Journal of Hazardous Materials 197 (2011) 49–59

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Simultaneous removal and evaluation of organic substrates and NH_3-N by a novel combined process in treating chemical synthesis-based pharmaceutical wastewater

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ARTICLE INFO

Article history:
Received 9 April 2011
Received in revised form 30 August 2011
Accepted 14 September 2011
Available online 17 September 2011

Keywords:
Chemical synthesis-based pharmaceutical wastewater
UASB
NHAR
Two-stage aerobic biological system
Amoxicillin

ABSTRACT

A full-scale novel combined anaerobic/micro-aerobic and two-stage aerobic biological process is used for the treatment of an actual chemical synthesis-based pharmaceutical wastewater containing amoxicillin. The anaerobic system is an up-flow anaerobic sludge blanket (UASB), the micro-aerobic system is a novel micro-aerobic hydrolysis acidification reactor (NHAR) and the two-stage aerobic process comprised cyclic activated sludge system (CASS) and biological contact oxidation tank (BCOT). The influent wastewater was high in COD, NH_3-N varying daily 4016–13,093 mg-COD L^{-1} and 156.4–650.2 mg-NH_3-N L^{-1}, amoxicillin varying weekly between 69.1 and 105.4 mg-amoxicillin L^{-1}, respectively; Almost all the COD, NH_3-N, amoxicillin were removed by the biological combined system, with removal percentages 97%, 93.4% and 97.2%, respectively, leaving around 104 mg-COD L^{-1}, 9.4 mg-NH_3-N L^{-1} and 2.6 ± 0.8 mg-amoxicillin L^{-1} in the final clarifier effluent. The performance evaluation of the wastewater treatment plant (WWTP) by mathematical statistic methods shown that at most of time effluent can meet the higher treatment discharge standard. In addition, the fate of amoxicillin in the full-scale WWTP and the amoxicillin removal rate of each different removal routes in UASB, NHAR, CASS, BCOT and final clarifier processes are investigated in this paper. The results show that biodegradation, adsorption and hydrolysis are the major mechanisms for amoxicillin removal.

1. Introduction

In recent years, the rapid development of chemical synthesis-based pharmaceutical industry of China leads the treatment of chemical synthesis-based pharmaceutical wastewater to emerge as an important concern. Chemical synthesis-based pharmaceutical wastewaters contain such a variety of organic and inorganic constituents including spent solvents, catalysts, additives, reactants and amounts of intermediates and products (amoxicillin), that with high COD and NH_3-N concentration, low biodegradation, bacterial toxicity and recalcitrance [1,2]. Therefore, effective removal of substances included in pharmaceutical residual effluents is a challenging task [3,4]. It has been reported that approximately half the pharmaceutical wastewaters containing lots of organic and inorganic pollutants such as antibiotics are discharged without any specific treatment; this behavior will leads large numbers of drugs enter into the natural environment and causes various environmental problems [5–7].

Amoxicillin, a semi-synthetic β-lactam antibiotic, is the most common featured pollutant in the fermentation and chemical synthesis-based pharmaceutical wastewater [8,9]. Its bacterial toxicity and recalcitrance may play an important role in decreasing the chemical oxygen demand (COD) removal efficiency in biological treatment systems [9]. Furthermore, from an environmental engineering point of view, the health effects of pharmaceutical wastewater containing amoxicillin on human and animals are unknown, and stricter discharge standard of water pollutants for pharmaceutical industry also needs an approach towards appropriate technology for the treatment of pharmaceutical wastewater containing amoxicillin.

The present studies to treat the chemical synthesis-based pharmaceutical wastewaters containing amoxicillin mainly focused on physical and chemical treatment, such as UV/ZnO photo-catalytic process [10], photo-Fenton process [11], ultrasonic process [12], advanced oxidation processes (AOPs) and so on [13–15]. Though high COD and amoxicillin removal rates were observed in these bench-scaled experiments, they are not suitable for full-scale

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doi:10.1016/j.jhazmat.2011.09.053

wastewater treatment plant (WWTP) due to their high cost [16,17]. Therefore, it is urgent to request a cost-effective and efficient technique to deal with the wastewater. Adopting biological processes as a treatment alternative of the chemical synthesis-based pharmaceutical wastewaters should be an economical choice.

In this study, a practical application of up-flow anaerobic sludge blanket (UASB) process (a pre-treatment unit), novel micro-aerobic hydrolysis acidification reactor (NHAR), two-stage aerobic biological system composed cyclic activated sludge system (CASS) and biological contact oxidation tank (BCOT), and a final clarifier as a combined biological wastewater treatment technique was used for upgrading and retrofitting a full-scale WWTP in Inner Mongolia of China to meet the higher treatment discharge standard due to a change of legal framework in 2010. Several aspects were considered when employed the anaerobic/micro-aerobic and two-stage aerobic biological system to treat the chemical synthesis-based pharmaceutical wastewaters.

(1) High COD concentration in pharmaceutical wastewaters makes anaerobic/micro-aerobic process become a potential candidate [18,19]. Moreover, UASB (anaerobic process) with simple design, easy construction and maintenance, low operating cost, ability to withstand fluctuations in pH, temperature and influent substrate concentration [20–22]. It should be a wise option to employ UASB treat the high-strength wastewater in this WWTP. NHAR (micro-aerobic process) can provide a favorable condition for the subsequent aerobic processes, especially the micro-aerobic condition can enhance the physiological metabolizability of facultative hydrolytic and acidogenic bacteria, and aerating stirring can improve the gas–liquid–solid exchange condition [23].

(2) Although anaerobic digestion and hydrolysis acidification were effective means for decreasing the organic content in pharmaceutical wastewaters through the use of a consortium of heterotrophic microorganisms capable of utilizing a wide spectrum of substrates, in the absence of oxygen, high COD, NH_3-N and amoxicillin still reside in the effluent. To meet the purpose of direct discharge, the two-stage aerobic biological system which has a high potential of pollutants removal employed to treat the effluent of the anaerobic/micro-aerobic process.

(3) Up to now, no laboratory, pilot-scale or full-scale experiment has been reported exploiting combined anaerobic/micro-aerobic and two-stage aerobic process to treat pharmaceutical wastewaters containing amoxicillin, to authors' knowledge. Therefore, the suitability of using this type of system for the treatment of amoxicillin pharmaceutical wastewaters requires investigation.

(4) Last but not the least, the combined process has less energy consumption and the effluent can readily attain the discharge standards without only costly chemical or physical-chemical process.

(5) In addition, this research evaluates the performance of WWTP by mathematical statistic methods and investigated the fate of amoxicillin in the full-scale WWTP and quantified each removal rate of amoxicillin in UASB, NHAR, CASS, BCOT and final clarifier, respectively.

2. Materials and methods

2.1. Wastewater source and characteristics

As the largest pharmaceutical production bases in northwestern China, United Laboratories (Inner Mongolia) Co. Ltd. is located at Inner Mongolia China. The main products of this pharmaceutical enterprise are 6-APA (one of most important intermediates for amoxicillin synthesis) and amoxicillin. During 6-APA and amoxicillin manufacturing, many kinds of wastewaters were generated. Such as, the production of penicillin G sylvite which is the raw materials of 6-APA could generate the waste acid wastewater, ethanol stillage residue, filter cloth washing wastewater and low-strength wastewater which containing corn syrup, corn gluten meal and glucose; the production of 6-APA could generate phenyl acetic acid and wastewater containing 6-APA; the production of amoxicillin could generate amoxicillin workshop flush wastewater and amoxicillin liquid. So, the pharmaceutical wastewater was mixture of waste acid, phenyl acetic acid, stillage residue of ethanol, discharge wastewater of amoxicillin workshop and domestic sewage of staff, containing a variety of organic and inorganic constituents, such as spent solvents, catalysts, reactants and amounts of intermediates (6-APA) and products (amoxicillin).

In this paper, amoxicillin is carefully and further studied while 6-APA just regard as a kind of organic substrate without only further describe and study. The characteristics of the amoxicillin pharmaceutical wastewater are presented in Table 1. As shown in Table 1 the average influent flow, filtered COD, COD loading rate, NH_3-N, amoxicillin and pH of UASB are 2031.2 $m^3\,d^{-1}$, 7404 mg-COD L^{-1}, 15.6 kg-COD $m^{-3}\,d^{-1}$, 363.8 mg-NH_3-N L^{-1}, 92.2 mg-amoxicillin L^{-1} and 7.4, respectively. Because sedimentation tank, pre-aeration tank, buffer pool were designed between UASB and NHAR, UASB effluent water characteristics are different from NHAR influent. Table 1 demonstrate that the average influent flow, COD, COD loading rate, NH_3-N, amoxicillin and pH of NHAR are 11,150 $m^3\,d^{-1}$, 4518 mg-COD L^{-1}, 11.7 kg-COD $m^{-3}\,d^{-1}$, 212.1 mg-NH_3-N L^{-1}, 70.2 mg-amoxicillin L^{-1} and 6.7, respectively. The operational conditions of the combined processes are also shown in Table 1.

2.2. Combined anaerobic/micro-aerobic and aerobic process

A schematic flow chart of the combined anaerobic/micro-aerobic and two-stage aerobic biological wastewater treatment system is shown in Fig. 1. The UASB, NHAR and BCOT are new-built processes which are based on the primary process. Four types of wastewater are separately treated through the preliminary and primary treatment steps. First, the waste acid wastewater, after the treatment of flocculation sedimentation pool (I) and buffer pool (I) is fed to the UASB, and the effluent of UASB flows into the sedimentation tank (I), pre-aeration tank and buffer pool (II) in sequence. Second, the high concentration wastewater mixed of ethanol stillage residue, phenyl acetic acid and amoxicillin liquor is fed to the four-effect evaporator (four-effect evaporator is one of evaporator which composed by four stage evaporators. When operation, after pressurize the vapors generated by the first evaporator is transferred to the second evaporator as its heat source, the vapors generated by the second evaporator is transferred to the third evaporator, and the vapors generated by the third evaporator is transferred to the last stage evaporator as its heat source) employed in reusing the amoxicillin and other materials, the effluent of four-effect evaporator enters buffer pool (II). Third, after treated in the sedimentation tank, the filter cloth washing wastewater is introduced into the buffer pool (II). Last, after mixed with the domestic wastewater, corn syrup, corn gluten meal and glucose wastewater, fermentation and amoxicillin workshop flush wastewater, the low-strength concentration wastewater is introduced to grid screen and water-collecting well, the effluent of which also enters the buffer pool (II). Finally, the mixed effluent from the buffer pool (II) is treated by the biological treatment technology: NHAR, CASS and BOCT. The effluent of NHAR flows to CASS, and then the effluent of the CASS is inputted to the BCOT process. Finally, the

Table 1
Influent wastewater characteristics and the operational conditions of the combined processes.

	UASB	NHAR	CASS	BCOT	Final clarifier
Influent wastewater characteristics					
Soluble COD/mg-COD L^{-1}	4016–13,093	2398–11,505	1868–9951	230–1768	63–1589
Average soluble COD/mg-COD L^{-1}	7404	4518	3872	415	183
NH_3-N/mg-NH_3-N L^{-1}	156.4–650.2	145.2–318.5	164.4–351.4	70.6–175.6	2.9–126.9
AverageNH_3-N/mg-NH_3-N L^{-1}	363.8	212.1	227.9	112. 4	18.1
Amoxicillin/mg-amoxicillin L^{-1}	69.2–105.4	48.7–92.2	34.9–67.5	7.1–28.3	1.3–5.5
Average amoxicillin/mg-amoxicillin L^{-1}	92.2	70.2	56.1	17.7	3.4
Operational conditions					
Dimension (m)	38 × 8.6 × 6	48 × 15 × 6	56 × 23 × 6	56 × 23 × 6	φ20 × 5
Volume (m^3)	1960.8	4320	6900	6900	628
HRT (h)	23.5	9.3	14.9	14.9	1.35
Flow (m^3 d^{-1})	2031.2	11,150	11,150	11,150	11,150
Ph	5.6–8.3	5.3–7.1	6.8–7.6.	7.0–7.8	7.1–7.6
T (°C)	30–38	11–32	11–32	11–32	11–32
DO (mg L^{-1})	0.2–0.3	0.4–1.2	1.5–2.5	1.5–2.5	ND[a]

[a] No-detect.

sludge and wastewater are separated by the final clarifier, and the effluent is directly discharged into natural waters.

2.2.1. UASB process

The UASB reactor is a new-built anaerobic biological treatment process which employed in treating the wastewater with high COD and amoxicillin concentrations in the condition of high COD loading rate ranging between 12.57 and 21.02 kg m^{-3} d^{-1} and wide pH between 5.6 and 8.3. The UASB reactor has a volume of 1960.8 m^3, height of 38 m, length of 8.6 m, and width of 6 m, ten sludge ports are arranged along the height of the reactor, the first one at 2 m above the UASB bottom and the others distribute equally along the height of the reactor. The reactor contains a three identical conical gas solids separator at the top of the tank with a height of 3.8 m.

2.2.2. NHAR process

As the first stage of anaerobic digestion process, the traditional hydrolysis acidification reactor (THAR) is designed mainly for providing the substrates for the methanogen. In this study, NHAR which was an improved UASB without the three identical conical gas solids separator employed as an independent process for providing a favorable condition for the subsequent aerobic processes. The NHAR is also a new-built treatment process, and compared with the THAR, it has more effectively in organic substrates removal and wastewater biodegradability improvement, leading as the following reasons:

(1) Facultative anaerobes are the dominated bacterium of the hydrolysis acidification reactor. The presence of oxygen will reduce biochemical reaction activation energy value of facultative anaerobes, and more energy would be obtained by facultative anaerobes under a micro-aerobic condition of DO ranging between 0.4 and 1.2 mg L^{-1} and ORP between −30 and +80 mv. The micro-aerobic condition in the NHAR can promote the facultative anaerobes growth and reproduction, increase the sludge activity and strengthen the efficiency of

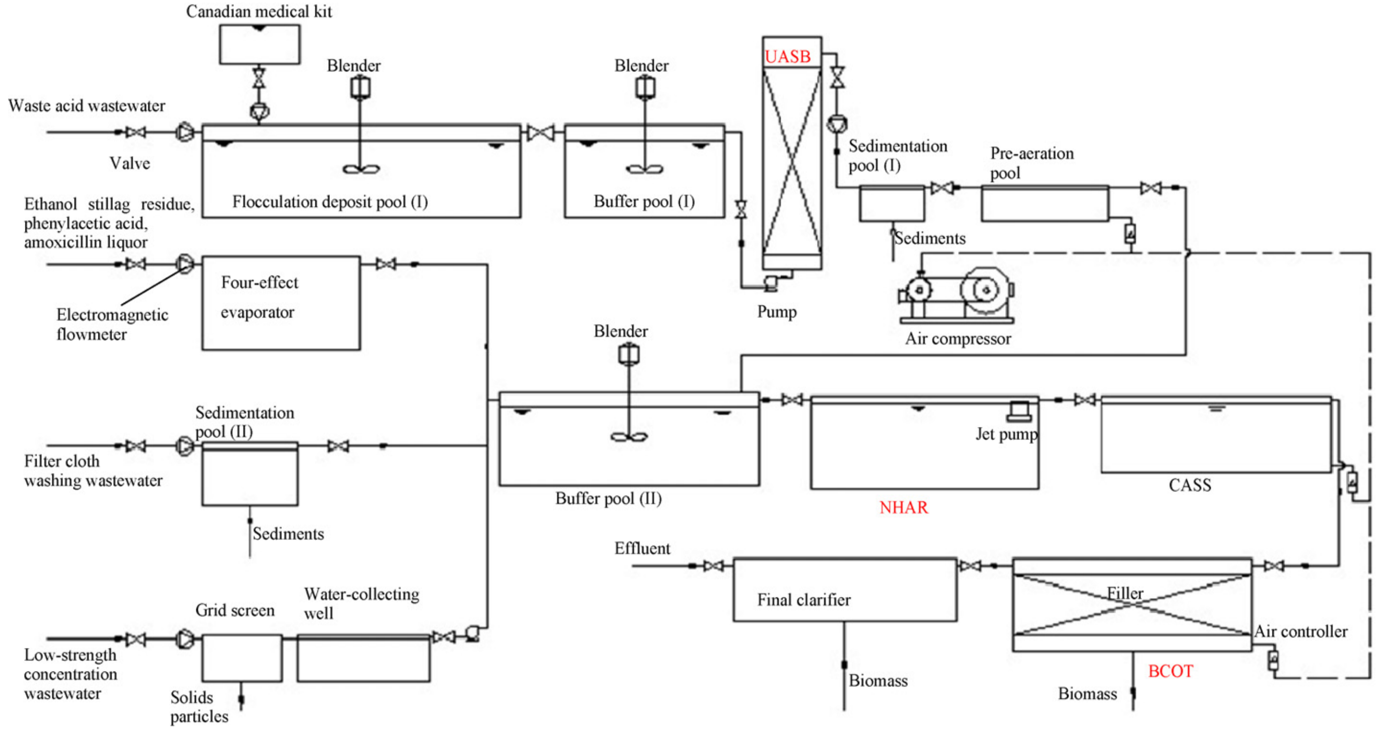

Fig. 1. Process flow diagram of the full-scale WWTP of United Laboratories Co. Ltd. (UASB, NHAR and BCOT were the new-built processes.)

wastewater acidification. What's more, the value of −30 mv to +80 mv ORP which achieved by aeration is most suitable for facultative anaerobe growth and acidification.

(2) On the other hand, aerating stirring have many advantages, such as, improve the gas–liquid–solid hydraulic conditions, enhance the mass transfer between hydrolysis sludge and organic substrates, accelerate the organic substrates transfer to the microbial cells from wastewater, increase the spread strength of organic substrates to activity sludge, improve the concentration gradient of organic substrates, also evacuate the VFA which generate in the hydrolysis acidification process to the liquid phase to reduce the feedback inhibition, thereby enhancing the rate of biochemical reactions.

There are two NHARs, and each has dimensions of 48 m × 15 m × 6 m, with an average HRT of 9.3 h.

2.2.3. CASS process

The CASS, which is the former treatment process, provides aerobic treatments in the WWTP. As a result, the CASS has great capability to minimize the organic substrates in the treatment of wastewater. After upgrading and retrofitting the WWTP, there are eight CASS reactors, and each reactor has dimensions of 50 m × 23 m × 6 m. When the system is under the normal operation, five CASS pools work, three CASS pools idle, holding a working volume of approximately 6900 m^3 and a HRT of 14.9 h.

2.2.4. BCOT process

The primary aerobic system of the WWTP were 12 CASS reactors, and the performance of the primary process demonstrated that the primary aerobic biological process (CASS) has a well performance of COD removal (the average effluent COD concentration was 313 $mg\,L^{-1}$ with an 88.7% COD removal percentage), while the performance of the NH_3-N removal was unsatisfactory (the average effluent NH_3-N concentration was 214.7 $mg\,L^{-1}$ with a 48.7% NH_3-N removal percentage). In order to improve the performance of aerobic biological system in this full-scale WWTP to meet the higher treatment discharge standard due to a change of legal framework in 2010, the two-stage aerobic biological system are considered to make the aerobic biological system be operated at its optimum level.

BCOT, the second-stage of the two-stage aerobic biological system, was new-built, a completely mixed and continuously operated process. There are four series of BCOTs in parallel with dimensions of 50 m × 23 m × 6 m, providing a total working volume of 6900 m^3 and a HRT of 14.9 h.

In the two-stage aerobic biological system, CASS is expecting to reduce the COD mainly as the heterotrophic aerobic bacteria dominated, and the BCOT is expecting to remove NH3-N mainly as the autotrophic aerobic bacteria dominated. What's more the two-stage aerobic biological system can make the aerobic microbe more diversity than one aerobic step.

What's more, lots of studies reports employ the two-stage aerobic biological systems in different wastewater treatment, such as coke plant wastewater [24], domestic wastewater [25], and PTA wastewater [26].

2.2.5. Final clarifier

As the quality of effluent from BCOT is still far from the required standard for discharge, two final clarifiers (φ20 m × 5 m) are designed for sedimentation active sludge.

2.3. Analytical methods

The WWTP was daily sampled during the 109 days from May 1st to August 17th, 2010. UASB influent and effluent, NHAR influent, NHAR effluent, CASS effluent, BCOT effluent and final clarifier effluent were collected at fixed sampling sites between 10 and 11 am daily for the analysis of soluble COD (sCOD), NH_3-N (ammonium-nitrogen) and pH. Measurement of sCOD and NH_3-N was performed directly on the sampled slurry according to Standard Methods [27]. DO and pH were monitored daily by a hand-held oxygen meter (COM 381, Shanghai Light Industry Research Institute, China) equipped with a DO probe (COS 381, Shenzhen Futai Instrument Co. Ltd, China) and a portable pH meter (sensionl, HACH), respectively.

A high performance liquid chromatography (Prominence LC-20 A, SHIMADZM, from Waters) which equipped with a guard column (Waters Sunfire C18 (5 μm × 20 mm × 4.6 mm)), an Alltech C18 (250 mm × 4.6 mm) column and a Jasco ProSar/Dynamax UV detector was used to measure amoxicillin concentration in the wastewater. The mobile phase was a mixture of phosphate buffer (0.01 $mol\,L^{-1}$, pH 4.8) and acetonitrile (95:5 v/v) pumped at a flow rate of 1.3 $mL\,min^{-1}$ through the column. Peaks were monitored by UV absorbance at 229 nm with a sensitivity of 0.005 AUFS. Quantification of amoxicillin was obtained by comparison to the internal standard peak height ratios as a function of concentration [7].

2.4. Experimental

The experiment of amoxicillin fate investigation contain two parts: (1) to research which treatment processes in the full-scale WWTP are capable of removing amoxicillin from pharmaceutical wastewater and the removal efficiency of each treatment process; (2) to determine the removal routes of amoxicillin in the anaerobic/micro-aerobic and aerobic combined process by investigating both abiotic (hydrolysis and adsorption) and biotic (biodegradation) processes. Amoxicillin removal due to volatilization is considered insignificant due to its low volatility.

2.4.1. Amoxicillin removal in the full-scale WWTP

To study the removal of amoxicillin in the full-scale WWTP, 24-h composite samples were collected at Monday of each week of the experimental period from the WWTP at the following locations (Fig. 1): influent to UASB, effluent of UASB, influent to NHAR, influent to CASS (effluent of NHAR), influent to BCOT (effluent of CASS), influent to final clarifier (effluent of BCOT), and effluent of the final clarifier. One litre grab samples were collected at each of these locations. To prevent cross contamination of samples, the tubing was changed prior to each new sample collection.

All collected samples were placed on ice in a cooler and transported back to the lab. Upon arriving at the lab, they were immediately filtered, acidified, covered, and stored in a refrigerator for analysis. Each sample was done three parallel experiments for ensure the accuracy of the experiments.

2.4.2. Amoxicillin fate in the full-scale WWTP

As described above, batch experiments investigating amoxicillin degradation mechanisms were done once a week during the experiment period.

2.5. Hydrolysis experiments

Fifteen flasks were separated into three parallel groups and the five flasks of each group, the five flasks of each group were dosed amoxicillin solution which solvent is in sterile, aerated deionized water and concentration same with the influent of UASB, NHAR, CASS, BCOT, and final clarifier, respectively. They were kept in test glass tubes covered by aluminum foil and the experiment was operated in the dark to avoid photolysis. The temperature was maintained constant at 25 °C. Amoxicillin hydrolysis was determined by measured the concentration of amoxicillin in sterile, aerated deionized water at appropriate intervals (the corresponding HRT of each treatment process) and analyzed by HPLC in order to monitor the amoxicillin decay. The reduction of amoxicillin in these flasks was contributed by hydrolysis.

2.6. Biodegradation and adsorption experiments

Another thirty flasks were separated into three parallel groups. Ten flasks of each group were marked with "$UASB_1$", "$NHAR_1$", "$CASS_1$", "$BCOT_1$", "Final $clarifier_1$", "$UASB_2$", "$NHAR_2$", "$CASS_2$", "$BCOT_2$" and "Final $clarifier_2$", respectively. 200 mL suspension of sludge was removed from each treatment process, and then injected to its corresponding flask.

The DO of each flask with subscript number "1" was kept at the same condition with its corresponding process, such as $UASB_1$ was kept at an anaerobic condition, $NHAR_1$ was kept at a micro-aerobic condition, $CASS_1$ and $BCOT_1$ were kept at aerated and stirred condition over the whole investigation period in order to ensure microbes of each process maintained the original biological activity, and the temperature was maintained constant at 25 °C by means of water bath. After the suspension of sludge was injected, 100 mL sodium azide was added to these flasks with subscript number "2", these flasks were maintained in the same condition with flasks with subscript number "1". Aliquots of the homogeneous suspension were withdrawn at different reaction time (the corresponding HRT of each treatment process) and filtered through cellulose disks before the HPLC analysis to monitor the amoxicillin decay.

The removal of amoxicillin determined by killing the microbial population in bioreactor with 100 mg L^{-1} sodium azide was attributed to hydrolysis and adsorption. The decay rates in the flasks with subscript number "1" were attributed to hydrolysis, sorption, and biodegradation. The difference of the removal rates between the bioreactors with sodium azide and aerated deionized water (i.e., hydrolysis) were attributed to sorption and that between the bioreactors with and without the sodium azide addition was attributed to biodegradation [7].

3. Results and discussion

3.1. UASB performance

UASB is anaerobic biological reactor in which the anaerobic digestion contributes to the pollutants removal, and the organic pollutants are degraded by microbial populations through multiple degradation steps contain hydrolysis/fermentation, acetogenesis and methanogenesis. Figs. 2 and 3a illustrated the influent, effluent COD, NH_3-N concentration and COD, NH_3-N removal efficiency of UASB during the experiment period. COD was detected every day during the 109 days, while NH_3-N was detected once 3 days from the 33rd day until the combined system began to run on the 93rd day, and a total of 20 groups of data about NH_3-N in each unit are illustrated in this paper.

As shown in Figs. 2 and 3a, we can observe results as follows: the fluctuation of pollutants in influent varied between 4016 and 13,093 mg-COD L^{-1} and between 156 and 650 mg-NH_3-N L^{-1}, with average COD and NH_3-N concentration at 7504 and 363 mg L^{-1}, respectively. From Fig. 2a, we can observe that, in the initial 53 days, UASB influent COD concentration fluctuated substantially from 4726 to 13,093 mg-COD L^{-1}; paralleling to this variation, the UASB effluent varied from 2175 to 5936 mg-COD L^{-1} and COD removal percentage from 29% to 71%, with an average of 49.6%. At days 54–58, there were more organic residual solvents, butyl, toluene, and mycelium at low pH in flocculation enter into sedimentation pool (I) and buffer pool (I) as more 6-APA and amoxicillin were product in theses day. The average values of influent, effluent and COD removal percentage were 7709 mg-COD L^{-1}, 6372 mg-COD L^{-1} and 15.2% at Stage 2, respectively. It is worth to mention that the effluent COD decreased from Day 69 to Day 110, varied between 2260 mg-COD L^{-1} and 4653 mg-COD L^{-1} despite the UASB influent concentration stabilized at a average 6523 mg-COD L^{-1}. This indicated that the UASB has potential for treating high-strength pharmaceutical wastewater. The COD removal efficiency in this UASB is lower than in another UASB study reporting COD removal percentage in range of 86.2%–91.6% in treating herbal pharmaceutical wastewater at HRT fluctuating between 33 and 43 h [28].

It is significant to point out that almost during the full-scale experiment the effluent NH_3-N concentration (423.7–821.7 mg-NH_3-N L^{-1}) was higher than the influent, with an average removal efficiency of −68.75% (Fig. 3a). This outcome was resulted from the breakdown of the total organic nitrogen in the raw wastewater under anaerobic condition.

3.2. NHAR performance

It is reported that hydrolysis/acidification is the rate limiting step of anaerobic digestion performed by the facultative hydrolytic and acidogenic bacteria; in the previous study hydrolysis and acidification reactor was employed to wastewater treatment as an initial process of anaerobic digestion. In this paper, we used a micro-aerobic hydrolysis and acidification reactor as the pretreatment unit to enhance the biodegradability of pharmaceutical wastewater, offer good substrate for subsequent aerobic treatment and optimize the hydrodynamic condition of the reactor [23].

Though NHAR can remove some pollutants by the biodegradation and interception of sludge and sedimentation of large particles, NH_3-N and the COD removal efficiency decreased compared with UASB. The influent concentrations fluctuated from 2398 to 11,505 mg-COD L^{-1} and from 145.2 to 318.5 mg-NH_3-N L^{-1}, the effluent varied from 1858 to 9951 mg-COD L^{-1} and from 164.4 to 351.4 mg-NH_3-N L^{-1} (Figs. 2 and 3b). The average COD removal percentages were 7.6%, −24.7% and 20%, and corresponding organic loading rates (OLR) 4.78 kg-COD m^{-3} d^{-1}, 3.64 kg-COD m^{-3} d^{-1} and 2.75 kg-COD m^{-3} d^{-1}, respectively in the three stages. During the full-scale experiment NH_3-N removal rate was −9.3%. The increase of NHAR effluent NH_3-N provided sufficient nitrogen to the follow-up aerobic operations, made up for the poverty of nitrogen in the pharmaceutical wastewater, and thus avoided adding nitrogen to wastewater to meet the need of the following aerobic process.

The average BOD_5/COD ratio of the wastewater was raised from 0.20 to 0.36 via NHAR treatment, but the THAR raised the ratio from 0.20 to 0.30 when it employed to treat this kind of pharmaceutical wastewater, which indicating that the biodegradability of the pharmaceutical wastewater could be greatly improved via the treatment of NHAR. The improvement of wastewater biodegradability could offer a favorable condition for the subsequent aerobic treatment process.

3.3. CASS performance

Though the NHAR greatly improved the biodegradability of pharmaceutical wastewater and some organic content were removed from the wastewater, lots of COD and NH_3-N still resides within NHAR effluent. To meet the purpose of direct discharge, a sub-sequential aerobic process is thus required. Recently, the aerobic treatment of pharmaceutical wastewater has been reported [29–32]. However, up until the present, no laboratory or pilot-scale experiments have been reported exploiting a process based on the CASS and BCOT technology to remediate high-strength

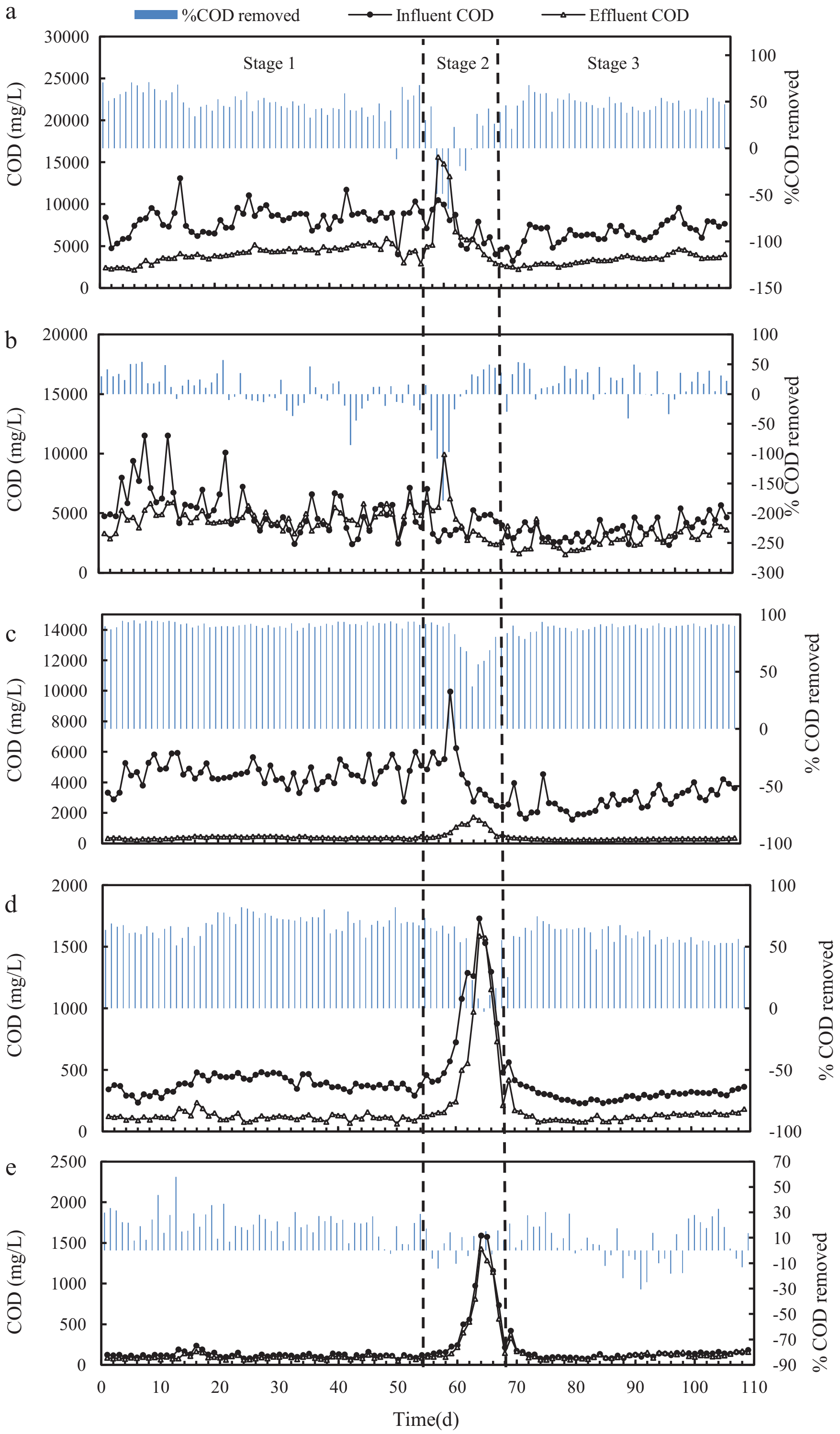

Fig. 2. (a) COD removal in the full-scale UASB during the 1–3 stages, (b) COD removal in the full-scale NHAR during the 1–3 stages, (c) COD removal in the full-scale CASS during the 1–3 stages, (d) COD removal in the full-scale BCOT during the 1–3 stages, (e) COD removal in the full-scale final clarifier during the 1–3 stages.

pharmaceutical wastewaters. We put forward to combining CASS and BCOT as a two-stage aerobic biological system to treat the pharmaceutical wastewater.

Performances of the CASS are illustrated in Figs. 2 and 3c. As shown in Fig. 2c, we can observe the results as follows; most of COD was removed in CASS, except Day 54 to Day 68 pollutants in influent, effluent and removal efficiency were kept at a stable level. CASS influent was the NHAR effluent which flocculated substantially from 1858 to 9951 mg-COD L^{-1} and 164.3 to 351.4 mg-NH_3-N L^{-1}; paralleling to this variation, the CASS effluent varied from 230 to 1768 mg-COD L^{-1} and 70.6 to 175.6 mg-NH_3-N L^{-1}, obtain COD removal percentage from 37% to 95% with an average of 88.4%, NH_3-N removal percentage from 35% to 65% with an average of 51.2%. The performances of CASS were significantly higher than reported previously. For example, Lapara et al. reported COD removal rate values of 38%–62% at temperature from 30 °C to 60 °C

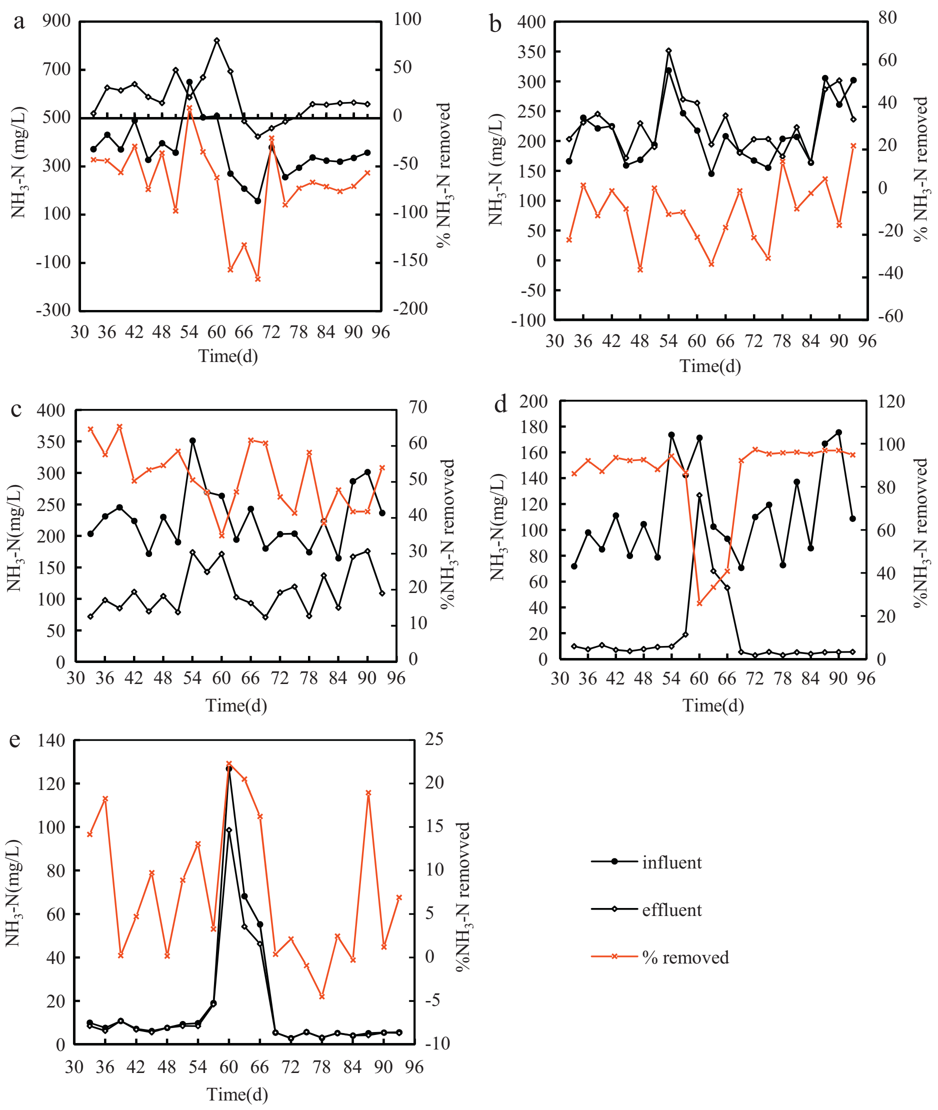

Fig. 3. (a) NH$_3$-N removal in the full-scale UASB during the experiment, (b) NH$_3$-N removal in the full-scale NHAR during the experiment, (c) NH$_3$-N removal in the full-scale CASS during the experiment, (d) NH$_3$-N removal in the full-scale BCOT during the experiment, (e) NH$_3$-N removal in the full-scale final clarifier during the experiment.

during the aerobic biological treatment of pharmaceutical wastewater [29]; Wu et al. reported values of 30%–60% COD removal rate in a combined process of micro-aerobic baffled reactor-biological contact oxidation treating printing and dyeing wastewater [33].

3.4. BCOT performance

As the second-stage system of the two-stage aerobic biological system, BCOT play a crucial role in pollutants removal. The results presented in Figs. 2 and 3d reveal that during the experiment, the BCOT influent concentration fluctuated from 230 to 1728 mg-COD L^{-1} and 70.6 to 175.6 mg-NH$_3$-N L^{-1}, with an average of 415 mg-COD L^{-1} and 112.4 mg-NH$_3$-N L^{-1}, the BCOT effluent varied from 63 to 1589 mg-COD L^{-1} and 2.9 to 126.9 mg-NH$_3$-N L^{-1}, COD removal percentage was fluctuated between −2.9% and 82% with an average of 61.8%. It is worth to mention that during the experiment BCOT obtained a high NH$_3$-N removal efficiency at 84.5%.

It is clear that, the COD removal efficiency of the first-stage aerobic biological unit was higher than that of the second-stage aerobic biological unit, while BCOT obtained a better NH$_3$-N removal efficiency (Figs. 2 and 3c,d). The reasons for this result are stated as follows. First, aerobic heterotrophic bacterium which has a very short generation time and a fast proliferation rate were the dominant species in the CASS reactor, while aerobic autotrophic bacterium with a longer generation time were the dominant species in BCOT; second, COD degradation heterotrophic aerobic bacteria and NH$_3$-N degradation autotrophic aerobic bacteria that compete DO and space fiercely in CASS; third, in the first stage of the aerobic system, the influent COD concentration is very high, that is, CASS is operated with a high organic loading rate, which offers a comfortable condition for the heterotrophic bacteria, while inhibits the removal of NH$_3$-N by restraining growth of nitrifying bacteria, so that high COD removal rate and low NH$_3$-N removal rate showed in CASS. On the contrary, the influent COD of the second stage of the aerobic system (BCOT) is low, while NH$_3$-N concentration in pharmaceutical wastewater was kept at a relatively high level, so from the perspective of biochemical reaction kinetics, the inhibitory effect of heterotrophic bacteria to autotrophic bacteria was weakened, the driving force of nitrification was strengthened, nitrifying bacteria got rid of slow proliferation rate and became the dominant species of BCOT, and therefore nitrification showed a strong effect.

Table 2
Amoxicillin concentration of each process during the experiment period.

	$C_{\text{amoxicillin max}}$ (mg L^{-1})	$C_{\text{amoxicilli ave}}$ (mg L^{-1})	$C_{\text{amoxicillin min}}$ (mg L^{-1})	SD
Influent to UASB	105.4	92.2	69.2	6.3
Effluent of UASB	86.1	73.5	51.6	10.5
Influent to NHAR	92.2	70.2	48.7	9.4
Influent of CASS	67.5	56.1	34.9	7.6
Influent of BCOT	28.3	17.7	7.1	4.2
Influent of final clarifier	5.5	3.4	1.3	2.8
Effluent of final clarifier	3.6	2.6	1.5	0.8

$C_{\text{amoxicillin max}}$, the maximal value of amoxicillin concentration during the experiment period; $C_{\text{amoxicillin max}}$, the average value of amoxicillin concentration during the experiment period; $C_{\text{amoxicillin max}}$, the minimal value of amoxicillin concentration during the experiment period; SD, standard deviation.

3.5. Final clarifier performance

The UASB + NHAR + CASS + BCOT obtained a perfect COD and NH_3-N removal efficiency of pharmaceutical wastewater during the full-scale experiment. Though the influent of this combined system was high in 7404 mg-COD L^{-1} and 363.8 mg-NH_3-N L^{-1}, the effluent decreased to 183 mg-COD L^{-1} and 18.1 mg-NH_3-N L^{-1}. Inspite of the excellent COD and NH_3-N removal rate, the effluent COD and NH_3-N concentration could not meet the higher pharmaceutical treatment discharge standard of China (COD < 120 mg L^{-1}, NH_3-N < 10 mg L^{-1}) which will be enforced in 2010, thus a final clarifier is needed to separate sludge and water. As shown in Fig. 2e, the effluent COD fluctuated from 51 to 1429 mg L^{-1}, and the corresponding COD removal rate was −30.7% to 58.0%, with an average of 13.2%. During Stage 1 and Stage 3, the average COD of the pharmaceutical wastewater was 118 and 129 mg L^{-1} after treated by this unit, giving an average COD removal rate of 20.8% and 6.2%, respectively, higher than 4.7% in Stage 2.

In general, the effluent NH_3-N of the final clarifier varied between 2.85 and 98.6 mg-NH_3-N L^{-1}, with an average removal efficiency of 7.5%, which demonstrated that the final clarifier also exhibited the ability to remove NH_3-N from pharmaceutical wastewater (Fig. 3e).

3.6. Evaluation of WWTP performance by mathematical statistic methods

It is an enormous challenge to make the effluent of a full-scale WWTP meet the discharge standard all the time as many reasons (failure operation of equipment, fluctuation of water characteristics and changes of operation conditions) will lead system work instability, thus the effluent pollutants concentration as well fluctuated irregularly. So evaluate the performance of a WWTP not only by the removal of pollutants, also can investigate the stability of the WWTP. The attainment rate, level below discharge and value below discharge standard were introduced to evaluate the performance of the full-scale WWTP. The attainment rate, level below discharge and value below discharge standard are defined as follows:

$$\text{Attainment rate} = \frac{D_{\text{att}}}{D_{\text{tot}}} \tag{1}$$

$$\text{Value below discharge standard} = C_{\text{observed}} - C_{\text{standard}} \tag{2}$$

$$\text{Level below discharge standard} = \frac{C_{\text{observed}} - C_{\text{standard}}}{C_{\text{standard}}} \times 100 \tag{3}$$

where D_{att} represent the days which the meet the discharge standard, D_{tot} is the days which the studied, C_{observed} represent the observed pollutant concentration, C_{standard} represent the discharge standard.

As shown in Figs. 4 and 5a,b, during the experiment, the attainment rate of COD and NH_3-N were 76.2% and 80%, which demonstrate that in the most of time the effluent can meet the standard and the WWTP has well system stability. Expect from Day 54 to Day 68, average values below discharge of COD and NH_3-N were 15.89 mg-COD L^{-1} and 1.89 mg-NH_3-N L^{-1}, average levels below discharge standard of COD and NH_3-N were 13.54% and 23.64%. This result illuminates that the system has the potential to upgrade the water quantity of effluent which higher than the existing discharge standard.

However, evaluating the performance of a full-scale WWTP by the above means is still not enough to us for a better operation of the system. It is essential to explore the pollutants removed quantity by each process of the WWTP. Consequently Figs. 4 and 5c reveals the pollutants removed quantity by each process of the combining process and the contribution rate of each process for the pollutants removal in the combining process. Figs. 4 and 5c show that CASS contributes most both COD and NH_3-N removal.

3.7. Fate of amoxicillin in the wastewater treatment plant

Wastewater was sampled weekly before and after each unit of the combined process throughout the full-scale WWTP for amoxicillin removal evaluation. The results and analysis of the performance of amoxicillin removal are presented in Tables 2 and 3. As tables shown, amoxicillin appeared to be removed in every process, especially in the two-stage aerobic biological system. The amoxicillin concentrations of the wastewater entering the UASB varied between 69.1 and 105.4 mg-amoxicillin L^{-1} with an average concentration of 92.2 mg-amoxicillin L^{-1}, and the effluent amoxicillin concentrations fluctuated from 51.6 to 86.1 mg-amoxicillin L^{-1} with an average removal efficiency of 20.2% during the stable stages in the full-scale experiment. The UASB can removed 52.8 kg amoxicillin d^{-1} with a cost of 11.9 US$ kg amoxicillin d^{-1}. Similar to the results seen from the UASB analysis, Table 2 also shows that the influent amoxicillin average concentrations of NHAR were 70.2 mg-amoxicillin L^{-1}, and the average effluent concentrations were 56.1 mg-amoxicillin L^{-1}, with average removal efficiency (20.4%) approximates to UASB. However, the cost of amoxicillin removal was higher than UASB.

It is obvious to observe that amoxicillin concentration decreased significantly through the two-stage aerobic biological system, which confirms that aerobic degradation is the primary factor in removing amoxicillin from pharmaceutical wastewater. The average concentration of amoxicillin leaving the NHAR

Table 3
Amoxicillin removal rate, quantities of removed amoxicillin and the cost for amoxicillin removal of each process.

	Amoxicillin removal rate (%)	Quantities of removed amoxicillin (kg d^{-1})	Cost (US$ kg-amoxicillin^{-1})
UASB	20.2	52.9	11.9
NHAR	20.4	157.6	21.2
CASS	68.2	428.8	19.6
BCOT	80.6	158.7	22.3
Final clarifier	25.7	9.3	3.7

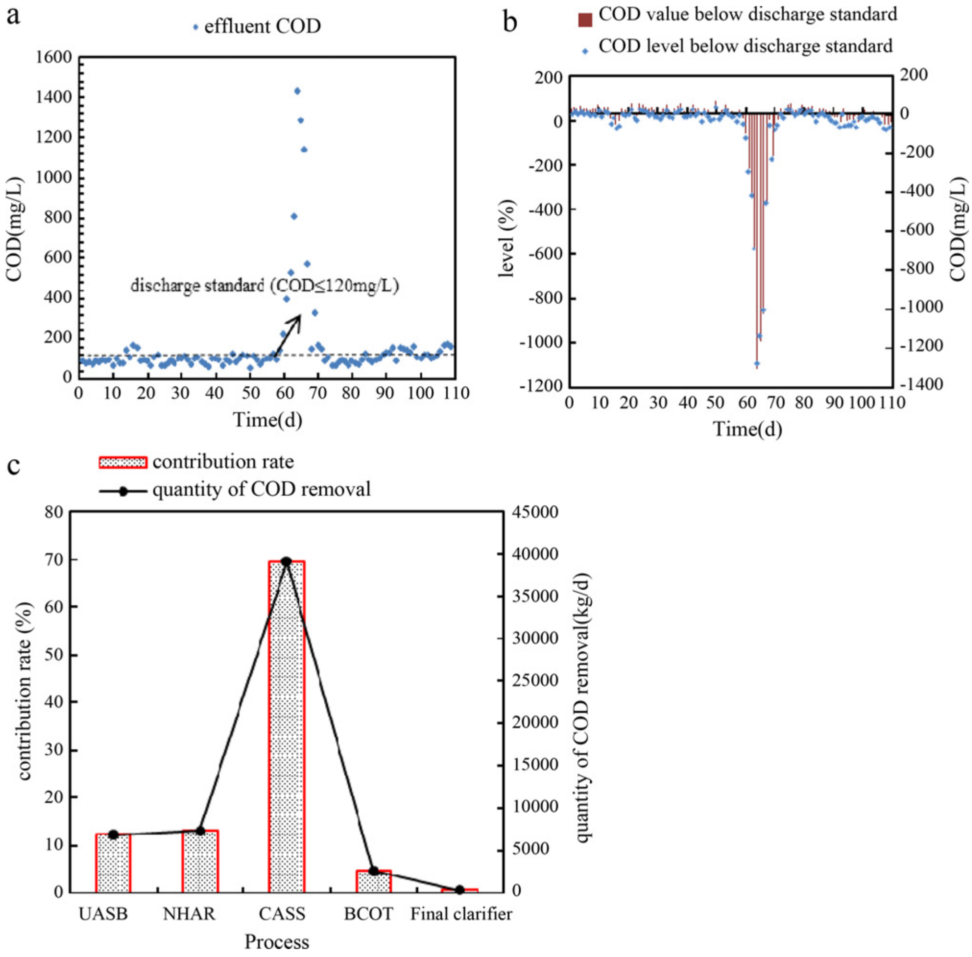

Fig. 4. (a) the state of COD reach discharge standard, (b) COD value and level below discharge standard, (c) removed COD quantity and contribution rate of each process.

was 56.1 mg-amoxicillin L^{-1}, while after treatment by two-stage biological system, amoxicillin concentration was reduced to 3.4 mg-amoxicillin L^{-1}, with an average amoxicillin removal efficiency of 93.9%. As shown in Table 3, the removal rate of amoxicillin and the removed quantity of amoxicillin in the CASS process was very high. The average concentration in CASS decreased from 56.1 to 17.7 mg-amoxicillin L^{-1}, with a removal efficiency of 68.2% and quantity of 428.8 kg-amoxicillin d^{-1}. In the second stage of the aerobic biological system (BCOT), the amoxicillin concentration decreased from an average of 17.7 mg-amoxicillin L^{-1} to 3.4 mg-amoxicillin L^{-1}, with a removal of 80.6% which was the highest of the entire WWTP unit.

Table 4
The concentration of amoxicillin removed by each degradation mechanism in each process of the full-scale treatment plant during the experiment.

	Mechanism	$R_{\text{amoxicillin max}}$ (mg L^{-1})	$R_{\text{amoxicillin ave}}$ (mg L^{-1})	$R_{\text{amoxicillin min}}$ (mg L^{-1})	SD
UASB	Hydro.	6.0	4.8	2.6	0.9
	Biodegrad.	10.3	8.1	5.2	1.2
	Adsorp.	7.4	4.1	2.4	1.1
	Unkno.	5.0	2.3	1.0	0.8
NHAR	Hydro.	5.1	3.1	1.1	1.2
	Biodegrad.	9.8	6.0	2.7	1.2
	Adsorp.	8.2	4.1	2.4	1.7
	Unkno.	4.2	1.4	0.5	1.7
CASS	Hydro.	6.6	4.1	2.5	1.5
	Biodegrad.	31.3	21.9	12.4	8.5
	Adsorp.	15.6	9.9	4.8	4.7
	Unkno.	5.6	3.1	1.0	0.8
BCOT	Hydro.	4.2	2.3	0.5	1.1
	Biodegrad.	12.5	8.2	4.2	2.1
	Adsorp.	5.9	3.0	0.5	1.1
	Unkno.	4.7	1.4	0.5	1.6
Final clarifier	Hydro.	0.4	0.2	0.0	0.1
	Biodegrad.	0.4	0.2	0.1	0.1
	Adsorp.	0.3	0.2	0.0	0.0
	Unkno.	0.6	0.3	0.0	0.2

$R_{\text{amoxicillin max}}$, the maximal removed concentration; $R_{\text{amoxicillin ave}}$, the average removed concentration; $R_{\text{amoxicillin min}}$, the minimal removed concentration; SD, standard deviation; Hydro., hydrolysis; Biodegrad., biodegradation; Adsorb., adsorption; Unkno., unknown mechanism.

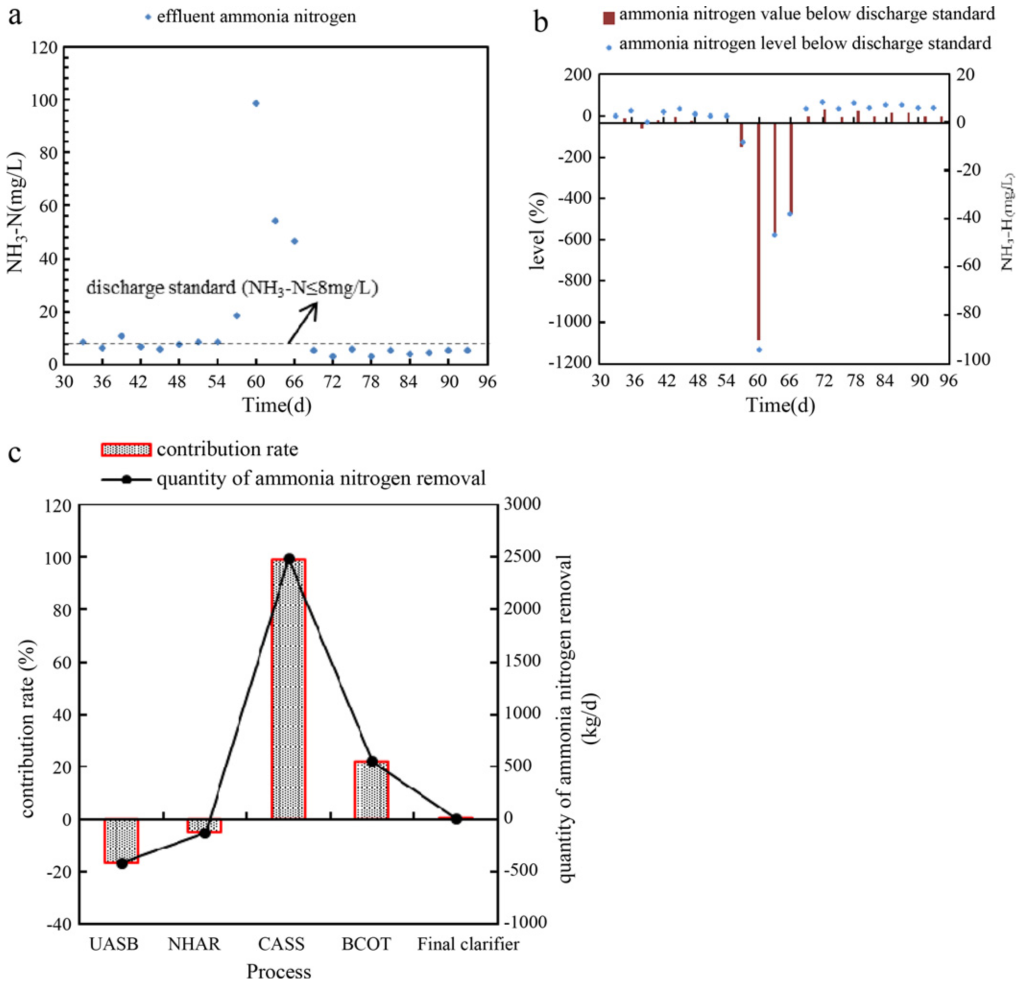

Fig. 5. (a) the state of NH_3-N reach discharge standard, (b) NH_3-N value and level below discharge standard, (c) removed NH_3-N quantity and contribution rate of each process.

It is noteworthy to point that the estimation of operating and maintenance costs has been made regarding for each process used for the treatment of this containing amoxicillin pharmaceutical wastewaters. Table 3 show that UASB and final clarifier have the lower cost than the aerobic biological processes (CASS and BCOT).

It is assumed that amoxicillin loss due to volatilization is negligible because of its low vapor pressure and Henry's constant, and believed that decrease in amoxicillin concentration is due to biodegradation, adsorption, hydrolysis and unknown mechanism [34,35].

Table 4 illustrates the concentration of amoxicillin removed by each degradation mechanism in each process of the full-scale WWTP during the experiment. Fig. 6 reveals the proportion of each mechanism to remove amoxicillin in UASB: 42.2% was contributed by biodegradation; adsorption and hydrolysis contributed 21.3% and 24.8%, respectively; and the proportion of NHAR was approximate to UASB. This result confirms that amoxicillin can be removed in anaerobic and micro-aerobic conditions by biodegradation, adsorption, and hydrolysis.

Clearly from Fig. 6 and Table 4, the data supported the fact that biodegradation, adsorption, hydrolysis of amoxicillin occurred within the two-stage aerobic biological system. The results for the mechanism investigation verify that biodegradation (56.0%, 55.3% in the CASS and BCOT process, respectively), adsorption (25.4%, 20.1% in the CASS and BCOT process, respectively), hydrolysis (10.5%, 13.4% in the CASS and BCOT process, respectively), unknown mechanism, further illuminate that biodegradation, adsorption, hydrolysis are the major mechanisms of amoxicillin removal.

The results demonstrated in Fig. 6 and Table 4 show that biodegradation is the most important mechanism for removing aqueous-phase amoxicillin in the bioreactors. The results also

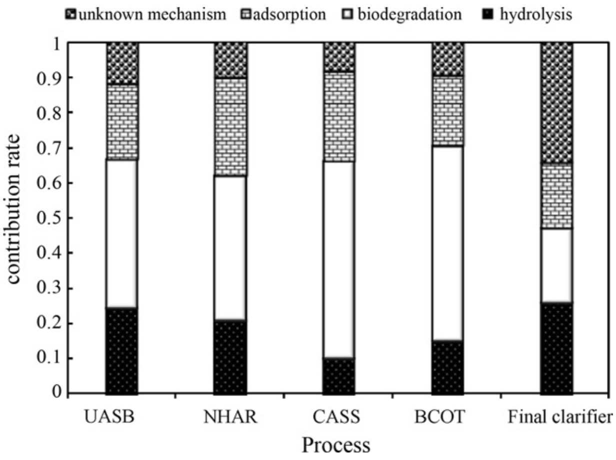

Fig. 6. The removal rate of amoxicillin of each different removal route in UASB, NHAR, CASS, BCOT and final clarifier process.

support the fact that biodegradation, hydrolysis and adsorption were responsible for degradation of amoxicillin.

4. Conclusion

A novel combined process was demonstrated at a full scale for the treatment of amoxicillin pharmaceutical wastewater. The anaerobic/micro-aerobic and aerobic biological system comprised an up-flow anaerobic sludge blanket (UASB), a novel micro-aerobic hydrolysis acidification reactor (NHAR), a cyclic activated sludge system (CASS) and a biological contact oxidation tank (BCOT). It is found that the UASB-NHAR (under the condition of DO from 0.4 to 1.2 mg L^{-1})-CASS-BCOT is one of the most promising technologies that can be employed to treat organic matter in chemical synthesis-based pharmaceutical wastewaters.

The evaluation of WWTP performance by mathematical statistic methods illustrate that the combined process has a well stability. The results for the mechanism investigation verify that the biodegradation, adsorption, hydrolysis and unknown mechanism such as photolysis are able to remove amoxicillin from the pharmaceutical wastewater in the UASB, NHAR, CASS and BCOT and final clarifier. This paper demonstrates that biodegradation is the major mechanism for amoxicillin removal and CASS process removed the most quantity of amoxicillin.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 50908062), the State Key Laboratory of Urban Water Resource and Environment (No. HIT-QAK201103), the Special Fund for Basic Scientific Research of Central Colleges (HEUCP101020) and Harbin innovation Science Foundation for Youths (2008RFQXS023). The authors are grateful to Research Center of Environmental Biotechnology in Harbin Institute of Technology for their technical and logistical assistance.

References

[1] Y.A. Oktem, O. Ince, P. Sallis, T. Donnelly, B.K. Ince, Anaerobic treatment of a chemical synthesis-based pharmaceutical wastewater in a hybrid up-flow anaerobic sludge blanket reactor, Bioresour. Technol. 99 (2008) 1089–1096.

[2] Z. Chen, N. Ren, A. Wang, Z. Zhang, Y. Shi, A novel application of TPAD-MBR system to the pilot treatment of chemical synthesis-based pharmaceutical wastewater, Water Res. 42 (2008) 3385–3392.

[3] J.A. Melero, F. Martínez, J.A. Botas, R. Molina, M.I. Pariente, Heterogeneous catalytic wet peroxide oxidation systems for the treatment of an industrial pharmaceutical wastewater, Water Res. 43 (2009) 4010–4018.

[4] C. Zwiener, F.H. Frimmel, Oxidative treatment of pharmaceuticals in water, Water Res. 34 (2000) 1881–1885.

[5] X.M. Lang, Pharmaceutical wastewater treatment with hydrolysis acidifying-UNITANK-BAF process. Ph.D. Thesis, Northeast University, China (2006) pp. 1–12.

[6] O.V. Enick, M.M. Moore, Assessing the assessments: pharmaceuticals in the environment, Environ. Impact Assess. Rev. 27 (2007) 707–729.

[7] J.H. Cunningham, L. Lin, Fate of amoxicillin in mixed-culture bioreactors and its effects on microbial growth and resistance to silver ions, Environ. Sci. Technol. 44 (2010) 1827–1832.

[8] A.G. Trovó, S.A.S. Melo, R.F.P. Nogueira, Photodegradation of the pharmaceuticals amoxicillin, bezafibrate and paracetamol by the photo-fenton process—application to sewage treatment plant effluent, J. Photochem. Photobiol. A. 198 (2008) 215–220.

[9] A. Wen, T. Hang, S. Chen, Z. Wang, L. Ding, Y. Tian, M. Zhang, X. Xu, Simultaneous determination of amoxicillin and ambroxol in human plasma by LC-MS/MS: validation and application to pharmacokinetic study, J. Pharm. Biomed. Anal. 48 (2008) 829–834.

[10] E.S. Elmolla, M. Chaudhuri, Degradation of amoxicillin, ampicillin and cloxacillin antibiotics in aqueous solution by the UV/ZnO photocatalytic process, J Hazard. Mater. 173 (2010) 445–449.

[11] E.S. Elmolla, M. Chaudhuri, Degradation of the antibiotics amoxicillin, ampicillin and cloxacillin in aqueous solution by the photo-fenton process, J. Hazard. Mater. 172 (2009) 1476–1481.

[12] V. Naddeo, S. Meriç, D. Kassinos, V. Belgiorno, M. Guida, Fate of pharmaceuticals in contaminated urban wastewater effluent under ultrasonic irradiation, Water Res. 43 (2009) 4019–4027.

[13] D. Vogna, R. Marotta, R. Andreozzi, A. Napolitano, M. d'Ischia, Kinetic and chemical assessment of the UV/H_2O_2 treatment of antiepileptic drug carbamazepine, Chemosphere 54 (2004) 497–505.

[14] D. Vogna, R. Marotta, A. Napolitano, R. Andreozzi, M. d'Ischia, Advanced oxidation of the pharmaceutical drug diclofenac with UV/H_2O_2 and ozone, Water Res. 38 (2004) 414–422.

[15] M. Ernst, M. Jekel, Advanced treatment combination for groundwater recharge of municipal wastewater by nanofiltration and ozonation, Water Sci. Technol. 40 (1999) 277–284.

[16] I. Akmehmet Balcıoglu, M. Ötker, Treatment of pharmaceutical wastewater containing antibiotics by O_3 and O_3/H_2O_2 processes, Chemosphere 50 (2003) 85–95.

[17] H. Tekin, O. Bilkay, S.S. Ataberk, T.H. Balta, I.H. Ceribasi, F.D. Sanin, F.B. Dilek, U. Yetis, Use of Fenton oxidation to improve the biodegradability of a pharmaceutical wastewater, J. Hazard. Mater. 136 (2006) 258–265.

[18] S. Chelliapan, T. Wilby, P.J. Sallis, Performance of an up-flow anaerobic stage reactor (UASR) in the treatment of pharmaceutical wastewater containing macrolide antibiotics, Water Res. 40 (2006) 07–516.

[19] A. Enright, S. McHugh, G. Collins, V. O'Flaherty, Low-temperature anaerobic biological treatment of solvent-containing pharmaceutical wastewater, Water Res. 39 (2005) 4587–4596.

[20] P.V. Vadlani, K.B. Ramachandran, Evaluation of UASB reactor performance during start-up operation using synthetic mixed-acid waste, Bioresour. Technol. 99 (2008) 8231–8236.

[21] J.A. Álvarez, I. Ruiz, M. Gómez, J. Presas, M. Soto, Start-up alternatives and performance of an UASB pilot plant treating diluted municipal wastewater at low temperature, Bioresour. Technol. 97 (2006) 1640–1649.

[22] C. Cronin, K.V. Lo, Anaerobic treatment of brewery wastewater using UASB reactors seeded with activated sludge, Bioresour. Technol. 64 (1998) 33–38.

[23] P. Qi, L. Ding, Y. Liu, Treating high strength antibiotic wastewater by microaerobic hydrolysis and acidification process, Environ. Sci. 26 (2005) 106–111.

[24] W. Xiao, Study on coke plant wastewater treatment using anaerobic/anoxic/two-stage aerobic biological filters, China Water & Wastewater 22 (2006) 93–96.

[25] B. Long, H. Liu, G. Xiao, H. Ren, X. Nie, Efficiency analysis of two-stage sbr for phosphorus nitrogen and organic substrate removal, Environ. Sci. 30 (2009) 106–111.

[26] G. Wang, Y. Han, Treatment PTA unit wastewater with pretreatment two-stage aerobic biological process, Petrol. Ref. Eng. 33 (2003) 35–38.

[27] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1995.

[28] S. Satyanarayan, A. Karambe, A.P. Vanerka, Herbal pharmaceutical wastewater treatment by a pilot scale upflow anaerobic sludge blanket (UASB) reactor, Water Sci. Technol. 59 (2009) 2265–2272.

[29] T.M. LaPara, C.H. Nakatsu, L.M. Pantea, J.E. Alleman, Aerobic biological treatment of a pharmaceutical wastewater: effect of temperature on COD removal and bacterial community development, Water Res. 35 (2001) 4417–4425.

[30] S. Bernard, N.F. Gray, Aerobic digestion of pharmaceutical and domestic wastewater sludges at ambient temperature, Water Res. 34 (2000) 725–734.

[31] D.S. Suman Raj, Y. Anjaneyulu, Evaluation of biokinetic parameters for pharmaceutical wastewaters using aerobic oxidation integrated with chemical treatment, Process Biochem. 40 (2005) 165–175.

[32] S. Moosvi, D. Madamwar, An integrated process for the treatment of CETP wastewater using coagulation, anaerobic and aerobic process, Bioresour. Technol. 98 (2007) 3384–3392.

[33] H. Wu, S. Wang, H. Kong, T. Liu, M. Xia, Performance of combined process of anoxic baffled reactor-biological contact oxidation treating printing and dyeing wastewater, Bioresour. Technol. 98 (2007) 1501–1504.

[34] M.P. Patrick, R.W. Lloyd, Patrick O'Keefe, Robert Sheridan, Thomas King, Chia-Yang Chen, Sources of pharmaceutical pollution in the New York city watershed, Sci. Total Environ. 39 (2008) 90–102.

[35] A. Roberto, C. Vincenzo, C. Claudia, C. Marcella de, G. Roberto Lo, M. Raffaele, Z. Ettore, Antibiotics in the environment: occurrence in Italian STPs, fate, and preliminary assessment on algal toxicity of amoxicillin, Environ Sci. Technol. 39 (2004) 6832–6838.