China every year (Ben et al., 2008). However, only a small fraction of the ingested antibiotics may be absorbed by the organisms and more than 85% run off through animal excretion and finally enter the environment (Halling-Srensen et al., 2002; Zhou et al., 2007). The antibiotics in the environment will enhance the bacteria resistance, threaten the ecological system and human health (Esiobu et al., 2002; Sarmah et al., 2006; Richardson and Ternes, 2011). There are some reports related to the serious domestic waters pollution problems and ecological security problems caused by veterinary antibiotics (Liu et al., 2006; Xu et al., 2006; Ye et al., 2007; Tong et al., 2009).

Jiaxing, a city located in the lowest reaches of Taihu lake basin, is a very important pig breeding base in the Yangtze River Delta Region. The groundwater quality is seriously deteriorated by the ADPW, and extensive use of veterinary antibiotics in pig breeding industry has aggravated the ecosystem risk. 10 veterinary antibiotics from 4 classes are found to commonly use in the pig farms in Jiaxing. And the pollution situation of these 10 antibiotics in 10 typical rural river sections and 21 urban river sections was investigated and compared, data of which might be useful for preventing and controlling the exogenous chemicals pollution in the near future.

3.2. Materials and methods

3.2.1. Analytical methods

The analytical method of antibiotics was described in 2.2.2.

3.2.2. Operation conditions

Based on the method reported by Kim et al. (2005), optimal experiments were conducted with the LC/MS/MS. Mass spectrometry analysis was conducted with electrospray positive ion mode, capillary ion source voltage of 4 kV, 120℃, carrier gas temperature of 350℃, carrier gas flow rate of 550 L/h. Single antibiotics standard of 1 mg/L was injected for a mass scan, based on which multiple reaction (MRM) mode was used for qualitative and quantitative analysis. Chromatographic analysis was carried out with Aglient eclipse XDB C18 chromatographic column (ϕ 4.6 mm × 150 mm, 5 μm) with column temperature of 30 ℃. Sample injection volume was 10 μL, and the mobile phase flow rate was 0.3 mL/min. It was reported that acetonitrile and 0.1% formic acid showed excellent efficiency on antibiotics separation (Tong et al., 2009; Tan et al., 2007). Therefore, 0.1% formic acid (A) and acetonitrile (C) was selected as the mobile phase in linear gradient elution. 10% C and 90% A were kept within 2 minutes and then changed to 40% C, 60% A from 2 to 8 minutes, 90% C, 10%A from 8 to 24 minutes, and 10%C and 90% A from 24 to 26 minutes, then re-balanced chromatographic column in 4 minutes.

3.2.3. Experimental methods

(1) Standard solutions

0.010 g of each antibiotic standard was accurately weighed and dissolved in methanol, then transferred to 100 mL brown volumetric flask to obtain 100 mg/L of standard stock solution. The standard curve was obtained by stepwise dilution of the standard stock solution to different concentrations. The above standard stock solution could be stored for one month at 4℃.

(2) Solid-phase extraction (SPE)

The SPE condition was optimized based on the method reported by Poole (2003): 500 mL of water sample was collected into 1 L of brown glass bottle, 0.2 g of EDTA was added, and the solution was stored in 4℃ for use within three days. Before SPE, water samples were filtered through 0.7 μm of GF/F glass fiber filter and then regulated to pH 3 with 6 M HCl. A HLB column was activated three times before use by washing with 2 mL of methanol, 2 mL of deionized water and 2 mL of HCl (pH 3) in sequence. Water sample of 500 mL was sucked through a HLB column at a flow rate of 5 mL/min, then the HLB column was vacuum dried for 30 min; 2 mL of 5% aqueous methanol followed with 4 mL of methanol was used for elution, and the eluate was collected in a 10 mL glass centrifuge tube, purged to nearly dry with nitrogen, then made up to 1 mL of volume to by methanol, and later transferred to a 2 mL brown sample bottle for measuring after filtrated with a PTFE needle filter.

(3) Quality control

Water samples from Jintang bridge, Nanren bridge and Duyu bridge with significant differences in total organic carbon (TOC) concentration (17.8 mg/L, 19.2 mg/L and 25.8 mg/L, respectively) were selected for confirming the adding recovery rate of SPE-LC/MS/MS method. Water samples were dosed with different concentrations of antibiotics, and the recovery rate was calculated with Equation (3-1):

$$\begin{array}{l}\text{Recovery}\\ \text{rate (\%)}\end{array} = \frac{\text{Antibiotic conc. in standard adding sample} - \text{antibiotic conc. in blank sample}}{\text{Standard adding amount}} \times 100 \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (3\text{-}1)$$

Each test was conducted in triplicates. Plus scalar control was 0.5 ~ 2 times as large as the background concentration in water samples (SEPA, 2002).

For quality control, parallel tests of a negative control (deionized water) sample and a positive control sample were set in each enrichment test at the same time. The sampling volume of a mixed standard sample injected into the analytical instrument was 10 times higher than the theoretical calculation quantity. The instrument detection limit (IDL) was defined as the concentration value when the signal-to-noise (S/N) was 3 and the instrument quantification limit (IQL) was defined as the concentration when S/N was 10. The detection limit analysis method (limit of detection, LOD) and limit of quantification (limit of quantification, LOQ) were calculated by the whole SPE-LC/MS/MS process with the enrichment factor of 500.

(4) Sampling sites and samples collection

River water samples taken from typical rural river sections and main urban river sections are shown in Table 3-1. Rural river water samples were collected from 10 river sections (Figure 3-1). Urban river water samples were taken from 21 regular monitoring section stations monitored by the local environmental protection agency (Figure 3-2).

The investigated rural river samples were collected from 10 sections in 7 villages with high pig breeding density (Henggang, Jinzhang, Nijia, Xihuangdai, Fengwan, Zhulin and Chenliang) in late August, 2012. All the rivers were significantly polluted by ADPW. The TOC concentration was 17.8 ~ 25.8 mg/L and the NH_4-N concentration ranged from 1.2 to 8.6 mg/L. The investigated urban river samples were taken from 21 routine monitoring sections by the local monitoring station of Environmental Protection Agency in early September, 2012. The urban rivers were not polluted as severe as the rural rivers by the pig breeding industry, with TOC concentration of 10.8 ~ 22.6 mg/L and NH_4-N concentration of 0.07 ~ 1.0 mg/L.

3.3. Results and discussion

3.3.1. SPE-LC/MS/MS analysis

The recovery rate of antibiotics was determined during the SPE optimization process (Fig. 3-3, a-d). Significant difference was not observed among the recovery rates of different water samples. The recovery rates of three river water samples ranged from 50 to 78%, lower than the deionized water of 62 to 106%. The recovery rate of the surface water was generally lower than deionized water, probably attributed to the competitive adsorption of organic matter in surface water onto the SPE column. Similar results were also reported by other researchers (Tan et al., 2007; Chen et al., 2011). With SPE, Tan et al. (2007) recovered 63 ~ 103%, and Chen et al. (2010) recovered 62 ~ 84% of antibiotics from surface water, also a little lower than that from deionized water (80 ~ 120%).

The standard curves depicted a linear correlation coefficient (R^2) of > 0.99 for all antibiotics in the test range. The instrument detection limit of mixed antibiotics standard samples was 0.03 ~ 3.5 μg/L. Instrument quantitative limit was 0.1 ~ 11.7 μg/L. The detection limit and quantification limit of the SPE-LC/MS/MS method with an enrichment factor of 500 were thereby calculated as 0.06 ~ 7 ng/L and 0.2 ~ 20.0 ng/L, respectively. And the RSD of three replicates was 0.3 ~ 7.1%.

3.3.2. Antibiotics pollution in typical rural river sections

The total concentrations of the ten target antibiotics ranged from 65.6 to 471.0 ng/L in the rural river samples, in which the tetracyclines accounted for more than 60% of the total concentration (Fig. 3-4, a-d). OTC and CTC concentrations were respectively 13.3 ~ 126.9 ng/L and 17.1 ~ 76.0 ng/L, significantly higher than the TC concentrations of 10.4 ~ 52.1 ng/L. Two antibiotics of sulfonamides were detected in all sampling sites except V5. The SMX concentration, ranging from 1.2 to 4.2 ng/L, was the lowest, while the SMD concentration in each sampling point varied greatly. The highest concentration was up to 161.2 ng/L in V9 with no detection in V5 and trace detection in V1, while the concentration of SMD in other 7 sections fluctuated from 10.7 to 47.7 ng/L. The detection frequency and concentration of ENR and CIP were both low. ENR was only detected in V10 with concentration of 1.6 ng/L and CIP was only detected in V1 and V9 with concentrations of 1.6 ng/L and 8.9 ng/L, respectively. However, the NOR detection frequency was high, which was detected in all samples except for V2, V4 and V8, with concentration ranged from 10.0 to 14.5 ng/L. Macrolides were detected in all sampling sites. TYL concentration was 6.25 ~ 14.68 ng/L, slightly higher than RTM concentration of 3.1 ~ 10.24 ng/L.

Antibiotics of tetracyclines, macrolides and sulfonamides are widely applied in pig-breeding industry. Tetracyclines like OTC and CTC are of the most commonly used. TYL, a kind of macrolide antibiotics, is also used frequently. The abovementioned results in this study are in agreement with the report of UCS. Quinolone antibiotics are mainly used to prevent and treat disease in poultry breeding (Huang et al., 2001), therefore, the quinolone antibiotics content in river water was relatively low in concentrated pig breeding area.

The rural river water in section V9 contained much higher concentrations of antibiotics than other sampling sites, especially for tetracyclines and sulfonamides, which could be contributed by the highly developed aquaculture and livestock farms nearby. In addition, it had been reported that sulfonamides and OTC were strongly hydrophilic and refractory, so their high concentrations in the rivers might be attributed to their stable thereby accumulative nature in the environment (Liguoro et al., 2003).

3.3.3. Antibiotics pollution in typical urban rivers sections

Veterinary antibiotics concentrations in main urban river monitoring sections are shown in Figure 3-5. Compared to rural river condition, the four groups of antibiotics were all detectable in the 21 samples but with less difference in total concentration ranging from 20 to 60 ng/L. Sites with total antibiotics concentration of below 30 ng/L accounted for 29%, namely six river sections (including the Yougang export, Yang miao bridge, Xincheng export, Luodong bridge, Southlake and Longfeng bridge). Sites with total antibiotics concentration ranging from 30 to 50 ng/L accounted for 24%, namely five river sections (including the Qixing, Wangjiangjing, Tanghui, South gate and Beiyun bridge). The remaining 10 river sections (48% of the total sampling sites) demonstrated a total antibiotics concentration of 50 ~ 62 ng/L each.

Tetracyclines antibiotics accounted for the largest proportion of 39 ~ 95% with concentrations less than 44.0 ng/L. The antibiotics concentrations of quinolones, sulfonamides and macrolides were less than 21.6 ng/L, 6.3 ng/L and 2.7 ng/L, respectively.

The antibiotics concentration was significantly higher in rural rivers than in urban rivers, suggesting that the piggery farms pollution in rural waters would probably be an important contributor to the antibiotics pollution in urban water environment.

3.4. Summary

A SPE-LC/MS/MS method was established for synchronous detection of ten veterinary antibiotics from river water. The recovery rate was 50% ~ 78%. The limit of detection and limit of quantification were 0.06 ~ 7 ng/L and 0.2 ~ 20 ng/L, respectively.

With the above method, pollution status by 10 commonly used veterinary antibiotics was investigated in both rural and urban rivers in Jiaxing city. Results revealed the total concentration of the ten antibiotics in urban rivers of Jiaxing ranged from 20.1 to 61.2 ng/L, similar to the reported data of Huangpu River, but the rural rivers were polluted more seriously than the urban rivers because the ADPW discharged directly from the high pig-breeding density villages. Tetracyclines and sulfonamides accounted for the major proportion in rural rivers while tetracyclines and quinolones were the main antibiotics in urban rivers. The regional ecological security problems should be paid more attention and ecological risk assessment is urgently needed at the same time to develop effective and harmless technologies for ADPW treatment.

Table 3-1 Sampling sites in typical rural and main urban river sections

No.	Urban river sites	No.	Urban river sites	No.	Rural river sites
1	Youchegang export	12	Duchuan creek	V1	Jinfeng bridge
2	Yangmiao bridge	13	Pinglake pond	V2	Duxu bridge
3	Qixin	14	Xiangjiadang	V3	Dongshui bridge
4	Jiaoshanmen bridge	15	Nijiahui	V4	Zhonghe bridge
5	Xincheng export	16	Huangtang bridge	V5	Jintang bridge
6	Luodong bridge	17	Baile bridge	V6	Nanren bridge
7	Wangjiangjing canal	18	Longfeng bridge	V7	Baojia bridge
8	Guanjing port	19	Tanghui	V8	Zhaojiali bridge
9	Shiyaoyang	20	Changzheng bridge	V9	Shenjiabang bridge
10	South gate	21	Beiyun bridge	V10	Zhulin intersection bridge
11	Southlake				

Figure 3-1 Rural river water sampling sites

V1. Henggang village Jinfeng Bridge; V2. Jinzhang village Duwei Bridge; V3. Jinzhang village Dongmu Bridge; V4. Nijia village Zhonghe Bridge; V5. Qifeng village Jintang Bridge; V6. Chenliang village Nanren Bridge; V7. Fengwan village Baojia Bridge; V8. Zhulin village Zhaojiali Bridge; V9. Zhulin village Shenjiabang Bridge; V10. Zhulin village Lukou Bridge.

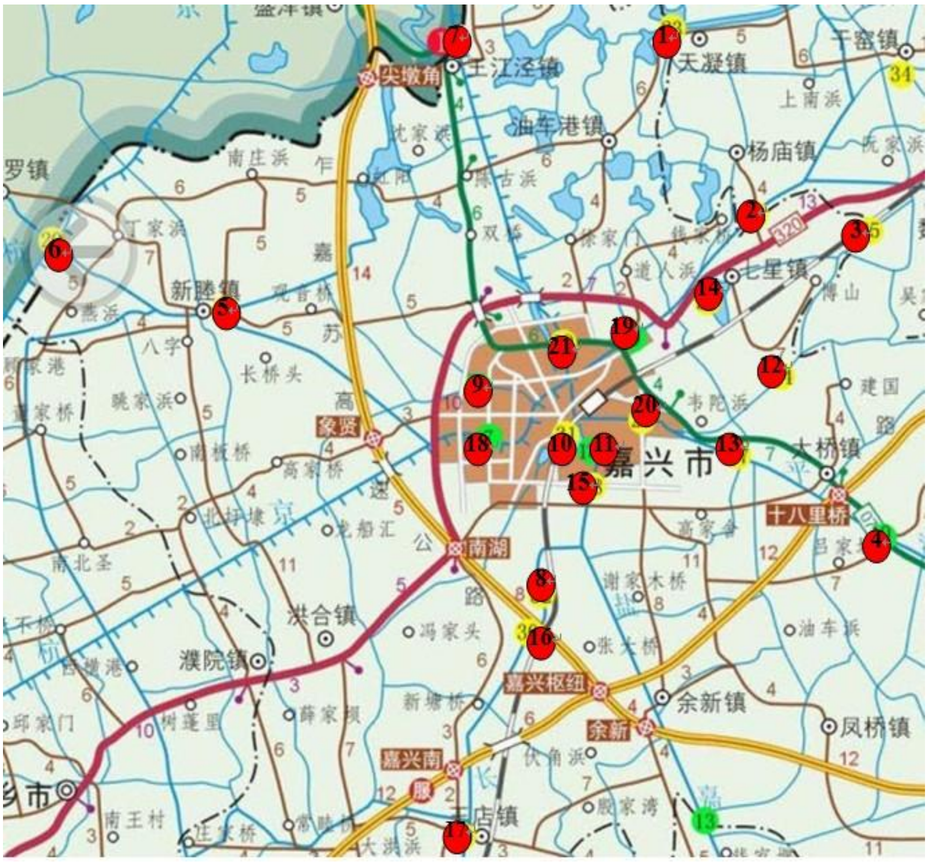


Figure 3-2 Urban river water sampling sites

1. Hongqizhen youchegang exports; 2. Sandiantang Yangmiao bridge; 3. Jiashantang Qixing; 4. Pinghutang Jiaoshanmen bridge; 5. Xintengtang Xinteng exports; 6. Xintengtang Luotengtang LuoDong bridge; 7. The canal river Wangjiangting; 8. Guanting harbor; 9. Shijiuyang; 10. The South gate ; 11. South lake center; 12. Jiashantang Duchuanbang; 13. Pinghutang Renzhongbang; 14. Sandiantang Xiangjiadang; 15. Haiyantang Nijiahui; 16. Changshuitang Mahuangtang Bridge; 17. Changshuitang Wangdianbaile Bridge; 18. The canal river Longfeng Bridge; 19. Sandiantang Tanghui; 20. Pinghutang Changzheng Bridge; 21. The canal river Beiyun bridge.

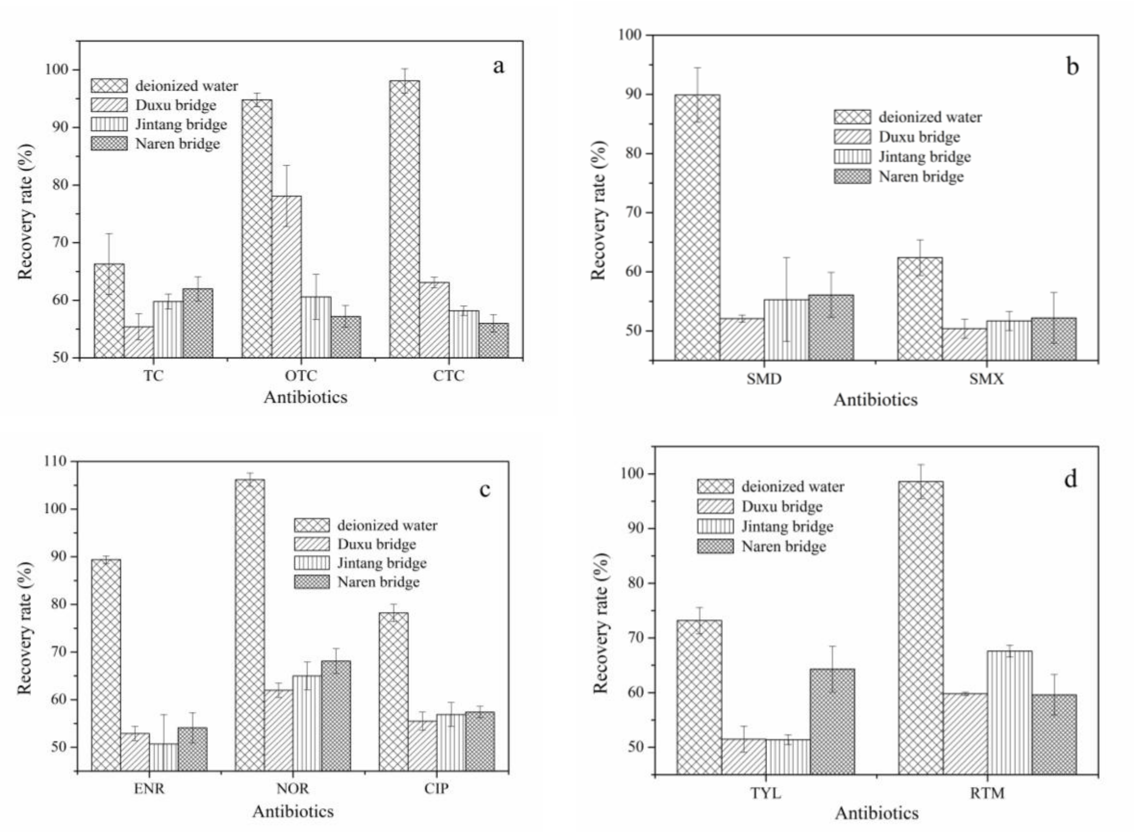


Figure 3-3 Recovery rates of (a) tetracyclines, (b) sulfonamides, (c) quinolones and (d) macrolides

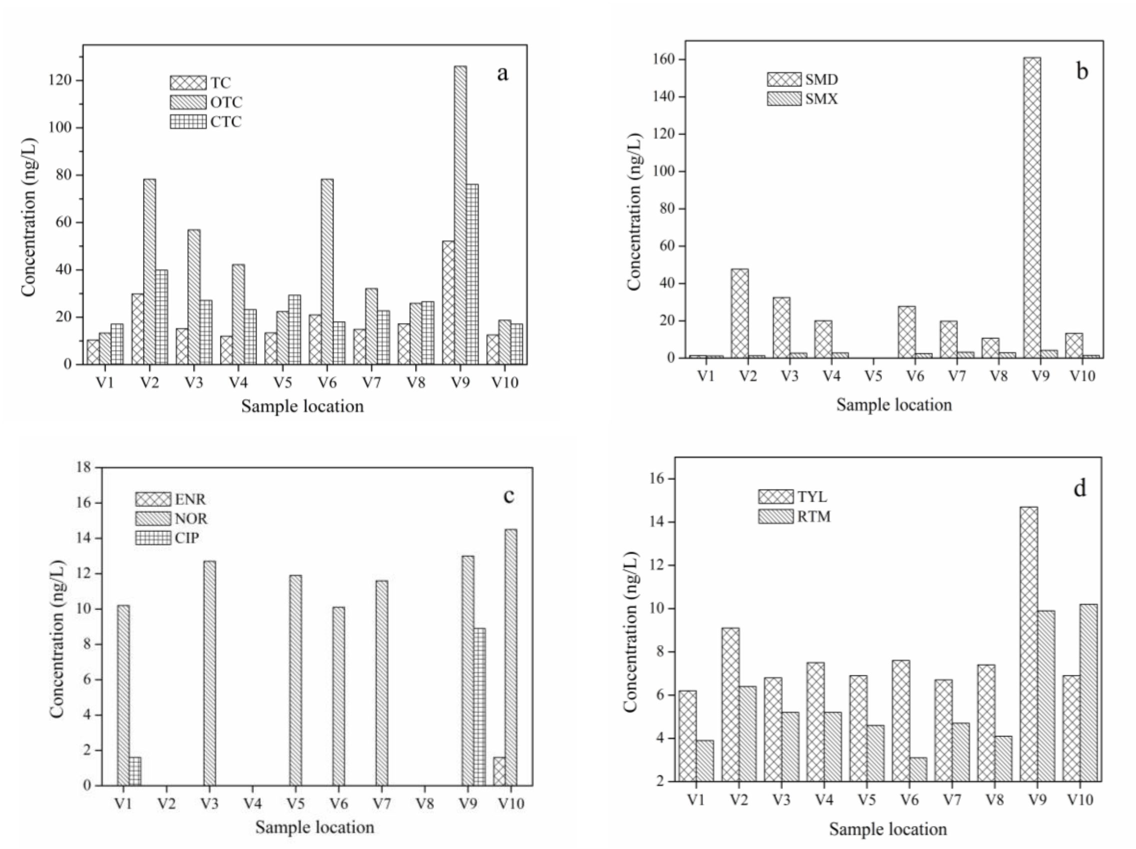


Figure 3-4 Concentrations of (a) tetracyclines, (b) sulfonamides, (c) quinolones and (d) macrolides in rural rivers

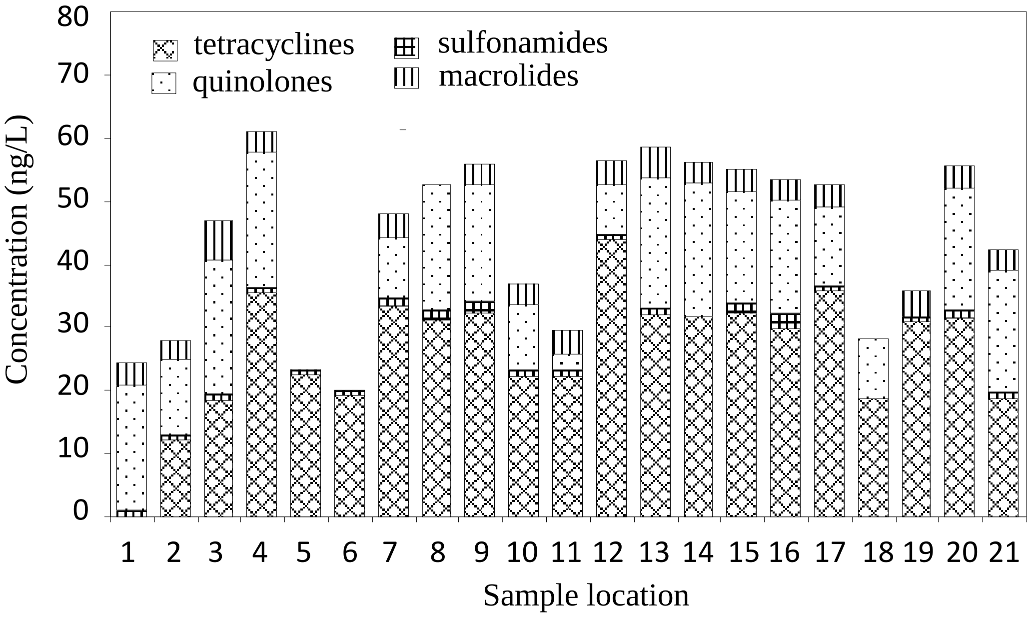


Figure 3-5 Antibiotics concentrations in main urban river sections

Chapter 4 Performance of SMBR for treating ADPW

4.1. Introduction

Wastewater pollution derived from pig-breeding industries has drawn growing concern recently in China (Tan et al., 2010). More than 460 million tons of ADPW has been annually discharged. Soil pollution problems by heavy metals or antibiotics resistance genes have been reported in lands fertilized with livestock excrement and ADPW without safe treatment (Wu, et al, 2010; Pan, et al., 2011). The increasingly stringent discharge standards in China signify the higher requirement for ADPW treatment technologies.

MBR is a combined technology of biological treatment and membrane separation. Thanks to the efficient interception of membrane filtration, the activated sludge concentration could be maintained very high in the reactor. As a consequence, MBR is well known for its advantages of high volumetric loading rates, compact volume, efficient nitrification performance, and stable, excellent effluent quality. MBR is also easy to automatically control, which gives the reactor additional advantages for treating ADPW in livestock and poultry farms (Prado et al., 2009).

In this chapter, a SMBR was applied to treat ADPW. Removal performance including COD, NH_4-N, heavy metals and antibiotics were investigated under gradually increasing volumetric loadings by gradually shortening the HRT. This study aimed to determine the suitable range of pollutant loadings with improved effluent quality by using SMBR to treat ADPW, providing a database for the design and operation of this technology.

4.2. Materials and methods

4.2.1. Experimental equipments

A completely mixed SMBR was applied, with length×width×height of 85×15×100 cm^3 and effective volume of 95 L (Figure 4-1). A PVDF flat sheet membrane module (Jiangsu Bigfu Membrane Technology Co. Ltd) was submerged inside, with effective filtration area of 0.1 m^2 and average pore size of 0.1 μm. The influent was continuously introduced into the system, from which the filtrate being continuously withdrawn. The filtration flux was maintained constant by regularly cleaning the membrane surface with tap water. Temperature of the mixed liquor was maintained 20 ~ 25℃ with a heater. The dissolved oxygen (DO) concentration was controlled no less than 5 mg/L and pH was between 8 ~ 9. The seed sludge was sampled from the Jiaxing Sewage Treatment Plant, with the initial concentrations of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the SMBR being 2.8 g/L and 2.1 g/L, respectively. No sludge was wasted from the MBR except sampling for the determination of nitrification activity.

Volumetric loading rates of COD and NH_4-N were increased by gradually shortening HRT from 12 to 8 d in run 1 (days 1 ~ 36), to 4 d in run 2 (days 37 ~ 57) and to 2.7 d in run 3 (days 58 ~ 72). Since the concentration of NH_4-N in the effluent arose in run 4 (days 73 ~ 95), HRT was extended to 4 d again in order to decrease the volumetric NH_4-N loading rate in order to maintain good nitrification performance of the reactor. HRT was shortened to 3 d in run 5 (days 96 ~ 119), and the volumetric NH_4-N loading increased again, as the effluent concentration of NH_4-N decreased to below 100 mg/L.

4.2.2. Raw wastewater quality

ADPW used in this study was sampled from a large-scale piggery farm in Southlake district of Jiaxing city, and the supernatant was stored at 10 ~ 15℃ after static precipitation. The concentrations of total COD, TN, NH_4-N and TP were 3,036 ~

9,160 mg/L, 1,733 ~ 2,439 mg/L, 1,233 ~ 1,774 mg/L and 59.1 ~ 80.8 mg/L, respectively. Soluble pollutants accounted for about 80%. The alkalinity was 40 ~ 60 mmol/L and pH was 7.65 ~ 8.12.

4.2.3. Analytical methods

COD, NH_4-N, TN, TP, alkalinity, MLSS and MLVSS were analyzed according to the national standard methods (SEPA, 2002). pH was determined with a pH meter (FE20, Mettler Toledo instrument co. ltd, Shanghai). Temperature and DO was detected with a desktop dissolved oxygen meter (YSI DO200, S.A.V Instrument Co. Ltd). Heavy metals, *i.e.* Cu and Zn were analyzed with a flame atomic absorption spectrometer (240AA Duo, Agilent Technologies Co. Ltd) after microwave digestion (MDS-10, Shanghai Sineo Microwave Chemistry Technology Co. Ltd) (SEPA, 2002). Antibiotics were analyzed with an internal standard method (Luo et al., 2011) using a liquid chromatography (LC, Waters e2695) coupled to a triple quadrupole-linear mass spectrometer (MS, waters TQ Detector) (Waters Science and Technology Co. Ltd., USA).

4.2.4. Nitrification capability of the activated sludge

The ammonia oxidation rate (ammonia utilization rate, AUR) and nitrite oxidation rate (nitrite utilization rate, NUR) were determined with batch tests at room temperature according to the literature (Yu et al., 2008). The specific ammonia oxidation rate (SAUR) and the specific nitrite oxidation rate (SNUR) were calculated as the ratios of AUR and NUR to MLVSS.

4.3. Results and discussion

4.3.1. Loading rates and removal performance of ammonia nitrogen

The volumetric loading rate of NH_4-N has an important influence on the pollutant removal performance of SMBR when treating ADPW. A too high NH_4-N loading rate would not only result in a decreased nitrification activity with resultant accumulation of high concentrated NH_4-N in the reactor, but also may exert its restraints upon the degradation activity of microorganisms towards other pollutants. The volumetric loading rate of NH_4-N was adjusted by HRT in this study, and its temporal change and the corresponding removal performance are shown in Figures 4-2 (a) and (b), respectively.

NH_4-N in the effluent was stable at below 10 mg/L in run 1 (days 1 ~ 36), when the loading rate was 0.13 ± 0.04 kg-NH_4-N/m^3 d and the influent NH_4-N concentration was 1,300 ~ 1,450 mg/L. The loading rate was gradually increased to 0.33 ± 0.07 kg-NH_4-N/m^3 d in run 2 (days 37 ~ 57) when the influent NH_4-N concentration was 1,049 ~ 1,496 mg/L. A removal rate close to 100% was achieved, with an effluent NH_4-N concentration below 5 mg/L. The effluent can not only meet the NH_4-N discharge limit of 80 mg/L in the existing discharge standard, but also meet the new discharge standard. The effluent NH_4-N concentration significantly increased to 403 mg/L in run 3 (days 58 ~ 72), when the loading rate was increased to 0.49 ± 0.07 kg-NH_4-N/m^3 d and the influent NH_4-N concentration fluctuated between 1,212 ~ 1,536 mg/L. The loading rate was then reduced again to 0.36 ± 0.04 kg-NH_4-N/m^3 d in run 4 (days 73 ~ 95), but the effluent NH_4-N concentration remained very high for a long period until day 87 when the concentration dropped to below 100 mg/L. In run 5 (days 96 ~ 119), the loading rate was increased again to 0.46 ± 0.09 kg-NH_4-N/m^3 d. The effluent NH_4-N concentration once increased to 319 mg/L at the beginning while soon dropped to 11 ~ 85 mg/L. The removal rate in run 5 was detected lower than those in runs 1 and 2 while higher than those in runs 3 and 4, but still remained over 90%. This observation indicated that the activated sludge could be acclimatized to high NH_4-N loading, which may result in an increased nitrification activity and enhanced resistance to shock loads.

The above results showed that SMBR could steadily produce the effluent with NH_4-N concentrations below the limit of the existing discharge standard and even the tentative standard when the volumetric load was remained lower than 0.33 ± 0.06 kg-NH_4-N/m^3 d. However, the nitrification rate significantly decreased, and the concentration of NH_4-N in the effluent greatly increased as the NH_4-N loading was increased to 0.49 ± 0.07 kg-NH_4-N/m^3 d. The reported volumetric loading rate of NH_4-N was 0.1 ~ 0.15 kg-NH_4-N/m^3 d for a conventional activated sludge process (Lei, 2012), which was only half of the endurable load of SMBR in this study.

4.3.2. Sludge concentrations and nitrification activity

The initial MLSS and MLVSS in the SMBR were 2.8 g/L and 2.1 g/L, respectively. Nearly no increase in sludge concentration was detected in run 1, owing to a long HRT and correspondingly low organic loading rates applied. After switched to run 2 and run 3, when HRT was shortened with resultant increased organic loading rate, the sludge concentration presented an exponential growth. MLSS and MLVSS amounted to 12 g/L and 9 g/L respectively on day 75. The sludge concentration tended to level off in run 4 and run 5.

Activated sludge was sampled respectively on day 1 (inoculation), day 33 (end of run 1), day 57 (end of run 2) and day 115 (end of run 5) to determine the nitrification activity as shown in Figure 4-3. Both the total nitrification activity and the specific nitrification activity greatly increased in each run compared to the seed sludge. The higher the NH_4-N loading rate was, the higher the increasing amplitude of nitrification activity. AUR and SAUR of activated sludge on day 57 were 0.401 kg-NH_4-N/m^3 d and 0.146 kg-NH_4-N/kg-MLSS d respectively, two or three times higher than the seed sludge. NUR and SNUR showed more significant increase than AUR and SAUR. NUR and SNUR were 0.622 kg-NO_2-N/m^3 d and 0.227 kg-NO_2-N/kg-MLSS d on day 57, respectively 6.3 and 4.8 times higher than the seed sludge. The corresponding data further increased by 32.3% and 48.8% on day 115, and a slight increase was also observed in AUR and SNUR. The nitrification activity of MBR on day 115 was equivalent to two to three times of the conventional active sludge (Zhan et al., 2008).

4.3.3. Loading rates and removal performance of COD

Temporal change in COD loading rates and removal efficiency in the SMBR are shown in Figure 4-4. The influent COD concentration fluctuated in the range of 3,036 ~ 9,160 mg/L, with no significant difference discerned among the runs. Nevertheless, both the volumetric loading rate and sludge loading rate of COD was low in run 1 (0.4 ± 0.1 kg-COD/m^3 d), increased sharply in run 2 (1.5 ± 0.8 kg-COD/m^3 d) and run 3 (2.8 ± 0.6 kg-COD/m^3 d), while decreased sharply in run 4 (1.4 ± 0.6 kg-COD/m^3 d), and dropped finally to a low level in run 5 (0.9 ± 0.2 kg-COD/m^3 d). The maximum volumetric COD loading rate of MBR was up to 3.2 kg-COD/m^3 d duration in this study, 5.3 times of those for conventional activated sludge processes (0.4~0.8 kg-COD/m^3 d). The sludge COD loading rate reached the maximum value of 1.1 kg-COD/kg-MLSS d in run 3 and was maintained in a range of 0.05 ~ 0.28 kg-COD/kg-MLSS d in runs 4 and 5, similar to those of conventional activated sludge processes (Pan et al., 2011). The effluent COD concentration was determined as 450 ± 225 mg/L, 380 ± 38 mg/L, 400 ± 80 mg/L, 460 ± 138 mg/L and 480 ± 96 mg/L, respectively in run 1 to run 5, showing no significant change with the COD loading rate. The removal rate remained 90% or above after 30 days of operation.

It was reported that refractory COD accounted for about 11% in the biogas slurry of piggery wastewater, thus the COD removal rate was usually 75 ~ 80% of conventional aerobic biological treatment processes (Dosta et al., 2008; Prado et al., 2009). The COD removal rate of SMBR in this study was higher than the conventional biological processes, although being operated at a much higher volumetric COD loading rate. Despite of high COD removal rates being achieved, there is a certain gap between the SMBR effluent COD and the regulated discharge limit of 400 mg/L in the discharge standard, say nothing of the limit of 100 mg/L in the new standard. It seems almost impossible to treat the ADPW and meet the discharge standard solely by biological treatment, considering the very high COD concentration and refractory components in the influent. Further advanced treatment is in need.

4.3.4. Removal of heavy metals

Cu and Zn are common growth promoting additives in pig feed, so they are frequently detectible in piggery wastewater. Concentrations of Cu and Zn were determined in the influent and effluent of SMBR on day 36 (end of run 1), 57 (end of run 2) and 74 (end of run 3), as shown in Figure 4-5. The influent concentrations of Cu and Zn were respectively 0.064 ~ 0.119 m/L and 0.162 ~ 0.565 mg/L. The effluent concentrations were greatly reduced, as low as 0.003 ~ 0.019 mg/L for Cu and 0.017 ~ 0.084 mg/L for Zn. The efficient removal of Cu and Zn should be mainly contributed bythe adsorption onto sludge flocs or uptake by the microorganisms, which also implied that heavy metals were possibly accumulated in the sludge. Heavy metal concentrations in the activated sludge were not determined in this study, and further investigation is required to confirm whether heavy metals accumulate or to what extent they could accumulate in sludge during a long period of operation. The accumulation of heavy metals in sludge might partially result in the decrease of MLVSS/MLSS ratio, from 0.73 in the start-up phase to 0.63 after running for 119 days. Accumulated heavy metals may inhibit the microbial activity in biological treatment (Xie, 2002), hence, they should be removed as much as possible by physicochemical pretreatment methods before entering a biological treatment process.

4.3.5. Removal of antibiotics

Ten typical veterinary antibiotics were determined on day 36 (end of run 1), 57 (end of run 2), 74 (end of run 3), and their removal rates by SMBR was evaluated, as shown in Table 4-1. Tetracyclines (including TC, OTC and CTC) summed up as high as 88,189 ~ 196,848 ng/L, accounting for 96.0 ~ 97.2% of the total concentration of the ten antibiotics. OTC was of the major one among the three tetracyclines, accounting for 93.3 ~ 94.5%; while TC and CTC were of the minor ones, but still with concentrations up to thousands of ng/L, higher than the concentrations of the other seven non-tetracycline antibiotics. SMD and CIP were detected in thousands of ng/L in each run, lower but close to the concentrations of TC and CTC. Concentrations of the left five antibiotics were below 600 ng/L, among which SMX and RTM were only detected in trace level, below 4.9 ng/L and 44 ng/L, respectively.

The total concentration of the ten antibiotics was removed by 92.2% in run1, 69.6% in run 2 and 61.3% in run 3. The removal rate tended to decrease under a shortened HRT or an increased organic loading rate condition, in which the removal performance of the three tetracyclines was mainly influenced. All of the three tetracyclines demonstrated a significant decrease in the removal rate when HRT was shortened. The removal rates of TC, OTC and CTC were respectively as high as 94.0 %, 93.2 % and 78.6 % in run 1 (HRT = 12 ~ 8 d), decreased to 69.0 ~ 74.7 % in run 2 (HRT = 4 d), and significantly dropped to 40.5 ~ 61.8 % in run 3 (HRT = 2.7 d). Removal of ENR presented a similar decreasing tendency with a shortened HRT, although the removal rate was much lower than tetracyclines in each run.

The removal rate of the other six antibiotics showed no significant change with HRT. Removal rates of two sulfonamides, namely SMD and SMX, always remained higher than 87.2% except for a low removal of SMX in run 1. CIP was stably removed by 75.6 ~ 82.1%. 71.1% of NOR was removed in run 2 and 85.8% in run 3 but much less removed in run 1. Two macrolides, TYL and RTM, were removed greater than 84.5% in run 1 and run 3 while much less removed in run 2. The reason is unclear at present that why the removal rate was low for SMX and NOR in run 1, and TYL and RTM in run 2. Analytical errors in trace concentrations might have some contribution to this phenomenon.

Compositions and concentrations of antibiotics in ADPW obtained in this study were in agreement with the literatures. A previous study on wastewater from six large-scale piggery farms in Hainan province showed that OTC, TC and SMD were of the most frequently detectable antibiotics and normally with high concentration (Han et al., 2012). They found that OTC was 100% detectable, with the maximum concentration of 71,750 ng/L. TC and SMD were respectively 63% and 83% detectable, with the corresponding concentrations up to 24,830 ng/L and 17,690 ng/L. Chen et al. (2010) conducted a research on ADPW from a large-scale pig farm in Tiaoxi base. Their results showed that tetracyclines was dominant in the veterinary antibiotics pollution, with the highest monomer concentration being 13,650 ng/L. Three sulfonamides (including sulfadiazine, sulfamethoxazole and sulfadimidine) were also detectable, in which sulfadimidine was dominant with a maximum concentration of 675.4 ng/L. Other detectable antibiotics were at trace level.

Previous studies (Sahar et al., 2011; Dorival-García et al., 2013) indicated that conventional activated sludge processes could remove 50 ~ 71% of tetracyclines, 27 ~ 72% of sulfonamides, 15 ~ 60% of quinolones and 46 ~ 78% of macrolides from wastewater polluted with ng/L level of antibiotics. SMBR in this study behaved better than the conventional activated sludge systems on removal of tetracyclines, sulfonamides and macrolides. The efficient removal of antibiotics by SMBR has also been reported. Sahar et al. (2011) claimed that antibiotics removal by SMBR was 15 ~ 42% higher than that by conventional activated sludge system. The results of Göbel et al. (2007) indicated that 80% of sulfamethoxazole could be removed by SMBR while only 60% by a conventional activated sludge system. Prado et al. (2009) obtained 79 ~ 89% of tetracycline removal in a SMBR. The improvement in removal efficiency may be attributed to the long sludge retention time in SMBR, resulting in better growth of effective microorganisms for decomposing refractory pollutants. Moreover, the lipophilic antibiotics in the wastewater could be adsorbed onto the high concentration of activated sludge in the SMBR and thereby caused a further decrease in antibiotics concentration in the effluent. Mass balance study is necessary to make clear the removal mechanisms of antibiotics in the SMBR in this study.

Longer sludge retention time (SRT) is believed to benefit the removal of antibiotics. Göbel et al. (2007) treated the wastewater containing trimethoprim, clarithromycin and dehydro-erythromycin in a MBR. The removal rate was found to be 50% at SRT of 16 ± 2 d or 33 ± 3 d, while increased to 90% at SRT of 60 ~ 80 d. Prado et al. (2009) reported that the MBR removal rate of tetracycline was 89% or higher at SRT of 30 d or longer, decreased to 85% at SRT of 10 d, and further dropped to 78% when SRT was shortened to 3 d.

It should be noted that although a high removal rate might be achieved in the SMBR, the remained concentration of antibiotics in the effluent is still much higher than the water environment threshold (10 ng/L) by EU. Ecosystem risk should be considered with more attention to the treated ADPW and its safety when discharged into receiving water bodies.

4.4. Summary

Pollutant removal performance of the SMBR is much more influenced by the volumetric loading rate of NH_4-N rather than that of COD. The SMBR could achieve effluent with NH_4-N concentrations steadily below the limit of the discharge standard and even below the new tentative standard when the NH_4-N loading rate was remained not higher than 0.33 ± 0.07 kg-NH_4-N/m^3 d. However, the nitrification rate was significantly decreased, and the concentration of NH_4-N in the effluent would be greatly increased as the NH_4-N loading rate increased to 0.49 ± 0.07 kg-NH_4-N/m^3 d.

Heavy metals of Cu, Zn were possibly accumulated in the SMBR after a long-term operation. So they should be removed as much as possible by physicochemical pretreatment before entering the biological treatment process.

Tetracyclines were the dominant antibiotics in the raw wastewater, which was removed by 94.0% at a long HRT of 8 ~ 12 d in the SMBR. Shorter HRT to 2.7 d had less obvious influence on the effluent concentration of NH_4-N and COD, but decreased the removal rate of tetracyclines significantly. Mass balance, migration and transformation of antibiotics, and the impact factors should be further studied in order to clear the removal mechanisms of antibiotics in the SMBR.

Table 4-1 Removal of antibiotics at different HRT conditions

Antibiotics	Influent concentrations (ng/L)			Effluent concentrations (ng L)			Removal（%）		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
TC	2578	2704	3229	154	683	1692	94.0	74.7	47.6
OTC	87235	82689	185937	5899	25654	71029	93.2	69	61.8
CTC	3656	2786	7682	783	838	4572	78.6	69.9	40.5
SMD	1446	1297	977	161	166	14	88.9	87.2	98.6
SMX	4.9	3.2	4.4	2	0.2	0.1	59.2	93.8	97.7
ENR	131	131	118	82	90	100	37.4	30.9	15.0
CIP	2066	1857	3537	405	333	862	80.4	82.1	75.6
NOR	77	219	583	52	63	83	32.5	71.1	85.8
TYL	87	97	396	4.5	27	62	94.8	72.5	84.5
RTM	22	13	44	2.9	6.4	1.4	86.8	48.8	96.8
Sum of concentrations	97303	91796	202507	7545	27861	78415			
Total removal (%)							92.2	69.6	61.3

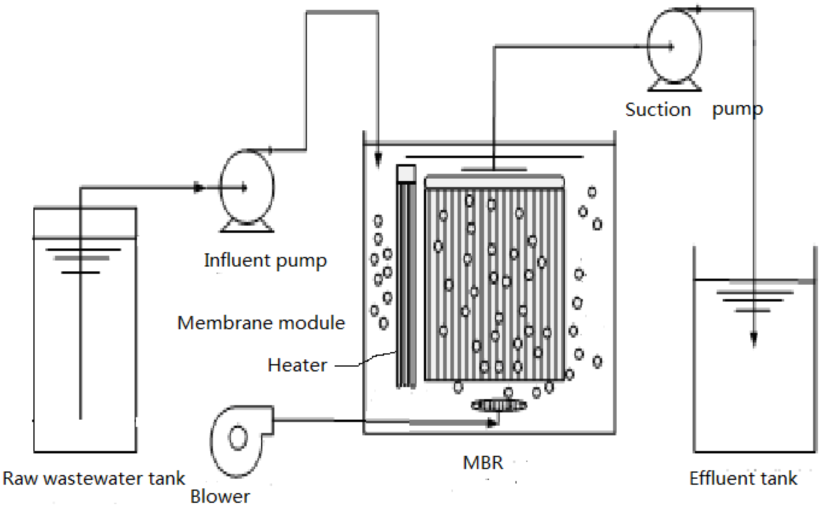
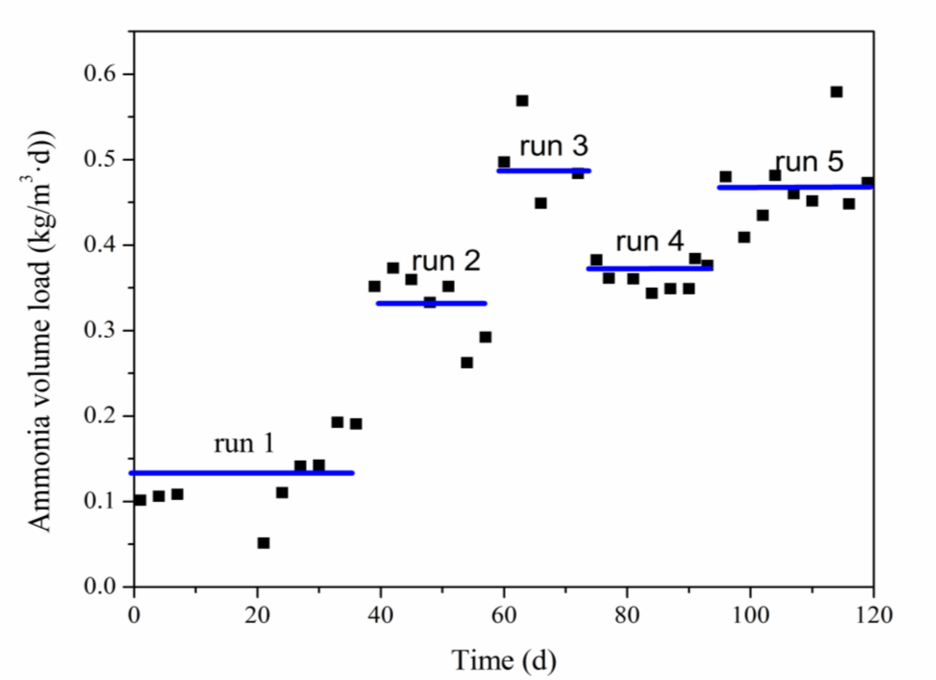
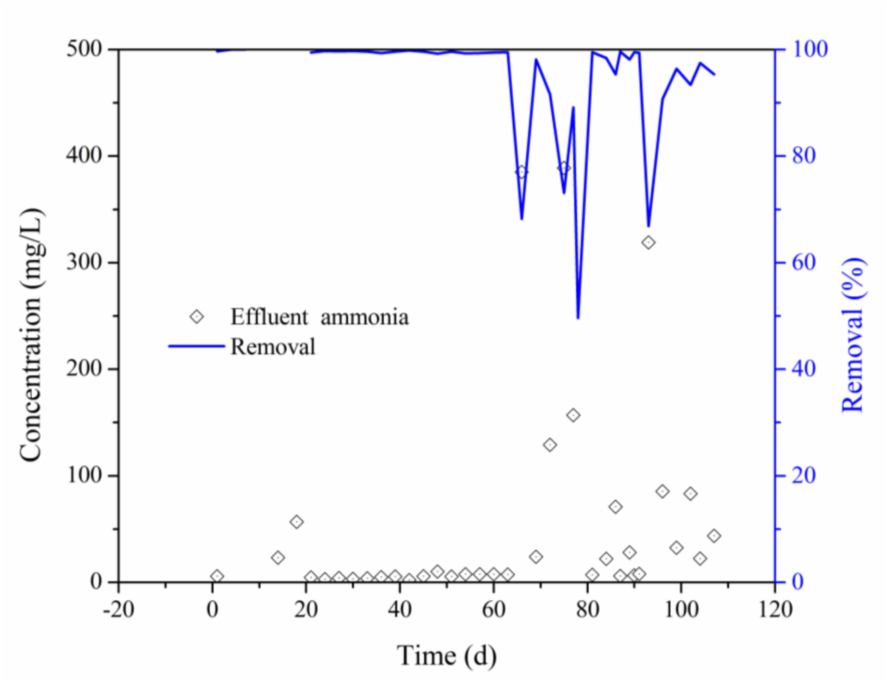


Figure 4-1 Schematic diagram of SMBR for treating ADPW



(a) Volumetric loading rate of NH_4-N



(b) Removal performance of NH_4-N

Figure 4-2 Variations of volumetric loading rate and removal performance of NH_4-N in the SMBR

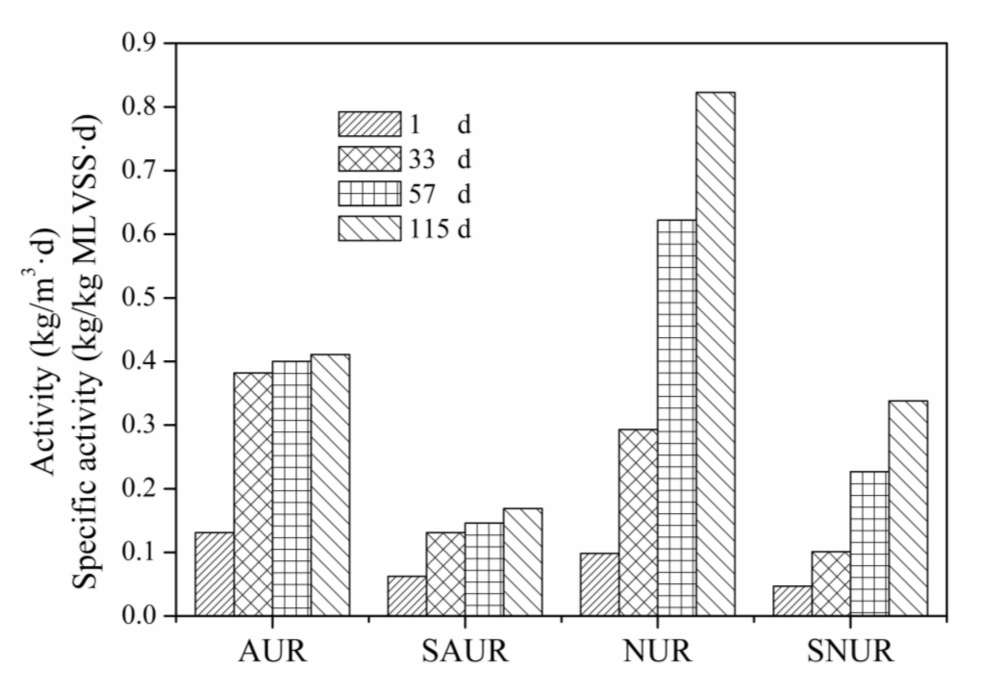


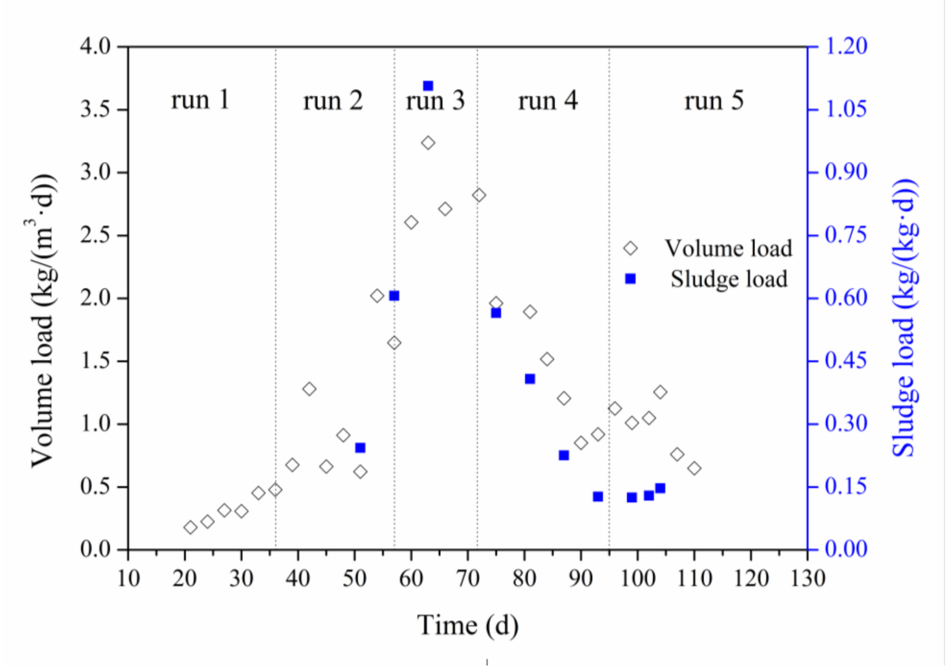
Figure 4-3 Variations in nitrification activity of the activated sludge

AUR: ammonia oxidation rate

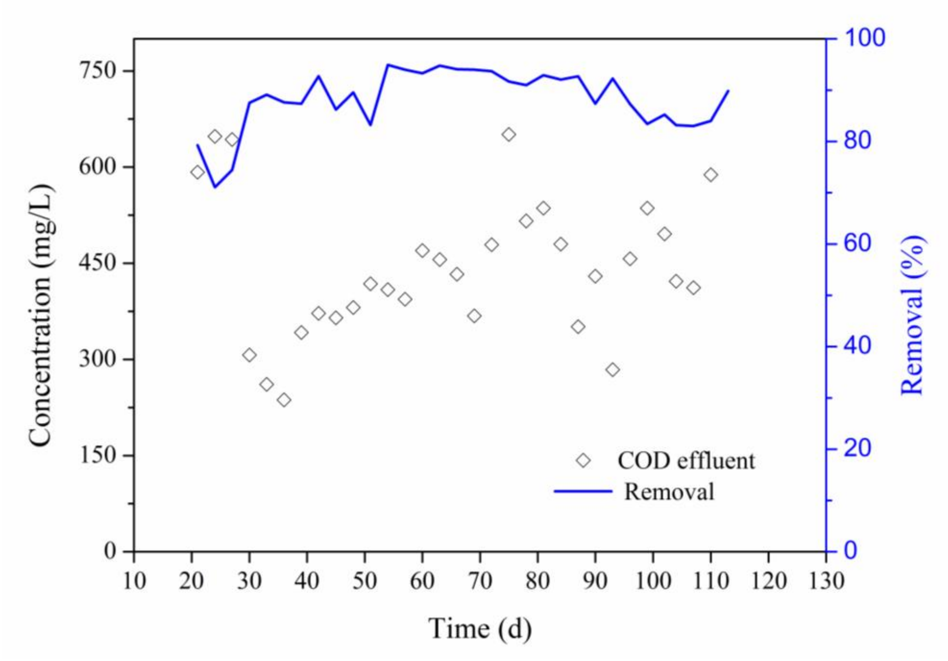
SAUR: specific ammonia oxidation rate

NUR: nitrite oxidation rate

SNUR: specific nitrite oxidation rate



(a) Volumetric loading rates and sludge loading rates of COD



(b) COD removal

Figure 4-4 Variations of COD loading rates and removal performance in the SMBR

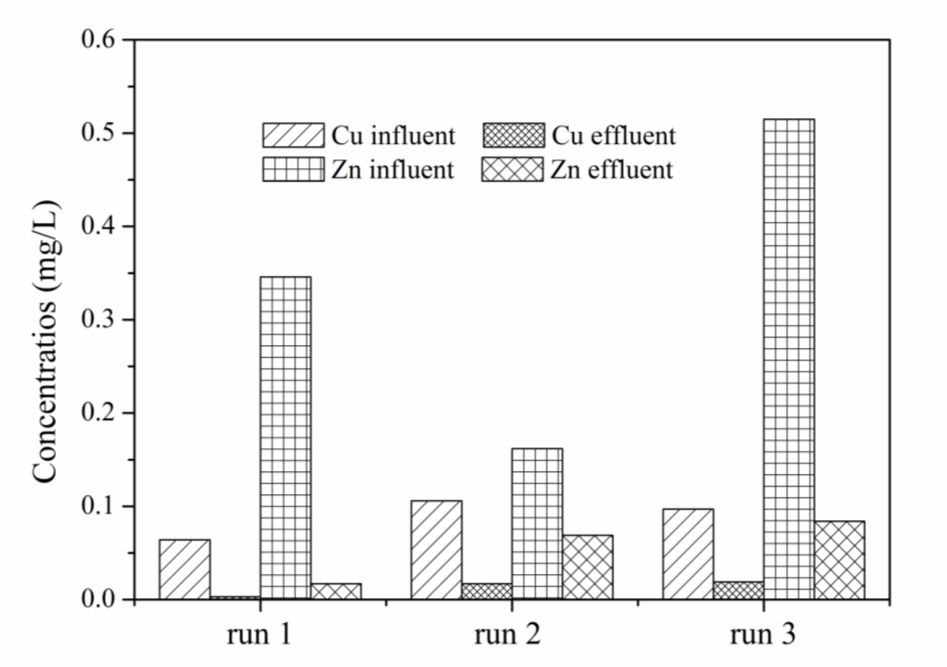


Figure 4-5 Removals of Cu and Zn

Chapter 5 Performance of IASBRs for treating ADPW

5.1. Introduction

After anaerobic digestion of swine wastewater, high concentrations of nutrients still remain in the effluent, ADPW. Nitrogen and organic matter contained in the ADPW can cause water pollution and eutrophication without proper treatment. Thus, removal of organic matter, nutrients and solids from the ADPW is necessary before it is discharged to water bodies, and therefore efficient treatment technologies are required.

A novel wastewater treatment technology, intermittently aerated sequencing batch reactors (IASBR), was developed in the Department of Civil Engineering, NUI Galway. IASBRs are considered to be an efficient technology for the treatment of wastewaters with low COD/TN ratios. Nitrogen can be removed efficiently using IASBR technology (Guo et al., 2008; Katsogiannis et al., 2003), and efficient partial nitrification is also observed in IASBRs (Jiang et al., 2009; Cheng et al., 2001; Zeng et al., 2008). The major advantage of using IASBR technology to achieve partial nitrification is no need for the precise control of DO and temperature in the reactor (Ciudad et al., 2007; Pambrun et al., 2008). However, partial nitrification by this approach is sensitive to operational upsets, and long-term stable partial nitrification in IASBRs is likely unreliable when treating wastewater with fluctuating influent characteristics.

In this chapter, the pollutant removal performance was compared in IASBRs under three operation modes, in order to optimize the operation conditions of IASBR. The feasibility of nitrogen and phosphorus removal using the integrated IASBR reactor was also discussed.

5.2. Materials and methods

5.2.1. Experimental equipments

Reactors 1# and 2# were made of stainless steel cylinders (Fig. 5-1a), with a diameter of 20 cm and a height of 45 cm, making a working volume of 10 L. Reactor 3# (Fig. 5-1b) had a diameter of 16 cm and a height of 20 cm, making a working volume of 4 L. Stirrers were used during the non-aeration phase at a stirring rate of 60 rpm, while aerators were used during the aeration phase at an aeration rate of 2 L/min. Peristaltic pumps, controlled by time switches, were used to feed and discharge the wastewater at designed intervals.

5.2.2. Operational conditions

Reactor 1# (Fig. 5-2a): 8h per cycle comprising fill (1 min), four alternative non-aeration (40 min)/aeration (65 min), settle (55 min), and draw/idle (5 min), with the HRT of 10 d and the SRT of 30 d. Reactor 2# (Fig. 5-2b): 8h per cycle comprising fill (1 min), four alternative non-aeration (50 min)/aeration (50 min), settle (75 min), and draw/idle (5 min), with the HRT of 10 d and the SRT of 30 d. Reactors 1# and 2# were simultaneously operated in spring to autumn. They were supplied with the same influent wastewater and the water temperature of 20 ~ 32°C. ADPW in all the reactors was diluted in the initial stages to start up the reactors under lower NH_4-N loading, and then dilution multiple was gradually reduced to raise the COD and NH_4-N loading, until eventually the original ADPW was used as influent after day 23.

Reactor 3# (Fig. 5-2c): 12h per cycle comprising fill (1 min), four alternative non-aeration (50 min)/aeration (120 min), settle (35 min), and draw/idle (5 min), with the HRT of 7.5 d and no sludge discharging during the testing periods. The running temperature was below 15℃ in the former 44 days and was about 20℃ afterwards. Reactor 3# was operated separately from Reactor 1# and 2# in autumn to winter with much higher pollutant concentration in the influent wastewater. and the water temperature in the reactor was as low as 8 ~ 18°C. ADPW was pumped into the reactor without any dilution from the start-up.

5.3. Results and discussion

5.3.1. Performance of Reactors 1# and 2#

(1) Removal of nitrogen

The influent TN concentration is shown in Figure 5-3. The volumetric loading and sludge loading of NH_4-N in the influent was mostly in a range of 0.03 ~ 0.12 kg-NH_4-N/m^3 d and 0.01~0.03 kg-NH_4-N/kg-MLSS d, respectively, except for days 36 ~ 40 and days 65 ~ 71 when the volumetric NH_4-N loading abruptly increased from 0.09 ~ 0.12 kg-NH_4-N/m^3 d to 0.16 kg- NH_4-N/m^3 d.

The removal rate of TN greatly varied with the influent TN volumetric loading (Fig. 5-3). 16 ~ 55% of TN was removed in Reactor 1# before day 70 and 22 ~ 58% of TN was removed in Reactor 2# before day 78. A sharp decrease in the TN removal rate was observed during days 40 ~ 48 and days 65 ~ 71, suggesting that a volumetric NH_4-N loading of over 0.12 kg-NH_4-N/m^3 d may be too high for efficient TN removal. The accumulation of NO_2-N in the reactors and a low denitrification rate could be attributed to the insufficiency of carbon source, as well as the fluctuation of pH in the reactor within one cycle of operation.

The concentrations of NH_4-N, NO_2-N, and NO_3-N in the effluent of Reactors 1# and 2# are shown in Figure 5-4. In Reactor 1#, NO_3-N was absolutely dominant in the inorganic nitrogen during days 22 ~ 40. This indicated that nearly complete nitrification could be achieved in the reactor although the denitrification performance was not desirable. Then the NO_2-N continuously increased from 4 to 770 mg/L, becoming the dominant inorganic nitrogen species in the effluent as the TN volumetric loading increased to 0.16 kg-N/m^3 d after day 40. The effluent NH_4-N concentration rose from

10 to 51 mg/L before day 71, and decreased to below 10 mg/L on day 80. In Reactor 2#, the effluent NH_4-N concentration was low in the first 9 days, increased to 284 mg/L on day 11, decreased on day 13 and finally stabled at a level of 20 ~ 50 mg/L. The effluent NO_3-N gradually increased in days 10 ~ 25, while continuously decreased in days 26 ~ 86.

Compared to Reactor 1#, there were some differences in Reactor 2#: i) The effluent NH_4-N stabled at 20 ~ 50 mg/L, much higher than that of Reactor 1# (below 5 mg/L); ii) The effluent NO_2-N concentration began to increase on day 23 and reached up to more than 600 mg/L on day 50, also much higher than that of Reactor 1#. The accumulation of NH_4-N and NO_2-N in Reactor 2# showed that a prolonged non-aeration time may create an adequate environment for shortcut nitrification.

(2) Removal of organics

COD concentrations in both reactors were shown in Fig. 5-5. And the COD volumetric loading was 0.08 ~ 0.2 kg-COD/m^3·d in the first 22 days, and 0.4 kg-COD/m^3·d in days 23 ~ 34, 0.97 kg-COD/m^3·d in days 35 ~ 40, and then decreased to 0.25 kg-COD/m^3·d after day 41; the sludge loading of COD was 0.03 ~ 0.05, 0.09 ~ 0.13 and 0.3 kg-COD/kg-MLSS·d, correspondingly.

The effluent COD almost remained below 400 mg/L before day 40 in Reactor 1# and before day 24 in Reactor 2#. Afterwards when the effluent NO_2-N concentration become increasing, the effluent COD concentration continuously increased most probably due to the existence of interference from NO_2-N reduction in the determination of COD. No further analysis would be carried out on the COD removal since the COD index could not well represent the organic amount under the large interference of NO_2-N in this study.

(3) Removal of total phosphorous

Polyphosphate accumulating organisms (PAOs) in the sludge release phosphorus under non-aeration condition while uptake under aeration condition. By alternately running the activated sludge under non-aeration and aeration conditions, dominant growth can be attained for PAOs that excessively accumulate phosphate, thus the phosphorus can be removed efficiently through discharging the surplus sludge.

The phosphorus removal performance of Reactors 1# and 2# is shown in Fig. 5-6. The TP concentration in the influent fluctuated significantly between 29.1 ~ 176.1 mg/L while the effluent TP was stabled at 20 ~ 50 mg/L in both reactors. The removal rate fluctuated with the TP concentration in the influent, ranging from 5.0 to 82.0% in Reactor 1# and from 11.9 to 85.1% in Reactor 2#. This indicated that the two systems were unable to establish a stable phosphorus removal environment. DO was mainly above 0.5 mg/L during the non-aeration phase (Figure 5-8), which might be a significant factor affecting the performance of PAOs.

(4) Sludge characteristics

Figure 5-7 presents the change of concentration and settling property of the activated sludge. In Reactor 1#, as the sludge discharge began from day 10, sludge settlement ratio (SV_{30}) and sludge volume index (SVI) rapidly decreased and then gradually reached a stable state after day 35. The sludge concentration changed in the similar trend: the initial MLSS was about 7,000 mg/L, then rapidly decreased and finally stabled at 2,400 ~ 3,000 mg/L (MLVSS = 2,000 mg/L) after day 35.

In Reactor 2#, the SV_{30} and SVI decreased rapidly during the first 25 days and then tended to be stable. The sludge concentration varied with the same trend of SV_{30} and SVI, which decreased rapidly during the first 25 days from an initial MLSS of 4,000 mg/L and finally stabilized at 2,000 ~ 3,000 mg/L. The corresponding MLVSS concentration was 1,500 ~ 2,000 mg/L.

(5) Removal of antibiotics

The 10 antibiotics in the influent and effluent of Reactors 1# and 2# were determined on days 18 and 40, and their removal rates were evaluated, as shown in Table 5-1.

On day 18, the removal rates of TC, OTC in Reactors 1# and 2# were respectively 57.14%, 90.63% and 75.71%, 50.00%, while the detected effluent CTC concentration was higher than that of influent concentration, which might be related to the short running time and insufficient acclimation of the seed sludge. By comparison, the removal rates of TC, OTC, CTC by Reactors 1# and 2# were correspondingly as high as 91.27%, 88.44%, 86.13% and 84%, 84%, 93.60% on day 40, with the CTC removal performance largely improved to more than 85%, indicating the cumulative adsorbed CTC on sludge was gradually removed with the sludge during the operation period. The effluent SMD concentrations sharply decreased with the removal rates by Reactors 1# and 2# of respectively 99.30% and 99.10% while SMX was not detected on day 18. Removal rates of quinolones (including ENR, CIP, NOR) by Reactors 1# and 2# were correspondingly 100%, 76.14%, 61.18% and 100%, 77.19%, 55.29%. The macrolides (including TYL and RTM) were not detected in this experiment. By comparison, on day 40, the removal rates of SMD by Reactors 1# and 2# were respectively 99.83% and 99.90% while SMX was still not detected. The removal rates of CIP and NOR by Reactors 1# and 2# were correspondingly 88.00%, 92.00% and 87.33%, 92.00%, and ENR was not detected. The RTM was not detected in both influent and effluent, and the removal rates of TYL were 86.00% and 87.00% by Reactors 1# and 2#, respectively.

(6) Variation of DO, pH and ORP in a typical cycle

In the aeration phase, pH decreases when nitrifying bacteria oxidizes NH_4-N into NO_2-N or NO_3-N, which is then reduced to nitrogen by denitrifying bacteria in non-aeration phase, leading to the increase in pH value. Oxidation reduction potential (ORP) is the index represents the potential of oxidative materials in the system, which should be higher when concentration of DO, NO_2-N and NO_3-N in the system is high, and lower on the contrary. Real-time monitoring of the variation of pH, DO and ORP values in different stages of IASBR process can help to know the system operation status and effect of nitrification and denitrification.

The variation of pH, DO and ORP values in a typical cycle on day 161 in Reactor 1# is shown in Figure 5-8 (a). The DO value increased rapidly to greater than 0.5 mg/L in 1 to 2 minutes when the process was switched from non-aeration phase to aeration phase. In addition the peak of DO was the lowest in the first aeration phase, and then gradually increased in the second, third and fourth aeration phases. This observation is mainly due to the decrease of NH_4-N and organic matters, thus the reduction of oxygen consumption in the system. When the process was switched from aeration phase to non-aeration phase, DO value decreased slowly, so the actual non-aeration time (the length of time when DO was at least less than 0.5mg/L in this study) was less than the setup time (40 min), more than 37 min in the first and second aeration phase, but only 25 min and 2 min in the third and fourth non-aeration phase. DO could be quickly used up when aeration finished because large amount of organics still remained in the system resulting in large amount of oxygen consumption in the first two non-aeration phase, but relatively higher concentrations of DO still were detected in the system and longer time needed to consume the remaining oxygen when aeration finished in the last two non-aeration phase, leading to a shorter non-aeration time.

The pH value depicted an evident downtrend with the increase of operation time in the first three aeration phase ($DO \geq 0.5mg/L$), but it rose in the fourth aeration phase, probably due to the CO_2 escape through air stripping. And the pH value remained almost the same in the non-aeration phase ($DO \leq 0.5mg/L$), showing that denitrifying performance was not good enough, possibly resulted from lacking rigorous anoxic environment and the lower COD/TN ratio in the reactor.

ORP changed obviously in a different trend as DO values. ORP rose in aeration phase probably because of the existence of DO and oxidized nitrogen forms (NO_2-N, NO_3-N), and it gradually decreased in non-aeration phase probably resulted from the consumption of DO and denitrification of oxidation nitrogen. As the increase of ORP in aeration phase was by far more than the decrease in non-aeration phase, a rising trend was observed in the entire process of IASBR, with the maximum value of 210 mv and the minimum value of 27 mv. This observation indicates that the operation mode should be improved because ORP is better to remain below 0 mv for a good non-aeration performance (Wang and Peng, 2009).

The results showed that the aeration phase of Reactor 1# performed well to generate the nitration reaction, but denitrification reaction did not worked well even in the first non-aeration phase, let alone the later periods of the process. Non-aeration conditions should be improved by adjustment of the operation mode, such as prolonging non-aeration duration and reducing the aeration rate.

The variation of pH, DO and ORP value within a typical cycle on day 146 in Reactor 2# is shown in figure 5-8 (b). The variation of DO was similar to Reactor 1#, the actual non-aeration time in all four non-aeration phases could remain more than 30 minutes because the longer setup non-aeration duration (50 min) than that of Reactor 1#, and the non-aeration duration in the first two non-aeration phase was longer than that in the last two.

The pH value reflected an evident downtrend with the increase of operation time in all the four aeration phases, which means a good performance of nitrification. And the pH value remained almost the same in the non-aeration phase, signaling that denitrifying performance should be strengthened.

The variation of ORP showed a rising trend in the entire process of IASBR, with the maximum value of 170 mv and the minimum value of 87 mv.

The results showed that the aeration phase of Reactor 2# performed well to generate the nitrification reaction, but still, denitrification reaction should be strengthened although it had more than 30 minutes of non-aeration duration in all the non-aeration phase.

5.3.2. Performance of Reactor 3#

The results in section 5.3.1 revealed that the operation condition needed to optimize. The following study aimed to investigate the possibility of achieving better removal of TN and COD by extending both non-aeration and aeration period.

(1) Removal of nitrogen

TN concentration in the influent was shown in Figure 5-9. More than 85% of the TN was NH_4-N. The volumetric load and sludge load of the NH_4-N was stabled at 0.15 ~ 0.25 kg-NH_4-N/m^3 d and 0.05 ~ 0.10 kg-NH_4-N/kg-MLSS d during days 0 ~ 32, decreased to 0.05 ~ 0.10 kg-NH_4-N/m^3 d and 0.02 ~ 0.05 kg-NH_4-N/kg-MLSS d during days 33 ~ 64 with a prolonged HRT, and basically maintained at 0.10 kg-NH_4-N/m^3 d and 0.03 kg-NH_4-N/kg-MLSS d after day 65.

The TN removal is also shown in Figure 5-9, and the changes in the effluent concentration of NH_4-N, NO_2-N and NO_3-N are shown in Figure 5-10. The reactor was started with a high initial loading of NH_4-N, and the test was carried out in winter with water temperature between 10 ~ 15°C. The effluent concentration of NH_4-N remained high during the first 32 days, began to decrease after day 33 when the volumetric loading of NH_4-N was decreased, dropped to below 10 mg/L in days 50 ~ 70, and then slightly increased but still lower than 100 mg/L after day 71.

The effluent concentrations of NO_2-N and NO_3-N were extremely low (below 10 mg/L) before day 43. An abrupt increase in the NO_3-N concentration was observed in days 50 ~ 70, reaching the peak of 743 mg/L on day 67 when the NO_2-N remained at very low level. After day 71, the NO_3-N concentration sharply decreased while NO_2-N concentration increased accordingly. The NO_2-N became the major form of nitrogen in the reactor in days 80 ~ 100.

The removal rate of TN was 60 ~ 88% during days 0 ~ 40 and 90 ~ 100, suggesting that Reactor 3# was efficient for TN removal even under low water temperature conditions (8 ~ 18°C) and a much higher initial loading of NH_4-N. The removal rate of TN sharply decreased after day 40 and failed to remove any TN on day 72. This might be attributed to the veterinary drug (such as antibiotics) accumulated in the ADPW.

(2) Removal of organics

The influent COD concentration is shown in Figure 5-11. And the COD volumetric loading and sludge loading were 0.27 ~ 0.66 kg-COD/m^3·d and 0.11 ~ 0.27 kg-COD/kg-MLSS·d before day 33, 0.13 ~ 0.16 kg-COD/m^3·d and 0.04 ~ 0.06 kg-COD/kg-MLSS·d during days 34 ~ 86, 0.66 kg-COD/m^3·d and 0.14 kg-COD/kg-MLSS·d during days 87 ~ 95, and 0.28 kg-COD/m^3·d and 0.05 kg-COD/kg-MLSS·d during days 96 ~ 100.

The effluent COD concentration is also shown in Figure 5-11 with the removal rate of 55 ~ 84.0%. After day 71, the determined COD became much higher most probably owing to the large interference of increasing NO_2-N in the effluent. Nevertheless, the removal rate of the observed COD remained 60 ~ 80%, suggesting that the actual removal rate of organic pollutants should be much higher.

(3) Variation of pH in a typical cycle

The typical cycle on day 58 was selected to monitor the pH changes with time, as shown in Figure 5-12. The pH increased in the first and second non-aeration phases and decreased in every aeration section, suggesting that both non-aeration and aeration conditions were much better for efficient nitrification and denitrification than Reactors 1# and 2#.

5.3.3. Comparisons

All the three reactors run well in the first 40 days. In Reactor 1# and Reactor 2#, the volumetric loading and sludge loading of NH_4-N were respectively 0.03 ~ 0.12 kg-N/m^3·d and 0.01 ~ 0.03 kg-N/kg-MLSS d. NO_3-N was the absolutely dominant inorganic nitrogen species in the effluent, while NH_4-N and NO_2-N were both at very low levels. The removal rate of TN was 16 ~ 55% and 18 ~ 58% in Reactor 1# and Reactor 2#. The reactors were found vulnerable to shock loadings. Both of them showed a sharply decreased TN removal rate and a continuous, large accumulation of NO_2-N in the reactor right after days 36 ~ 40 and days 65 ~ 71 when the volumetric NH_4-N loading abruptly increased from 0.09 ~ 0.12 kg-N/m^3·d to 0.16 kg-N/m^3·d.

With longer aeration and non-aeration durations, Reactor 3# was found to well endure lower temperature and higher NH_4-N shock loadings. The reactor was started up in winter under temperature lower than 18 ℃ with a loading of NH_4-N of 0.15 ~ 0.25 kg/m^3 d. Although up to 600 mg/L of NH_4-N was found to accumulate in the reactor, no toxicity or activity inhibition was observed to the activated sludge, and 60 ~ 88% of TN removal was achieved. The above results revealed that the operational parameters of Reactor 3# were more feasible for ADPW treatment.

The NH_4-N loading of Reactor 3# was similar to literatures (Table 5-2), but its TN removal rate seemed a little lower. This might be mainly attributed to the difference in ADPW water quality used in these research works. The COD/TN ratio of the raw wastewater for IASBR in this study was mostly lower than 4, and even close to 1 ~ 2 in many days of operation, which was much lower than those in the literature (Table 5-2). As shown in Chapter 2, very low COD/TN ratio is a common characteristic for ADPW in Jiaxing. Therefore, how to increase the COD/TN ratio so as to improve the biodegradability of ADPW might be another approach for achieving high efficient TN removal in the treatment of ADPW, which will be included in the further study.

5.4. Summary

IASBR is a novel technology that is considered to be feasible for the treatment of wastewater characterized as high TN concentration and low COD/TN ratio. This chapter studied the pollutant removal performance of the IASBR under three operational conditions. It was found that the IASBR was effective to remove antibiotics. More than 84% of various typical veterinary antibiotics were removed from the bioreactor, and SMD was even removed more than 99%. A non-aeration duration of not shorter than 50 min and an aeration of 50 ~ 120 min could be more feasible for ADPW treatment. Reactor 3# set with such adjusted aeration-nonaeration operation was found to well endure lower temperature and higher NH_4-N shock loading conditions. The reactor was started up in winter at lower than 18 ℃ with a loading of NH_4-N of 0.15 ~ 0.25 kg/m^3 d. Although up to 600 mg/L of NH_4-N was found to accumulate in the reactor, no toxicity or activity inhibition was observed to the activated sludge with 60 ~ 88% of TN being removed.

Low COD/TN ratio of ADPW in Jiaxing, possibly the main factor of causing relatively lower TN and COD removal as well as a continuous accumulation of NO_2-N, has become a bottleneck for stable and efficient operation of IASBR process. Therefore, how to increase the COD/TN ratio so as to improve the biodegradability of ADPW has become a key step for further study. Also, it is worth further studying how to optimize the operational parameters, improve the removal performance of conventional pollution indexes and antibiotics under lower and normal temperatures, and the impact factors on stable running and thereby find countermeasures.

Table 5-1 Antibiotics in the influent and effluent of Reactors 1# and 2#

Date			**TC**	**OTC**	**CTC**	**SMD**	**SMX**	**ENR**	**CIP**	**NOR**	**TYL**	**RTM**
18 d	Influent (ng/L)		1750	3200	950	99300	ND	50	2850	850	ND	ND
	Effluent (ng/L)	Reactor 1#	750	300	1130	700	ND	0	680	330	ND	ND
		Reactor 2#	430	1600	1680	900	ND	0	650	380	ND	ND
	Removal rate (%)	Reactor 1#	57.14	90.63	-18.95	99.30		100.00	76.14	61.18		
		Reactor 2#	75.71	50.00	-76.84	99.10		100.00	77.19	55.29		
40 d	Influent (ng/L)		2750	2250	3750	58250	—	ND	3000	3500	2000	—
	Effluent (ng/L)	Reactor 1#	240	260	520	100	—	ND	360	280	280	—
		Reactor 2#	440	360	240	60	—	ND	380	280	260	—
	Removal rate (%)	Reactor 1#	91.27	88.44	86.13	99.83			88.00	92.00	86.00	
		Reactor 2#	84.00	84.00	93.60	99.90			87.33	92.00	87.00	

ND: Not detectable

—: Not analyzed

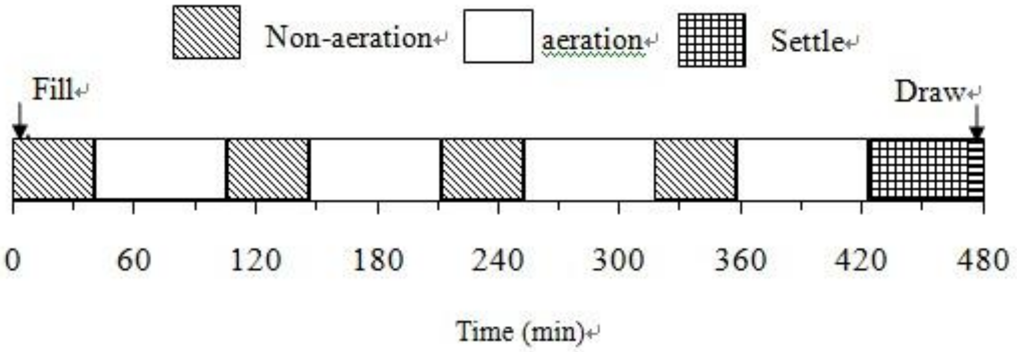
Table 5-2 Performance of piggery wastewater treatment processes in the literatures

Technology	Influent (mg/L)		Effluent (mg/L)		HRT (d)	SRT (d)	Ammonia loading ($kg/m^3 \cdot d$)	Organic loading ($kg/m^3 \cdot d$)	Removal rate (%)			References
	COD	NH_4-N	COD	NH_4-N					COD	NH_4-N	TN	
MBR	2268~5316	410~679	400~1100	<5	6	60			68.5~82.7	99.9		Yang and Cicek, 2008
MBR	6072	710	685	<1	3	30		0.96	88	99		Prado et al., 2009
MBR	4500±400	550±250	350±50	53.9±33.2	3.0~4.5	25~30	0.07~0.27	0.31 ~2.20	>93	~90		Prado et al., 2009
MBR	1401~6445	192	110~350	0.4	4.5	30	0.04	0.30~1.40	86	99		Prado et al., 2007
Upflow multi-layer bioreacotr	1500~13000	157~1417	292~545	~15	6~10	37.7~76.7	0.02~0.09	0.19~0.85	87~95	>95	90	An et al., 2007
Anoxic-MBR	6419	2560	1202	326~451	5.0~6.9		0.43	1.07	78~85	82~85	79~88	Kim et al., 2008
SBR		900		<5	0.5~3	11	0.30~1.80			>99		Obaja et al., 2005
SBR	1890±552	249±68	659~522	72.9~79.6	0.3	5.1	1.00±0.27	4.00±1.02	61~73	56~77		Morales et al., 2013
SBR	1325~7500	619~1616	1448±317	915±287	2~4	3~40	0.15~0.40	0.33~1.88	64±13	63±19		Scaglione et al., 2013
SBR		498~1018		71~174	1.5	30	0.04~0.08			>80		Wang et al., 2011
moving-bed SBR	500~2000	70~130	165~313	6~37	0.8	10	0.09~0.17	0.59~2.36	62~86	86~93		Sombatsompop et al., 2011
SBR	500~2000	70~130	166~484	9~66	0.8	10	0.09~0.17	0.59~2.36	61~84	75~87		
aerobic biological filter	973~2729	70.5~243.2	<400	<15	1		0.07~0.24	0.97~2.73	74.1~87.9	74.6~95.2		Wei et al., 2010
aerobic granular SBR	15932±2627	1823±496					0.96±0.27	7.30±0.68	88	97	70	Figueroa et al., 2011
SBR	387 ±145	519 ±134			2.5~5		0.05~0.18	0.03~0.24	76	96	80	Daverey et al., 2013

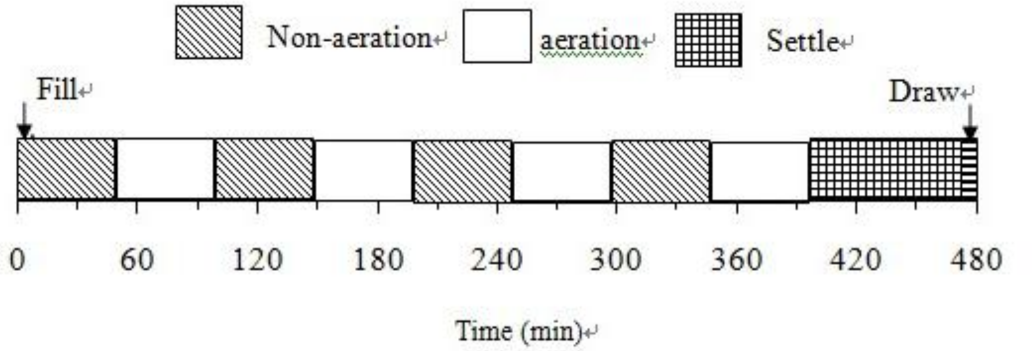
(a) Reactors $1^{\#}$ and $2^{\#}$

(b) Reactor 3#

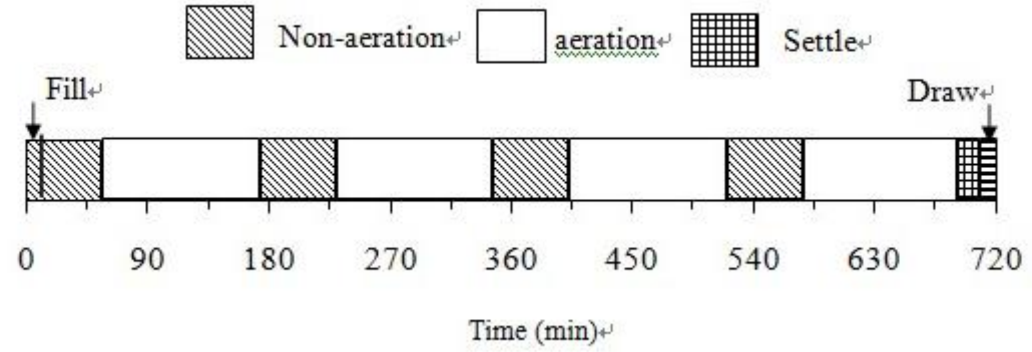
Figure 5-1 Photos of three IASBRs in the study



(a) Reactor 1#（40/65min）

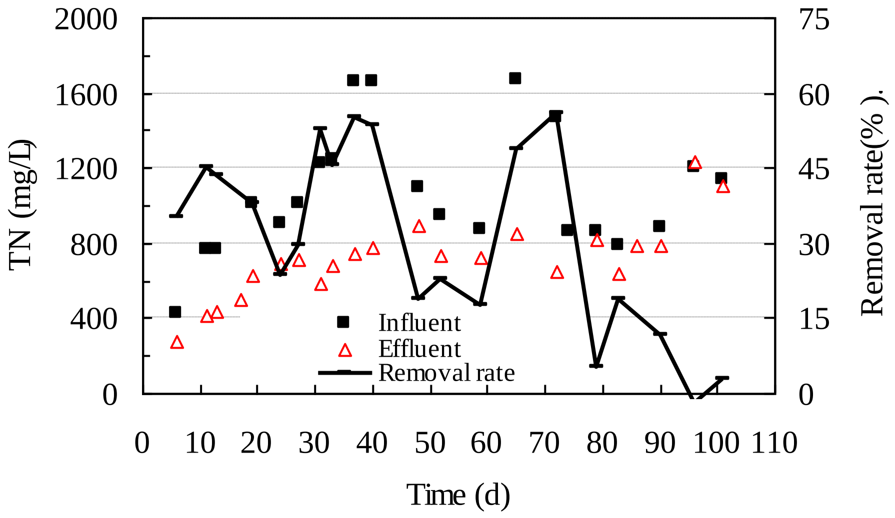


(b) Reactor 2#（50/50min）

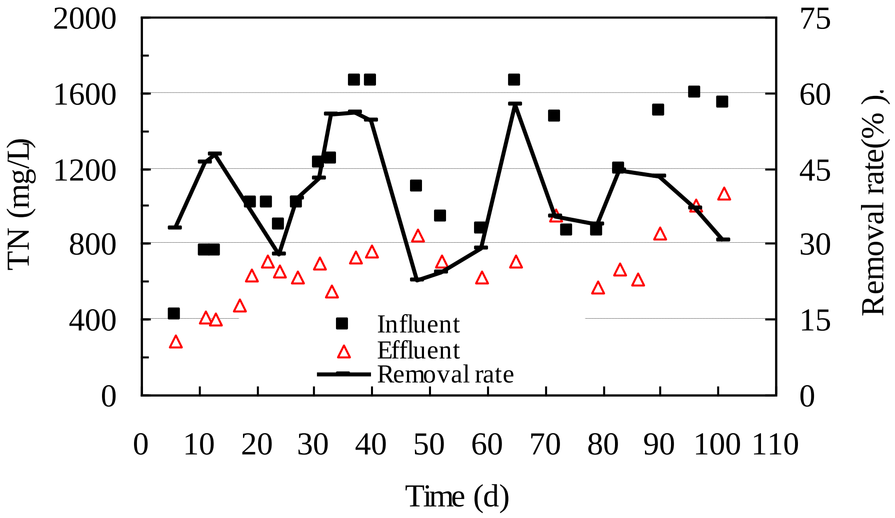


(c) Reactor 3#（50/120min）

Figure 5-2 Operation modes of the three IASBRs

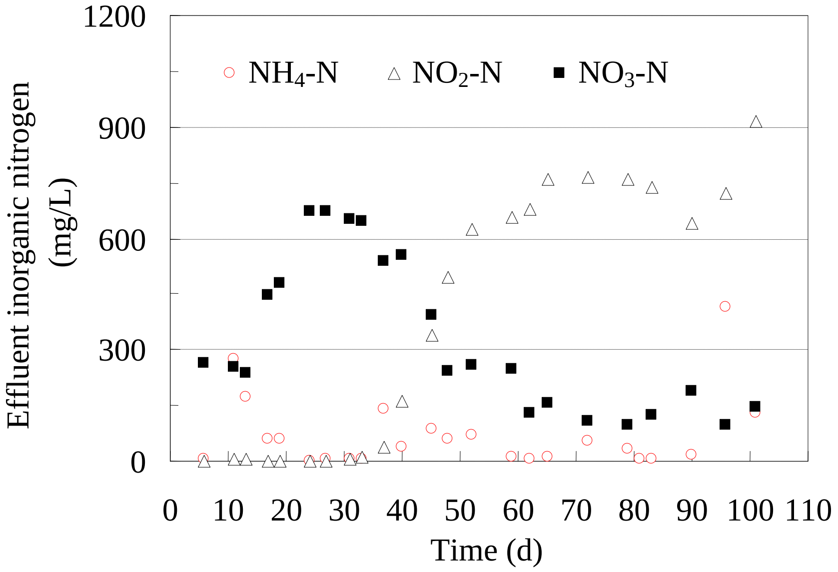


(a) Reactor 1#

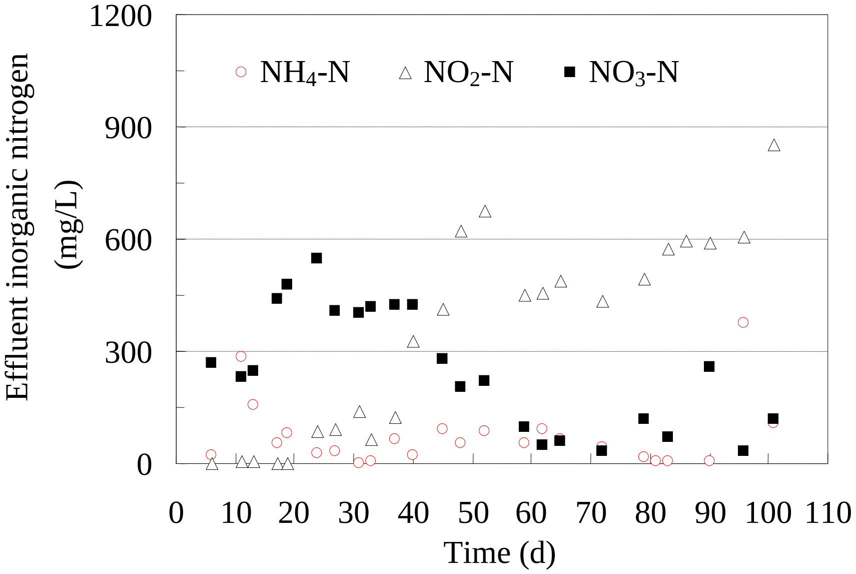


(b) Reactor 2#

Figure 5-3 Total nitrogen in the influent and effluent of Reactors 1# and 2#



(a)



(b)

Figure 5-4 Inorganic nitrogen in the effluent of Reactors 1# (a) and 2# (b)

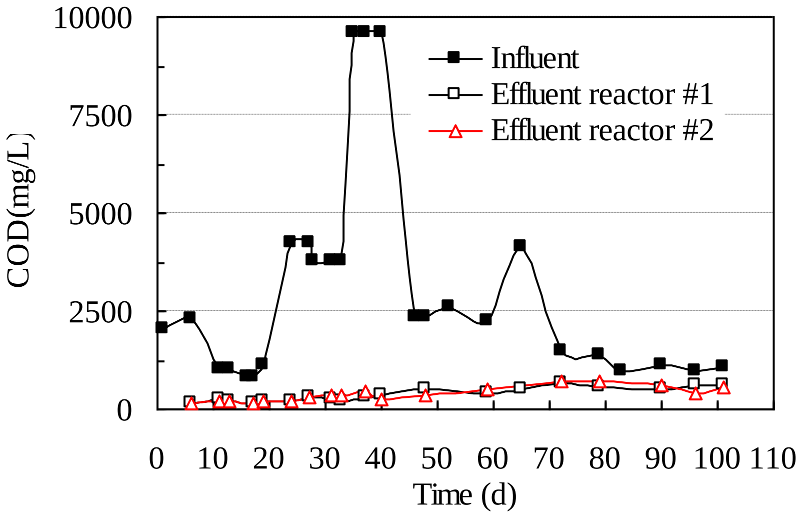


Figure 5-5 Change of COD in the influent and effluent of Reactors 1# and 2#

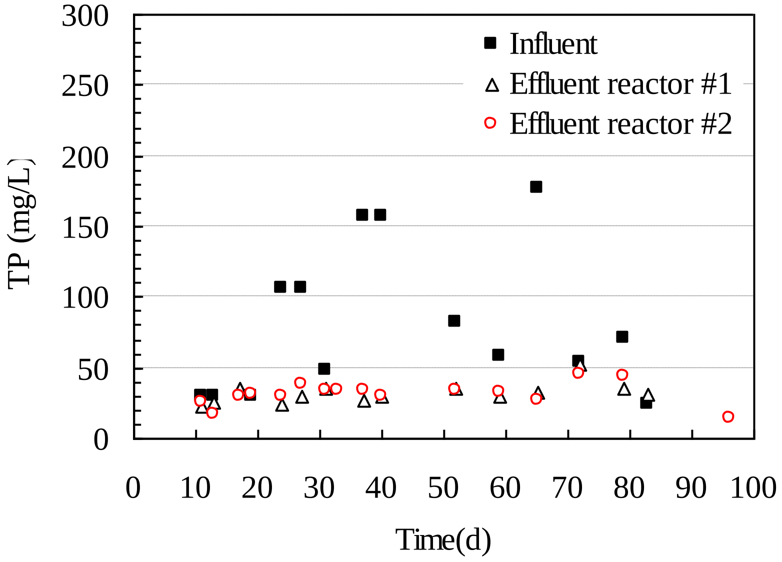
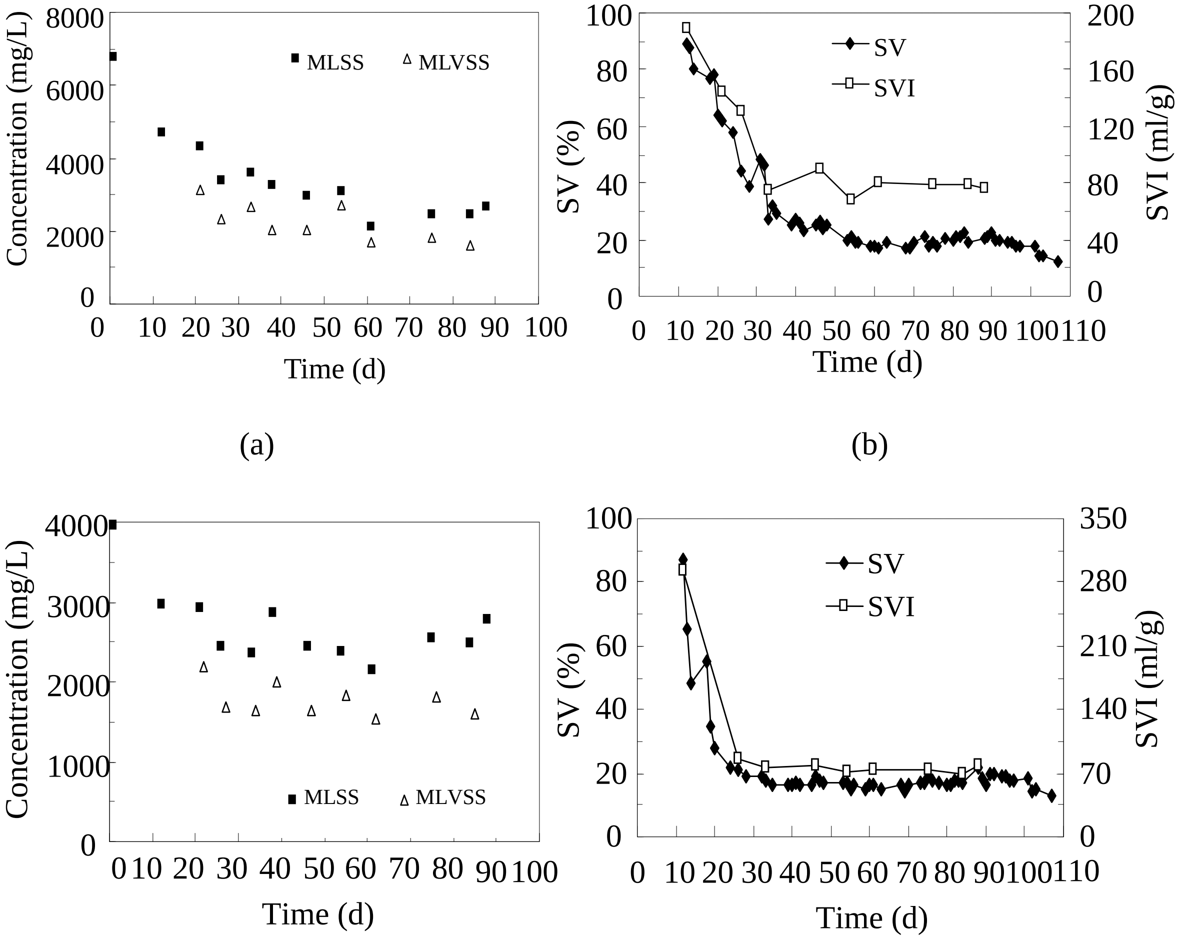


Figure 5-6 Total phosphorus in the influent and effluent of Reactors 1# and 2#



(a) (b)

(c) (d)

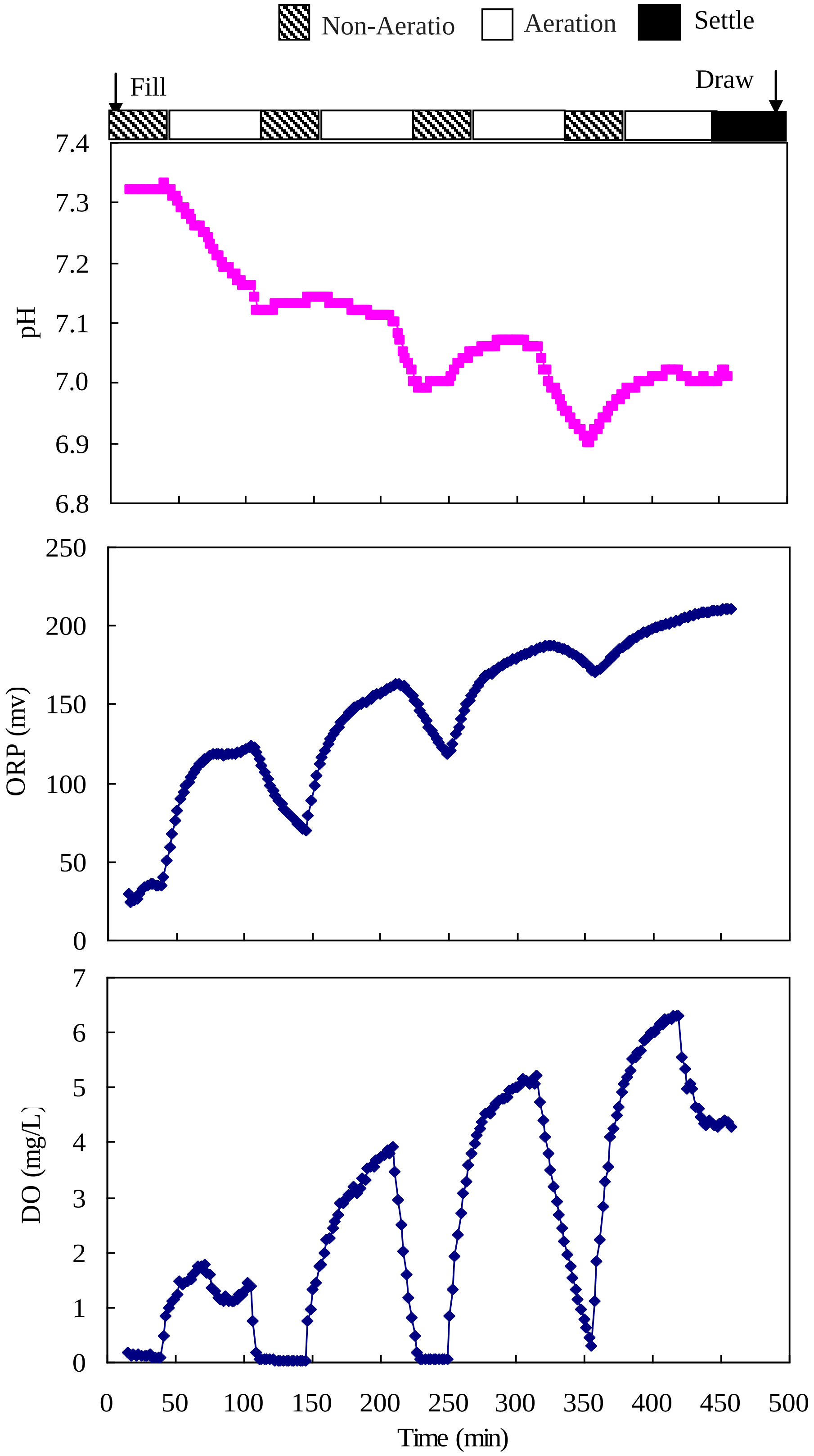
Figure 5-7 Concentration (a, c) and settling property (b, d) of the activated sludge in Reactor 1# (a, b) and Reactor 2# (c, d)

MLSS: higher mixed liquor suspended solids

MLVSS: mixed liquor volatile suspended solids

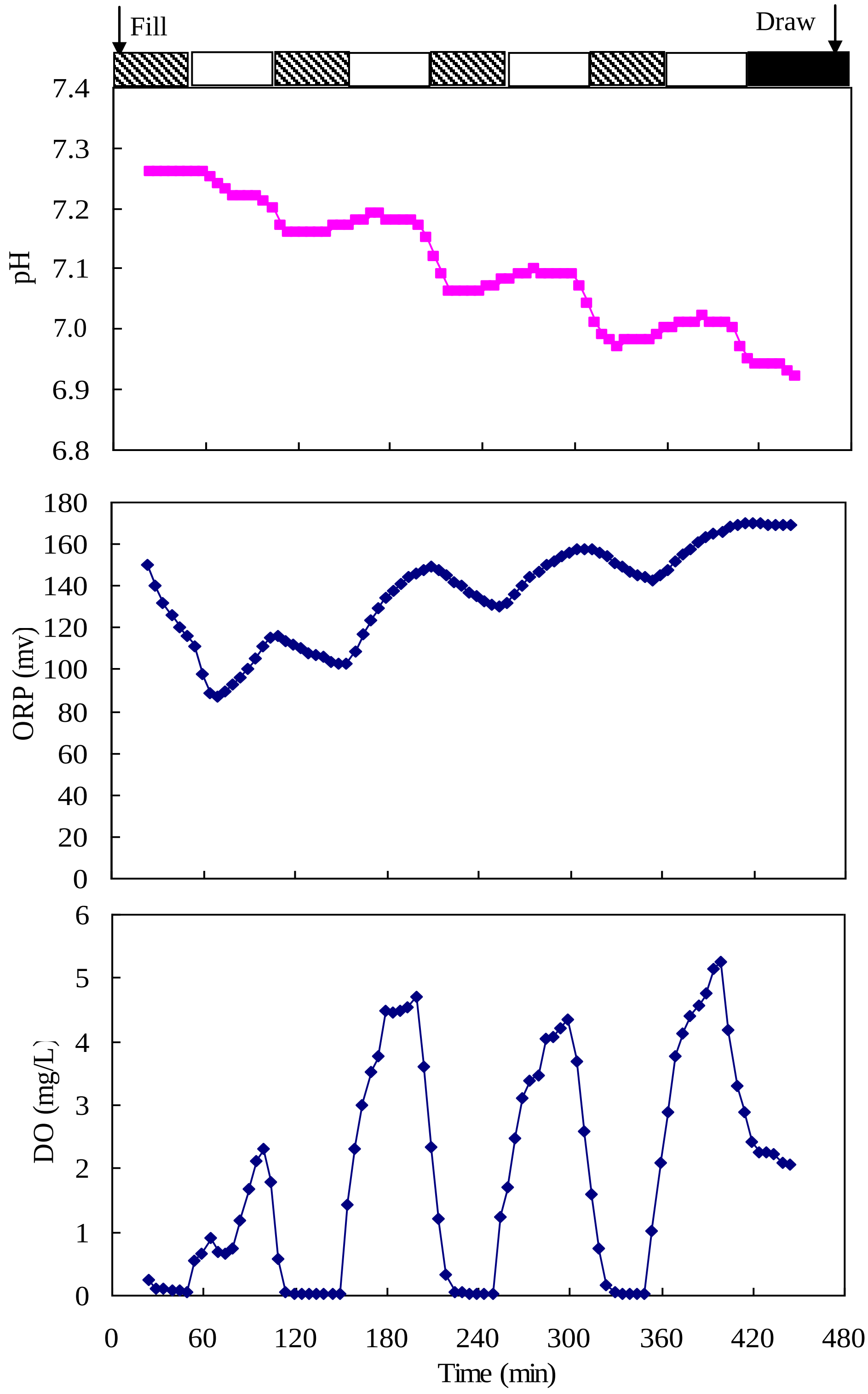
SV: sludge settlement ratio

SVI: sludge volume index



(a)

Figure 5-8 ORP, pH and DO profiles in a typical cycle of Reactors 1# (a) and 2# (b)



(b)

Figure 5-8 ORP, pH and DO profiles in a typical cycle of Reactors 1# (a) and 2# (b) (continued)

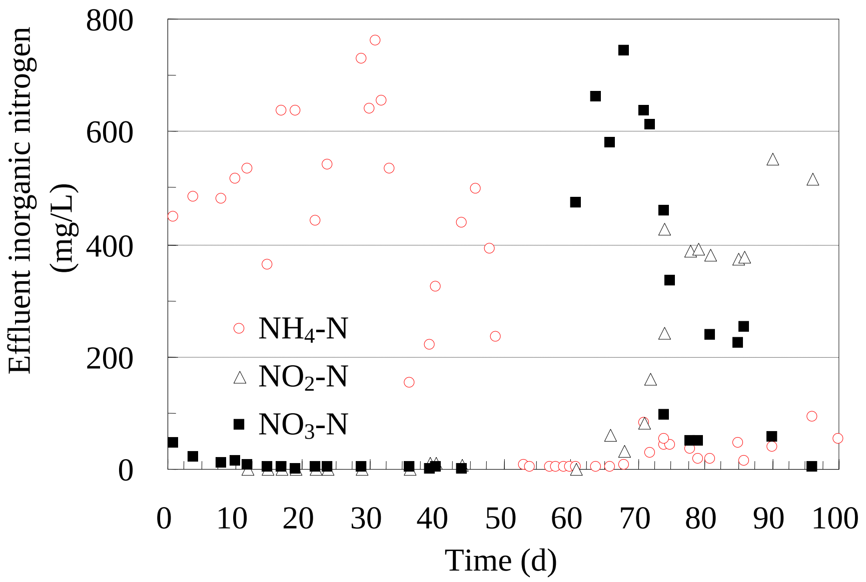


Figure 5-9 Inorganic nitrogen concentration in the effluent of Reactor 3#

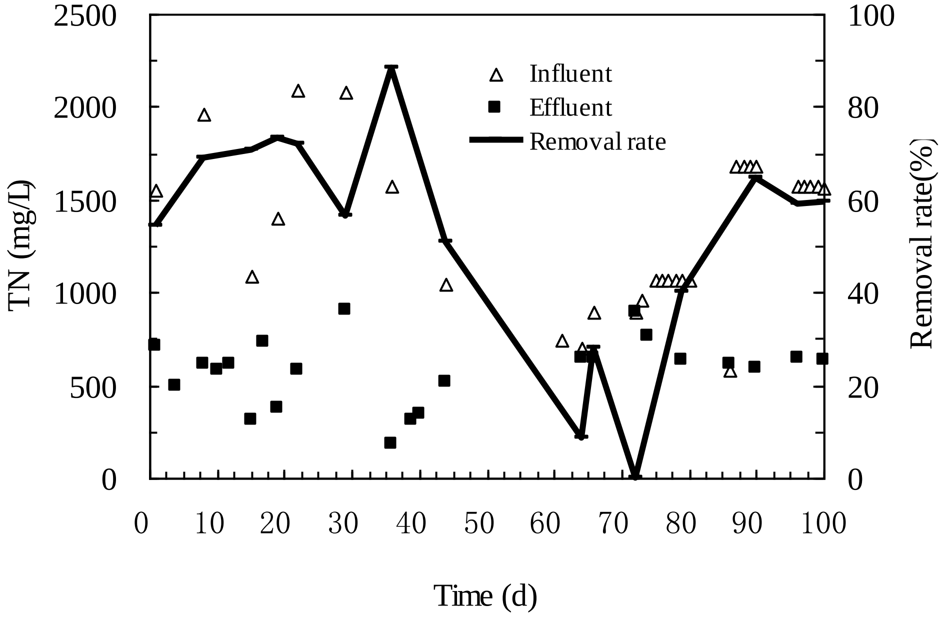


Figure 5-10 Total nitrogen in the influent and effluent of Reactor 3#

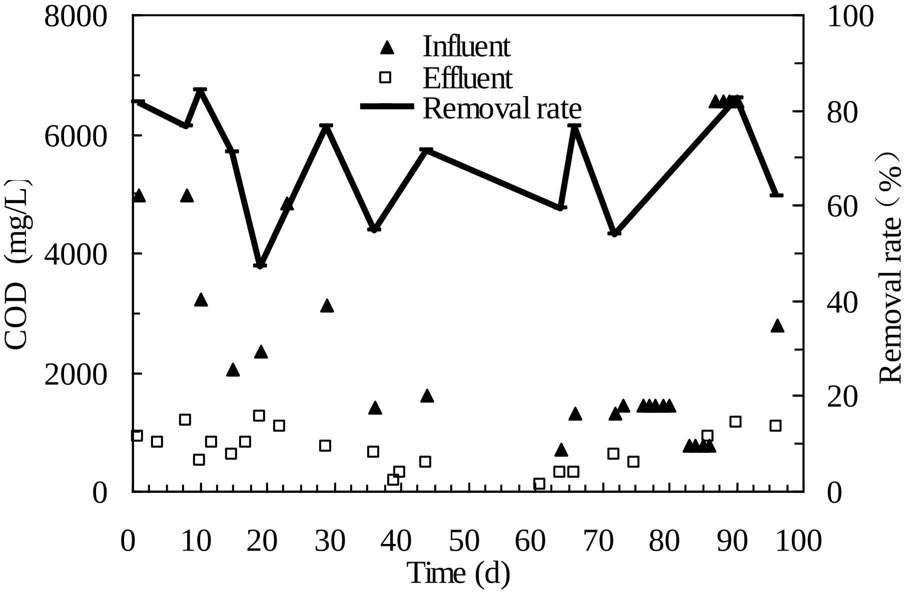


Figure 5-11 COD in the influent and effluent of Reactor 3#

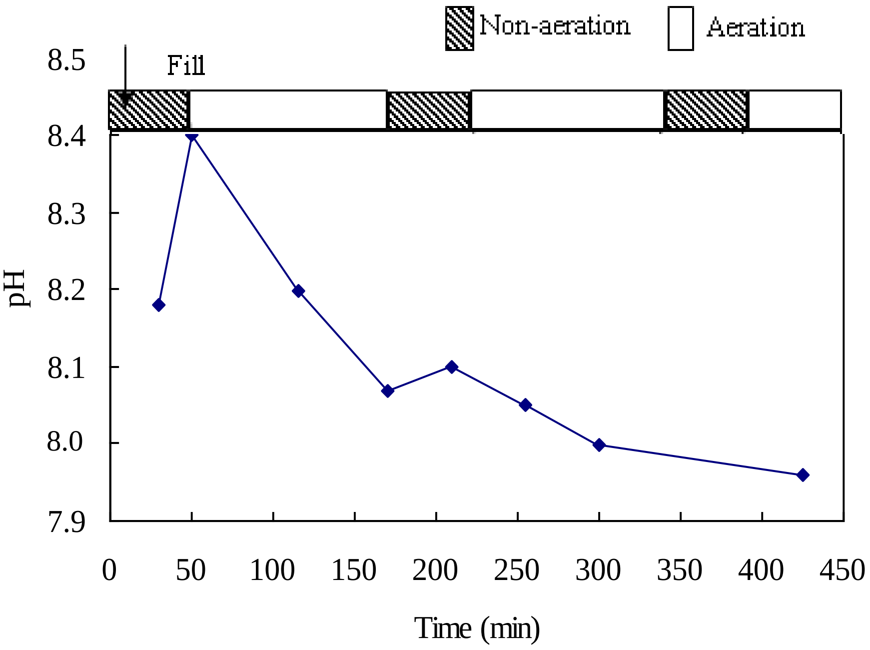


Figure 5-12 Change of pH in a typical cycle of Reactor 3#

Chapter 6 Conclusions and suggestions

Discharge of COD, nitrogen, phosphorus, and exogenous chemicals such as heavy metals and antibiotics from piggery farms has caused severe water pollution in China. It is in an urgent need to understand the pollution profiles of the piggery wastewater and develop efficient treatment technologies. The investigation on water quality of ADPW and pollution situation of surface water in Jiaxing, and the study on the effect and suitable operation parameters of SMBR and IASBR, can provide data and technical support for preventing and controlling water pollution from ADPW, especially the antibiotics pollution.

6.1. Conclusions

(1) ADPW quality varied in different scale pig farms, and COD, antibiotics and other indicators were fluctuated with season. The concentration of TN in ADPW was high, but the COD/TN ratio was low; the detected concentrations of Cu and Zn in ADPW were higher than Pb, Cd, Ni and Cr; and the antibiotics were also detected at high level.

(2) With the SPE-LC/MS/MS method, pollution statuses by 10 commonly used veterinary antibiotics were investigated in both rural and urban rivers in Jiaxing city. The rural rivers were polluted more seriously than the urban rivers. Tetracyclines and sulfonamides accounted for a larger proportion in rural rivers while tetracyclines and quinolones accounted for a large proportion in urban rivers.

(3) Pollutant removal performance of SMBR was much more influenced by the volumetric loading rate of NH_4-N rather than that of COD. The SMBR could achieve effluent with NH_4-N concentrations steadily below the limit of the discharge standard and even below the proposed limit of the tentative discharge standard when NH_4-N

loading rate was relatively low. Tetracyclines were the dominant antibiotics in the raw wastewater, which was removed by 94.0% under a long HRT of 8 ~ 12 d. Shortening HRT to 2.7 d would not much influence the effluent concentration of NH_4-N and COD, but a significant decrease was found for the removal rate of tetracyclines.

(4) IASBR is a novel technology that may be feasible for the treatment of wastewater with high TN concentration and low COD/TN ratios. It was found that the IASBR was effective to remove antibiotics. Greater than 84% of various typical veterinary antibiotics were removed from the bioreactor, and SMD was even removed more than 99%. 60 ~ 88% of TN could be removed with a not very high NH_4-N loading rate. A non-aeration duration of longer than 50 min and an aeration duration of 50 ~ 120 min could be more effective for the treatment of ADPW.

6.2. Future research and suggestions

The regional ecological security problems should be paid more attention and ecological risk assessment was urgently needed at the same time to develop the effective and harmless technologies for ADPW treatment. Mass balance, migration and transformation of antibiotics, and the impact factors should be further studied in order to clear the removal mechanisms of antibiotics in SMBR.

The concentration of TN in ADPW from Jiaxing was high, and the COD/TN ratio was low, so it is important to develop the processes with characteristic of less carbon source requirement such as shortcut nitrification and denitrification, anaerobic ammonia oxidation process etc. Also, it is worth further studying of the optimization of operational parameters of the IASBR, the removal performance of conventional pollution indexes and antibiotics, and the investigation of the impact factors on stable running and thereby finds countermeasures.

At last, COD, antibiotics and other indicators in ADPW were fluctuated with seasons, which should be fully taken into consideration in the process design for ADPW. Suspended solids in ADPW had impact on water quality, and the heavy metals of Cu, Zn possibly could be accumulated in the bioreactor during a long-term operation, so they should be removed as much as possible by physicochemical pretreatment before entering the biological treatment process, and a new treatment process with the combination of SMBR and IASBR may possess the potential for more stable and effective treatment of ADPW.

References

Aga DS, O'Connor S, Ensley S. Determination of the persistence of tetracycline antibiotics and their degradations in manure-amended soil using enzyme-linked immunosorbent assay and liquid chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry, 2005, 53: 7165-7171.

Amin M, Zillers J, Greiner J, Charbonneau S, Raskin L, Morgenroth E. Influence of the antibiotic erythromycin on anaerobic treatment of a pharmaceutical wastewater. Environmental Science & Technology, 2006, 40: 3971-3977.

An JY, Kwon J C, Ahn D W, Shin D H, Shin H S, Kim B W. Efficient nitrogen removal in a pilot system based on upflow multi-layer bioreactor for treatment of strong nitrogenous swine wastewater. Process Biochemistry, 2007, 42: 764-772.

Atkinson S. Research studies predict strong growth for MBR markets. Membrane Technology, 2006, 2: 8-14.

Ben WW, Qiang ZM, Adams C. Simultaneous determination of sulfonamides, tetracyclines and tiamulin in swine wastewater by solid-phase extraction and liquid chromatography-mass spectrometry. Journal of Chromatography A, 2008, 12: 173-180.

Binh CTT, Heuer H, Kaupenjohann M, Smalla K. Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids. FEMS Microbiology Ecology, 2008, 66: 25-37.

Boleas S, Alonso C, Pro J, Fernández C, Carbonell G, Tarazona JV. Toxicity of the antimicrobial oxytetracycline to soil organisms in a multi-species-soil system (MS center dot3) and influence of manure co-addition. Journal of Hazardous Materials, 2005, 122: 233-241.

Brown KD, Kulis J, Thomson B, Chapman TH, Mawhinney DB. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. Science of the Total Environment, 2006, 366: 772-783.

Carballa M, Omil F, lema JM, Llompartb M, García-Jaresb C, Rodríguezb I, Gómezc M, Ternesd T. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Research, 2004, 38: 2918-2926.

Cha JM, Yang S, Carlson KH. Rapid analysis of trace levels of antibiotic polyether ionophores in surface water by solid-phase extraction and liquid chromatography with iontrap tandem mass spectrometric detection. Journal of Chromatography A, 2005, 1056: 187-198.

Chen BM, Lu Z, Su R, Qian JY. Treatment of anaerobically digested effluent of piggery wastewater using two step-feed SBR method. Energy Environmental Protection, 2010, 24: 19-21.

Chen YS, Zhang HB, Luo YM. A preliminary study on the occurrence and dissipation of antibiotics in swine wastewater. Acta Scientiae Circumstantiae, 2010, 30: 2205-2212 (Chinese Journal).

Cheng J, Liu B. Nitrification/denitrification in intermittent aeration process for swine wastewater treatment. Journal of Environmental Engineering, 2001, 127: 705-711.

Cherlet M, Baere SD, Croubels S, Bacher PD. Quantitation of tylosin in swine tissues by liquid chromatography combined with electrospray ionization mass spectrometry. Analytica Chimica Acta, 2002, 473: 167-175.

Ciudad G, Gonzalez R, Bornhardt C, Antileo C. Modes of operation and pH control as enhancement factors for partial nitrification with oxygen transport limitation. Water Research, 2007, 41: 4621-4629.

Costanzo SD, Murby J, Bates J. Ecosystem response to antibiotics entering the aquatic environment. Marine Pollution Bulletin, 2005, 51: 218-223.

Cui ZF, Chang S, Fane AG. The use of gas bubbling to enhance membrane processes. Journal of Membrane Science, 2003, 221: 1-4.

Daverey A, Hung N T, Dutta K, Lin J G. Ambient temperature SNAD process treating anaerobic digester liquor of swine wastewater. Bioresource Technology, 2013, 141: 191-198.

D’Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB,Poinar HN, Wright GD. Antibiotic resistance is ancient. Nature, 2011, 477: 457-461.

Dorival-García N, Zafra-Gómez A, Navalón A, González-López J, Hontoria E, Vílchez JL. Removal and degradation characteristics of quinolone antibiotics in laboratory-scale activated sludge reactors under aerobic, nitrifying and anoxic conditions, Journal of Environmental Management, 2013, 120: 75-83.

Dosta J, Rovira J, Galí A, Macé S, Mata-Álvarez J. Integration of a Coagulation/Flocculation step in a biological sequencing batch reactor for COD and nitrogen removal of supernatant of anaerobically digested piggery wastewater, Bioresource Technology, 2008, 99: 5722-5730.

Esiobu N, Armenta L, Ike J. Antibiotic resistance in soil and water environments. International Journal of Environmental Health Research, 2002, 12: 133-144.

Figueroa M, Val del Río A, Campos J L, et al. Treatment of high loaded swine slurry in an aerobic granular reactor, Water Science and Technology, 2011, 63: 1808-1814.

Göbel A, McArdell CS, Joss A, Siegrist H, Giger W. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies, Science of the Total Environment, 2007, 372: 361-371.

Guo J, Yang G, Fang F, Qin Y. Performance of completely autotrophic nitrogen removal over nitrite process under different aeration modes and dissolved oxygen. Frontiers of Environmental Science and Engineering in China, 2008, 2: 439-445.

Guo YN, Fang ZK, Hu JH, Xie HZ, Li LT, Ye ZY. Research development of treating wastewater containing heavy metals by chemical precipitation process. Industrial Water Treatment, 2011, 31: 9-13.

Halling-Srensen B, Sengelv G, Tjrnelund J. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Archives of Environmental Contamination and Toxicology, 2002, 42: 263-271.

Hamscher G, Sczesny S, Hoper H, Nau H. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Analytical Chemistry, 2002, 74: 1509-1518.

Han JL, He DC, Wang ZL, He YP, Xu ZC. Research of variety and residual characteristics of antibiotics of wastewater in industrial raising farm. Guangzhou Chemistry, 2012, 37: 27-30 (Chinese Journal).

Healy MG, Zhan XM, Rodgers M. Effect of aeration rate on nutrient removal from slaughterhouse wastewater in an intermittently aerated sequencing batch reactor. Journal of Water, Air and Soil Pollution, 2008, 192: 251-261.

Huang CH, Renew J E, Smeby K L. Assessment of potential antibiotic contaminants in water and preliminary occurrence analysis. Water Research, 2001, 120: 30-40.

Huber MM, Göbel A, Joss A, Hermann N, Löffler D, McArdell CS, Ried A, Siegrist H, Ternes TA, Gunten UV. Oxidation of pharmaceuticals during ozonation of municipal wastewater effluents: a pilot study. Environmental Science & Technology, 2005, 39: 4290-4299.

Institute for Prospective Technological Studies. Integrated pollution prevention and control: reference document on best available techniques in the slaughterhouses and animal by-products industries. European Commission, Seville, Spain, 2005 (Document).

Jiang L, Chen SY, Yang R, Ren ZY, Yin DQ. Occurrence of antibiotics in the aquatic environment of the Changjiang Delta, China. Environmental Chemistry, 2008, 27: 321-324.

Jiang P, Jin SY, Hao XZ, Zhou DM, Li LZ, Lv JL. Distribution characteristics of heavy metals in feeds, pig manures, soils and vegetables. Journal of Agro-Environment Science, 2010, 29: 942-947.

Jiang Y, Chen J, Wang B, Chen J. Effect of intermittentaeration on growth kinetics of nitrifiers and mechanism for NO-accumulation. Environmental Science, 2009, 30: 85-90.

Karthikeyan KG, Meyer MT. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. Science of the Total Environment, 2006, 361: 196-207.

Katsogiannis AN, Kornaros M, Lyberatos G. Enhanced nitrogenremoval in SBRs bypassing nitrate generation accomplished by multipleaerobic/anoxic phase pairs. Water Science and Technology, 2003, 47: 53-59.

Kennedy DG, McCracken RJ, Cannavan A, Hewitt SA. Use of liquid chromatography-mass spectrometry in the analysis of residues of antibiotics in meat and milk. Journal of Chromatography A, 1998, 812: 77-98.

Kim H S, Choung Y K, Ahn S, Oh H S. Enhancing nitrogen removal of piggery wastewater by membrane bioreactor combined with nitrification reactor. Desalination, 2008, 223: 194-204.

Kim S, Reichhorn P, James J N. Removal of antibiotics in wastewater: Effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. Environmental Science & Technology, 2005, 39: 5816-5823.

Kosutic K, Dolar D, Asperger D, Kunst B. Removal of antibiotics from a model wastewater by RO/NF membranes. Separation and Purification Technology, 2007, 53: 244-249.

Kraume M, Bracklow U, Vocks M, Drews A. Nutrients removal in MBRs for municipal wastewater treatment. Water Science and Technology: A Journal of The International Association on Water Pollution Research, 2005, 51: 391-402.

Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt FA, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. The Lancet Infectious Diseases, 2010, 10: 597-602.

Kümmerer K, Al-Ahmad A, Mersch-Sundermann V. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. Chemosphere, 2000, 40: 701-710.

Lei D. Performance of A new integrated A/O reactor for the treatment of synthetic rural sewage wastewater. MSc Thesis, Zhengzhou University, Zhengzhou, China, 2012: 28-29 (Chinese Thesis).

Li D, Yang M, Hu J, Ren L, Zhang Y, Li K. Determination and fate of oxytetracycline and related compounds in oxtetracycline production wastewater and the receiving river. Environmental Toxicology and Chemistry, 2008, 27: 80-86.

Li J, Elliott D, Nielsen M, Healy MG, Zhan XM. Long-term partial nitrification in an intermittently aerated sequencing batch reactor (SBR) treating ammonium-rich wastewater under controlled oxygen-limited conditions. Biochemical Engineering Journal, 2011, 55: 215-222.

Li JP, Healy MG, Zhan XM, Rodgers M. Nutrient removal from slaughterhouse wastewater in an intermittently aerated sequencing batch reactor. Bioresource Technology, 2008, 99: 7644-7650.

Li S, Li X, Wang D. Membrane (RO-UF) filtration for antibiotic wastewater treatment and recovery of antibiotics. Separation and Purification Technology, 2004, 34: 109-114.

Li SL, Deng LW. The macro policy of waste disposal and technical progress for livestock and poultry breeding in China, 2007. Swine Industry Science, 2008: 70-72.

Liguoro DM, Cibin V, Capolongo F. Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. Chemosphere, 2003, 52: 203-212.

Lindberg RH, Wennberg P, Johansson MI, Tysklind M, Andersson BA. Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in sweden. Environmental Science & Technology, 2005, 39: 3421-3429.

Liu CX, Dong YH, Wang H. Survey on the intensive farming in Jiangsu province of livestock and poultry excrement tetracycline class antibiotic residues. Journal of Agro-Environment Science, 2008, 27: 1177-1182.

Liu YC, Xu WH, Yu LL. Determination of trace Macrolides in water using solid-phase extraction and liquid chromatography with tandem mass spectrometry. Journal of Instrumental Analysis, 2006, 25: 1-5.

Luo Y, Xu L, Rysz M, Wang YQ, Zhang H, Alvarez PJJ. Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China, Environment Science & Technology, 2011, 45: 1827-1833.

Luo Y, Zhou QX. Antibiotic resistance genes (ARGs)-A new type of environmental pollutants. Journal of Environmental Sciences, 2008, 28: 1499-1505.

Morales N, Figueroa M, Fra-Vázquez A, Val del Río A, Campos J L, Mosquera-Corral A, Méndez R. Operation of an aerobic granular pilot scale SBR plant to treat swine slurry. Process Biochemistry, 2013, 48: 1216-1221.

Nygaard K, Lunestad BT, Hektoen H, Berge JA, Hormazabal V. Resistance to oxytetracycline, oxolinic acid and furazolidone in bacteria from marine sediments. Aquaculture, 1992, 104: 31-36.

Obaja D, Macé S, Mata-Alvarez J. Biological nutrient removal by a sequencing batch reactor (SBR) using an internal organic carbon source in digested piggery wastewater. Bioresource Technology, 2005, 96: 7-14.

Pambrun V, Paul E, Sperandio M. Control and modelling of partial nitrification of effluents with high ammonia concentrations in sequencing batch reactor. Chemical Engineering and Processing, 2008, 47: 323-329.

Pan X, Qiang Z, Ben W, Chen M. Residual veterinary antibiotics in swine manure from concentrated animal feeding operations in Shandong Province, China. Chemosphere, 2011, 84: 695-700.

Poole CF. New trends in solid-phase extraction. Trends in Analytical Chemistry, 2003, 22: 362-373.

Prado N, Ochoa J, Audic J L, Amrane A, Meinhold J. Semi-industrial-scale Process for Dilute Swine Wastewater Treatment Using a Submerged Membrane Bioreactor (MBR) with Direct Reuse of Treated Water. International Journal of Chemical Reactor Engineering, 2007, 5: 68-75.

Prado N, Ochoa J, Amrane A. Biodegradation by activated sludge and toxicity of tetracycline into a semi-industrial membrane bioreactor. Bioresource Technology, 2009, 100: 3769-3774.

Prado N, Ochoa J, Amrane A. Zero Nuisance Piggeries: Long-term performance of MBR (membrane bioreactor) for dilute swine wastewater treatment using submerged membrane bioreactor in semi-industrial scale. Water Research, 2009, 43: 1549-1558.

Richardson SD, Ternes TA. Water analysis: emerging contaminants and current issues. Analytical Chemistry, 2011, 83: 4614-4648.

Sachera F, Lange FT, Brauch H, Blankenhorn I. Pharmaceuticals in groundwaters analytical methods and results of a monitoring program in Baden-Wu ̈rttemberg, Germany. Journal of Chromatography, 2001, 938: 199-210.

Sahar E, Messalem R, Cikurel H, Aharonic A, Brennera A, Godehardtd M, Jekeld M, Ernste M. Fate of antibiotics in activated sludge followed by ultrafiltration (CAS-UF) and in a membrane bioreactor (MBR). Water Research, 2011, 45: 4827-4836.

Sarmah AK, Meyer MT, Boxall BA. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere, 2006, 65: 725-759.

Scaglione D, Tornotti G, Teli A, Lorenzoni L, Ficara E, Canziani R, Malpei F. Nitrification denitrification via nitrite in a pilot-scale SBR treating the liquid fraction of co-digested piggery/poultry manure and agro-wastes. Chemical Engineering Journal, 2013, 228: 935-943.

Sombatsompop K, Songpim A, Reabroi S, Inkong-ngam P. A comparative study of sequencing batch reactor and moving-bed sequencing batch reactor for piggery wastewater treatment. Maejo International Journal of Science and Technology, 2011, 5: 191-203.

Shi LJ, Liu HF, Hao JC. Experimental study on treatment of livestock and poultry farm wastewaters using a hybrid baffled reactor. Environmental Science & Technology, 2011, 1: 148-151.

Song CF, Shan SD, Zhang MX, Wen XH. Concentration and determination of composition of biogas slurry. Transactions of the CSAE, 2011, 12: 256-259.

State Environmental Protection Administration. Monitoring and analysis method of water and wastewater, The 4th Edition. China Environmental Science Press, Beijing, 2002. 238-239; 252-256; 260-263; 266-269; 345-356 (Chinese Book).

Sui QW, Dong HM, Zhu ZP, Huang HK. Present status of biogas effluent treatment technology research and application. Journal of Agricultural Science and Technology, 2011, 13: 83-87.

Tan JH, Tang CM, Yu YY. Simultaneous analysis of multiple classes of antibiotics in urban river water by high performance liquid chromatography. Chinese Journal of Chromatography, 2007, 25: 546-549.

Tan Y, Li DS. Pig farming in large scale and environmental conservation: dilemma and choice—based on America's experience. Chinese Agricultural Science Bulletin, 2010, 26: 20-22 (Chinese Journal).

Tang WW, Gui WC, Zeng XP. Improvement of preparation of PFAS and its effect on treating heavy metal in wastewater. Journal of Shenzhen University (Science & Engineering), 2011, 28: 276-282 (Chinese Journal).

Ternes TA, Joss A, Siegrist H. Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environmental Science & Technology, 2004, 38: 392A-399A.

Tong L, Li P, Wang YX. Analysis of veterinary antibiotics residues in s wastewater and environmental water samples using optimized SPE-LC/MS/MS. Chemosphere, 2009: 4: 1090-1097.

Tuan XL, Munekage Y. Residues of selected antibiotics in water and mud from shrimp ponds in mangrove areas in Vietnam. Marine Pollution Bulletin, 2004, 49: 922-929.

Wang H, Pei WZ, Li XD, Hao C, Zeng KM. Removing nitrogen from low-C/N -piggery-wastewater using shortcut nitrification/denitrification-ANAMMOX. Environmental Science, 2009, 30: 815-821.

Wang L, Zhu J, Miller C. The stability of accumulating nitrite from swine wastewater in a sequencing batch reactor. Applied Biochemistry and Biotechnology, 2011, 163: 362-372.

Wang R, Liu TZ, Wang T. The outcome of antibiotics in the environment and its ecological toxicity. Acta Ecologica Sinica, 2006, 26: 265-270.

Wang XL, Peng YZ. The A^2/O biological treatment technology for denitrification and phosphorus removal and its application. Science Press, Beijing, China, 2009 (Chinese Book).

Wang YX. Research on the use of farmland disposal technology by biogas slurry and soil environmental effects. Zhejiang Normal University, 2010: 60-100 (Chinese Thesis).

Wei X M, Lina C, Duana N, Peng Y X, Ye Z Y. Application of aerobic biological filter for treating swine farms wastewater. Procedia Environmental Sciences, 2010, 2: 1569-1584.

Wu N, Qiao M. Tetracycline residues and tetracycline resistance gene pollution in soil: A Review. Asian Journal of Ecotoxicology, 2010, 5: 618-627.

Wu WE, Ge HG, Zhang KF. Biological treatment of wastewater. Chemical Industry Press publishing Center of Environmental Science and Engineering, 2003, 179-181.

Xie B. The Effect of Cu and Zinc ions on activated sludge microbes and its analysis with molecular biological technique. MSc Thesis, Donghua University, Shanghai, China, 2002: 70-100.

Xu WP, Chen TB, Liu JL, He YQ. Environmental pollution, comprehensive prevention and control tactics of the scale and intensify poultry farming. Environmental Science, 2004, 25: 105-108.

Xu WH, Zhang G, Zou SC. Occurrence and seasonal changes of antibiotics in the Victoria Harbour and the Pearl River, south China. Environmental Science, 2006, 27: 2459-2462.

Xun XY. Status and countermeasures of biogas slurry in large-scale pig farm. Jiangsu Agricultural Sciences, 2010, 6: 206 (Chinese Jounral).

Yamamoto T, Takaki K, Koyama T, Furukawa K. Novel partial nitritation treatment for anaerobic digestion liquor of swine wastewater using swim-bed technology. Journal of Bioscience and Bioengineering, 2006, 102: 497-503.

Yang CH, Zeng GM, Chen XC, Yang X. Study on the disposal technology of piggery wastewater. Environmental Engineering, 2002, 20: 19-21.

Yang W, Cicek N. Treatment of swine wastewater by submerged membrane bioreactors with consideration of estrogenic activity removal. Desalination, 2008, 231: 200-208.

Ye JP, Zou SC, Zhang G, Xu WH. Characteristics of selected antibiotics in the aquatic environment of the Pearl River Delta, south China. Ecology and Environment, 2007, 16: 384-388.

Ye Z, Weinberg H S. Trace Analysis of Trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry. Analytical Chemistry, 2007, 79: 1135-1144.

Yu LF, Wang SW, Guo TC, Peng DC. Nitrifiers accumulation with reject water and bio-augmentation for nitrification of sewage at short SRT. Environmental Science, 2008, 29: 332-337.

Zeng W, Peng Y, Wang S, Peng C. Process control of an alternating aerobic-anoxic sequencing batch reactor for nitrogen removal via nitrite. Chemical Engineering and Technology, 2008, 31: 582-587.

Zhan XM, Healy MG, Li JP. Nitrogen removal from slaughterhouse wastewater in a sequencing batch reactor under controlled low DO conditions. Bioprocess and Biosystems Engineering, 2008, 32: 607-614.

Zhang HM, Zhang MK, Gu GP. Tetracycline class antibiotic residues in the soil livestock and poultry dung and farmland in the north area of Zhejiang province. Journal of Ecology and Rural Environment, 2008, 24: 69-73.

Zhang SQ, Zhang FD, Liu XM, Wang YJ, Zou SW, He XS. Determination main harmful composition in excrement of scale livestock and poultry fee. Nutrition and Fertilizer Science, 2005, 11: 822-829.

Zhao LJ. The contribution and strategy of developing biogas engineering of Beijing rural. China Biogas, 1998: 40-42.

Zhao M. A/O-MBR process for treating digested piggery wastewater. Industrial Water Treatment, 2012, 32: 27-29.

Zhou QX, Luo Y, Wang MM. Environmental residues and ecotoxicity of antibiotics and their resistance gene pollution: A Review. Asian Journal of Ecotoxicology, 2007, 2: 243-251.

Zhou X, Liu JP, Zhang XS. Large-scale farms non-point source pollution investigation and countermeasures research. South Agricultural Machinery, 2009: 36-36.

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