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Pre-treating amoxicillin contained wastewater with an anaerobic expanded granular sludge bed (EGSB)

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ABSTRACT

The characteristic of anaerobic expanded granular sludge bed (EGSB) treating antibiotic wastewater that contained amoxicillin (AMX) was investigated. The performance of the reactor was characterized in terms of its COD, AMX removal efficiencies, and methane yield. After 241 d of operation, 85 and 80% removal efficiencies of COD and AMX were achieved, respectively. Furthermore, volumetric loading rate (VLR) effecting on EGSB system was evaluated by changing hydraulic retention time (HRT was switched to 14, 8, and 14 h, respectively). With HRT decreased to 8 h, COD and AMX removal efficiencies dropped obviously to 36.50 and 58.55%, respectively. When HRT was switched to 14 h again, AMX removal efficiency required three more days than COD to recover to around 80%. These results showed that EGSB reactor could resist the short-time shock of VLR and recover to high removal efficiencies of AMX and COD. According to AMX and COD removal efficiencies and methane yield, it was confirmed that AMX was unlikely to create problems in pre-treating AMX-contained wastewater with EGSB system.

Keywords: Antibiotic wastewater; Amoxicillin; EGSB; Volumetric loading rate; HRT

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1. Introduction

Antibiotic manufacturing industries produce a wide range of products which are used as human and animal medications [1]. The production process of these pharmaceutical products can be divided into three main stages: firstly research and development; secondly the conversion of organic and natural substances into bulk pharmaceutical substances or ingredients through fermentation, extraction, and/or chemical synthesis; finally the formulation and assembly of the final pharmaceutical product [2]. Large volumes of wastewater that streams from all the production processes containing high COD and residual antibiotic will make troubles to biological wastewater treatment processes [3]. Moreover, antibiotic wastewater has the characteristic of large fluctuations in water quality and quantity, especially the fluctuation of residual antibiotic. And bacterial toxicity and recalcitrance caused by antibiotic may also play an important role in decreasing the COD removal efficiency in antibiotic wastewater treating process. In recent years, the impact of the kind and concentration of antibiotics to bio-reactors have been investigated such

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as avilamycin, tetracycline, tylosin, and penicillin [1,4,5]. However, few study the effects of different amoxicillin (AMX) concentrations on long-term run of wastewater treatment bio-reactor.

Meanwhile, research has shown that low concentrations of antibiotic are detectable in municipal wastewater, surface water, and ground water, which may cause potential effects to environment, and even to the health of human beings [6–10]. In fact, the effluent from drug manufactures contains extremely high level of AMX (61–171 mg L^{-1}), although it has been proved AMX can be removed in an anaerobic conditions using UASB reactor [10], its removal efficiency was only about 26.2%. Expanded granular sludge bed (EGSB) bio-reactor, as a modified reactor of the traditional UASB [11], offers a potential solution for toxic substance. Sludge in the system is expanded granule and the effluent recycle is used to increase the upflow velocity and dilute the concentration of toxic in the reactor, therefore, EGSB reactor has large application in industrial wastewater treatment, especially in the treatment of containing toxic substances [12–14].

The aim of this research was to evaluate the impacting of AMX concentration on COD and AMX removal efficiencies in EGSB system. Meanwhile, the feasibility of using EGSB system as a pre-treatment option for treating AMX-contained wastewater was also to determine. In this study, fermented liquid taken from pharmaceutical factory was fed to EGSB, and different concentrations of AMX were introduced into the reactor. Furthermore, the performance of EGSB reactor with different volumetric loading rates (VLRs) by changing the HRT was also investigated.

2. Materials and methods

2.1. EGSB reactor and inoculated sludge

A laboratory-scale EGSB reactor with an internal diameter of 50 mm and height of 750 mm was constructed with Plexiglas for this study and the effective volume of the reactor was 1.47 L, as is showed in Fig. 1. A three-phase separator was installed at the top of reactor to keep the biomass within the reactor and collect gas. A peristaltic pump was used to control the influent rate. Liquid upflow velocity ($V_{\mathrm{up}} = 1.6$ m h^{-1}) was also controlled by inner recirculation with a peristaltic pump. The EGSB reactor was operated under mesophilic condition (35 ± 2°C) by water bath.

The seed sludge was collected from a full-scale municipal wastewater treatment plant (cyclic activated sludge system, CASS) with an average volatile suspended solid (VSS) content of 5.07 g MLVSS L^{-1}. Then, the inoculated sludge was activated with 800 mg L^{-1} glucose for 60 d in an anaerobic condition and an average VSS concentration increased to 15.05 g MLVSS L^{-1}. The sludge in the reactor was not discharged in the whole operation.

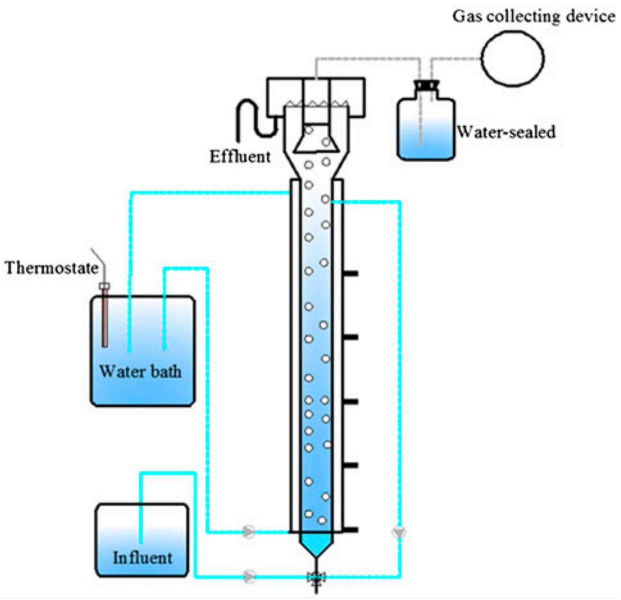

Fig. 1. Schematic diagram of the lab-experimental EGSB reactor.

2.2. Feed and run of the reactor

In order to investigate the removal characteristic of COD and AMX in the EGSB reactor, fermented liquid of antibiotic waste residue taken from pharmaceutical factory of Harbin, China, was stored at 4°C for reactor feed. The fermented liquid mainly contained about 20,000–30,000 mg L^{-1} of soluble COD (no AMX). The EGSB reactor ran for 304 d altogether. From the day 1 to 145, diluted wastewater of fermented liquid was introduced into the reactor by controlling the mean concentration of COD from 2,022.4 to 7,901.1 mg L^{-1} step by step. From the day 146 to 241, AMX was introduced into the system stepwise from the mean concentration 19.7 to 214.7 mg L^{-1} with the HRT of 20 h. Then, from the day 242 to 304, with stable influent concentration of COD and AMX, VLR was changed by controlling HRT. In the whole operation term, the influent pH value maintained at 7 ± 1. Other operational conditions details are summarized in Table 1.

Table 1
Summary of the EGSB operation parameters during whole operation stage

Stage	Operation mode	Mean SCOD concentration (mg L^{-1})	Mean AMX concentration (mg L^{-1})	HRT (h)	SCOD VLR (kg m^{-3} d^{-1})	AMX VLR (kg m^{-3} d^{-1})	Time (d)
1	Acclimation	2,022.4	0	20	2.43	0	1–15
		2,811.5	0	20	3.37	0	16–31
		4,057.8	0	20	4.87	0	32–57
		5,158.2	0	20	6.19	0	58–109
		6,651.9	0	20	7.98	0	110–123
		7,910.7	0	20	9.49	0	124–145
2	AMX effect and removal	7,797.8	19.7	20	9.36	-	146–165
		7,746.4	52.6	20	9.30	-	166–189
		7,901.1	90.4	20	9.46	-	190–215
		8,404.1	214.7	20	10.08	-	216–241
3	The effect of volumetric loading	8,084.7	193.3	14	13.9	0.33	242–270
		8,080.5	181.9	8	24.2	0.55	271–285
		8,057.6	203.6	14	13.8	0.34	286–304

2.3. Sampling and analysis

Analysis of COD was conducted in accordance with standard method for the examination of water and wastewater [15]. The biogas composition was estimated using a gas chromatograph (AGILENT7891A, Shanghai, China) fitted with a hydrogen flame detector and Poropak Q stainless steel column. The oven, injector, and detector temperatures were set as 140, 180, and 180℃, respectively, and nitrogen was used as the carrier gas.

AMX determination was syringe filtered through a 0.45-μm nylon membrane to remove biomass. Samples were stored at 4℃ until analysis. AMX concentration was measured using an HPLC (warers 1,525-2,996-tcm) equipped with a waters sun fire C18 (5 μm × 20 mm × 4.6 mm) guard column and a Jasco ProSar/Dynamax UV detector. The mobile phase was a mixture of ultrapure water, formic acid (0.1%), and methanol (40:30:30 v/v/v) pumped at a flow rate of 0.5 mL min^{-1} through the column. Peaks were monitored by UV absorbance at 254 and 240 nm with a sensitivity of 0.005 AUFS. Quantification of AMX was obtained by comparison with the external standard peak height ratios as a function of concentration. A calibration curve was prepared with five standards (0–100 mg L^{-1}), and the correlation coefficient ($r^2 = 0.999$) and method detection limit 0.277 mg L^{-1}) were determined. Water quality samples were preserved as indicated and analyzed within their holding time.

3. Results and discussion

3.1. Reactor performance

3.1.1. COD and AMX removal

From the day 1 to 145, COD was increased step by step from 2,022.4 to 7,901.1 mg L^{-1} by diluting fermented liquid without AMX. And COD removal results are showed in Fig. 2. When the concentration of COD was increased to 7,901.1 mg L^{-1}, the mean COD removal efficiency was about 88% and methane yield was stable at 0.24 L g^{-1} COD removed as shown in Table 2. Above results indicated that the startup stage of EGSB reactor was completed and microbial community in the system had high activity.

AMX (about 19.7 mg L^{-1}) was introduced into the reactor at the day 146 and the concentration was increased to 217.4 mg L^{-1} gradually. Fig. 3 shows AMX and COD removal results. COD removal efficiency remained around 85% from day 146 to 241. Compared with no AMX situation, the mean COD removal efficiency dropped from 89.16 to 84.70% mg L^{-1} as shown in Table 2 . It seemed that the concentration of AMX had minor influence on COD removal, even though in the high AMX concentration. Therefore, it was speculated that AMX concentration within around 200 mg L^{-1} had slight effect on the activity of microbial populations. But one important thing should be noticed that these results all happened under the condition that AMX concentration was

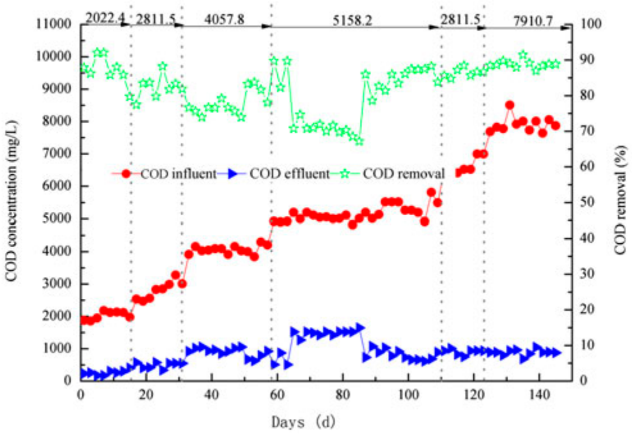

Fig. 2. Performance of the EGSB reactor for the removal of COD during the reactor acclimation 2,022.4, 2,811.5, 4,057.8, 5,158.2, 6,651.9, 7,910.7 (in mg L^{-1}) were on behalf of the average COD concentration in the influent.

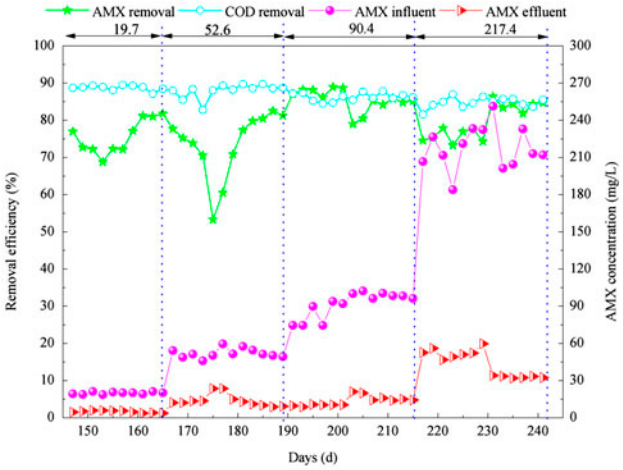

Fig. 3. Performance of the EGSB reactor for the removal of COD and AMX during the AMX increased. 19.4, 52.6, 90.4, 217.4 (in mg L^{-1}) were on behalf of the average AMX concentration in the influent.

increased step by step, and microbial community in the reactor might have adapted to the AMX-contained environment gradually. No matter what, for treating AMX-contained wastewater, it was concluded changing AMX concentration within around 200 mg L^{-1} was not a crucial negative factor to EGSB system.

The AMX removal efficiency was achieved to 80%, even the influent concentration of AMX reached to 214.7 mg L^{-1}. Compared with the UASB process [16], the AMX removal efficiency in EGSB reactor was significantly higher. However, it is worth noting that when the influent concentration of AMX was increased to 52.6 mg L^{-1}, there was a sharp drop of AMX removal to 53.3% then it increased to 80.8% slowly 10 d later. The same phenomenon also appeared at the day 153 (68.77% AMX removal) and 203 (79.01% AMX removal), and then it quickly recovered 8 and 4 d later, respectively. The possible reason was that the backflow of EGSB reactor led to the accumulation of toxic substances (metabolic intermediate) which inhibited the activity of microbial populations contributed to AMX biodegradation at first, whereas microbial population then adapted to the environment few days later, which led the AMX removal recover to previous levels. The phenomenon almost disappeared when the concentration of AMX was increased to 214.7 mg L^{-1}, and only in the beginning, there was a very slightly drop of AMX removal. It seemed that adverse effect was caused by AMX itself. On the whole, the removal of the AMX seemed to be harder and more susceptible than COD in the EGSB reactor.

Taken all together, these results showed that the EGSB consistently achieved typical COD reduction of 85% and AMX reduction of 80% at the stable stage. And AMX concentrations at or below 200 mg L^{-1} had minimal effect on reactor running. Meanwhile, it was inferred that changing of AMX concentration was not a crucial negative factor in treating the antibiotic wastewater that contained AMX.

Table 2
The performance of the laboratory-scale EGSB reactor during the study period

	Start-up	I	II	III	IV	V
Days	1–126	127–145	146–165	166–189	190–215	216–241
Influent AMX concentration (mg L^{-1})	0	0	19.7	52.6	90.4	214.7
Influent COD concentration (mg L^{-1})	1,800–7,500	8,000–8,500	8,000–8,500	8,000–8,500	8,000–8,500	8,000–8,500
Mean COD removal (%)	–	88.93 (1.00)	89.16 (0.51)	87.84 (1.96)	86.42 (1.21)	84.70 (1.37)
Mean methane yield (L g^{-1} COD removed)	–	0.24 (0.013)	0.26 (0.0034)	0.29 (0.024)	0.33 (0.0096)	0.30 (0.0190)

3.1.2. Methane yield

Methane yield was monitored from the day 146 to 241, and these results are showed in Fig. 4. According to the mass balance, methane yield accounting to 0.35 m^3 can be theoretically recovered from the digestion of 1 kg of COD removed [17]. In Fig. 4, it was evident that the greatest methane yield (average 0.33L g^{-1} COD removed) appeared at the term when the mean AMX concentration was increased to 90.4 mg L^{-1}. However, the methane yield dropped from 0.28 L g^{-1} COD removed at the 171 d to 0.25 g^{-1} COD removed at the day 175 d. In accordance with AMX removal efficiency (53.2%) in this period, it was likely that the methanogenic populations were also adversely affected by the accumulation of toxic substances from AMX or metabolic intermediates. With the highest AMX concentration (214.7 mg L^{-1}), methane yield also slightly dropped from the mean value 0.33 (at AMX 90.7 mg L^{-1}) to 0.27 L g^{-1} COD removed at the day 215, then recovered to 0.30 g^{-1} COD removed at the day 227. The above results were speculated that when the concentration of AMX increased to 52.6 mg L^{-1}, AMX produced inhibition for methanogenic population in the EGSB reactor, but the inhibition was slight and reversible. Meanwhile, the results showed that archaea had the very strong resistance to the AMX, even at the highest feed concentration around 200 mg L^{-1}. Previous research also confirmed that methanogens were active at the high Tylosin [1]. In a word, AMX was unlikely to create crucial problems in the treatment of antibiotic wastewater that contained AMX within anaerobic bio-reactor.

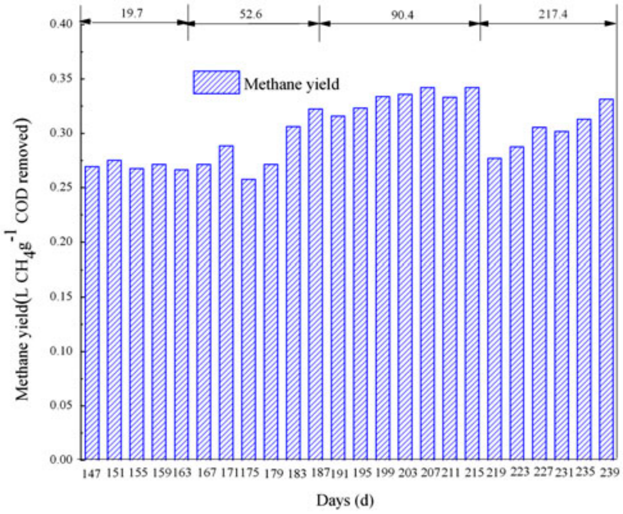

Fig. 4. The methane yield during the AMX increased.

3.2. The effect of VLR on the reactor

3.2.1. The removal of AMX and COD

Antibiotic wastewater has the characteristic of large fluctuations in water quality and quantity. Therefore, it was necessary to evaluate the resisting capacity to the shock of VLR, and provided reference data for the actual operation of antibiotic wastewater treatment.

The removal efficiencies of AMX and COD under different HRT of the reactor are shown in Fig. 5. When the HRT was 14 h, AMX removal efficiency dropped obviously. But it seemed that COD removal efficiency remains stable at around 83%. The result indicated that the high HRT had a disadvantage for removing of AMX, but it had no obvious effect on COD removal. However, when the HRT was decreased to 8 h (VLR to 24.2 kg COD m^{-3} d^{-1}), COD and AMX removal efficiencies decreased obviously to 36.50 and 58.55%, respectively. Running for 16 d, the performance of the EGSB did not improve, and the effluent contained a mass of floc sludge which affected the operation function of the system. Possible reason of poor AMX and COD removal was that huge loss of sludge in the reactor led to the poor performance of reactor, especially for AMX removal, for sludge has certain capacity about adsorption to antibiotic [18]. However, when the HRT was back to 14 h (VLR to 13.8 kg COD m^{-3} d^{-1}), AMX removal efficiency required three more days than COD to recover to about 80%. Thus, these results indicated that there were no permanent inhibitory effects from the short-term shock of VLR (short HRT).

3.2.2. Methane yield

Methane yield in each HRT period with different VLRs is showed in Fig. 6. When HRT decreased to 8 h (VLR to 24.2 kg COD m^{-3} d^{-1}), the methane yield reached to 0.35 L g^{-1} COD removed. This result was just the opposite of AMX and COD removal efficiencies. It showed that changing to low HRT did not affect the activity of methanogenic population in the reactor. It was guessed high upflow velocity could accelerate the transformation in organism which led to higher methane yield in 8 h than in 14 h. When HRT was increased to 14 h (VLR to 13.8 kg COD m^{-3} d^{-1}) again, compared with COD and AMX removal efficiencies, methane yield dropped. There were two main reasons for this phenomenon: (1) the amount of sludge was decreased; (2) low upflow velocity was unfavorable to the transformation in organism. The above results proved that the EGSB reactor had strong

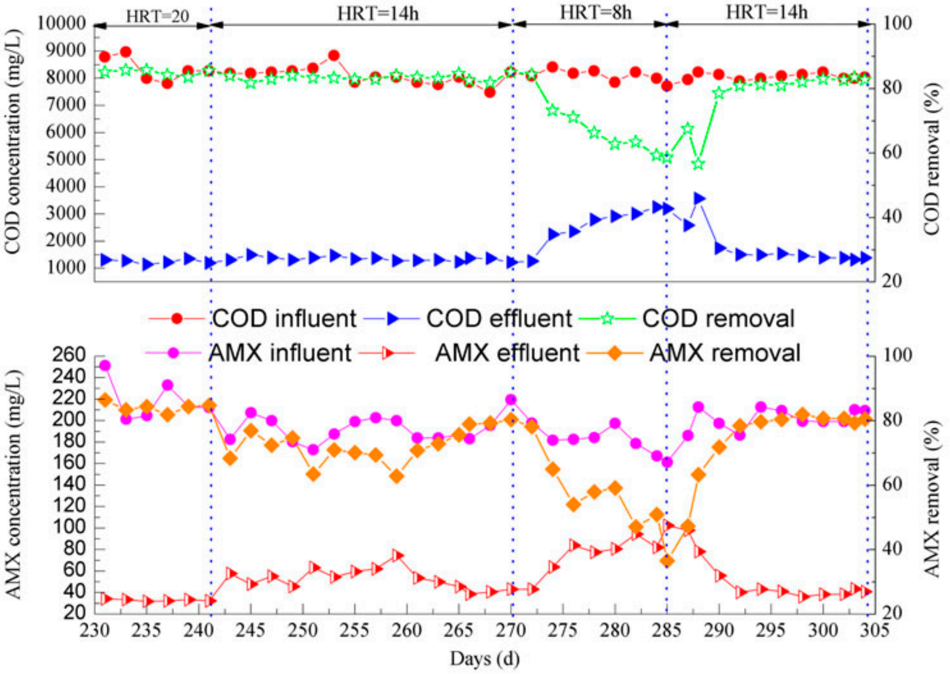

Fig. 5. Performance of the EGSB reactor for the removal of COD and AMX during the shocking loading.

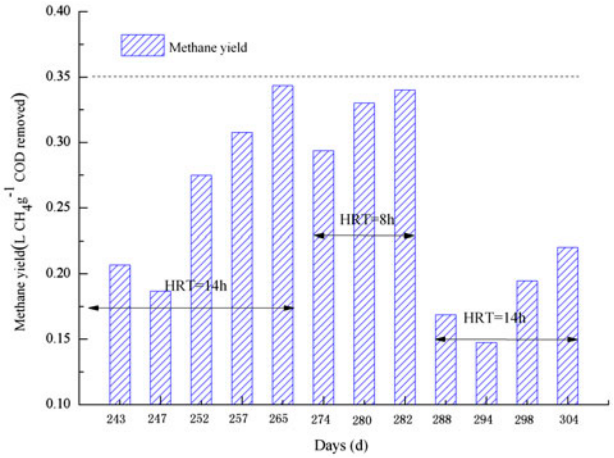

Fig. 6. The methane yield during shocking loading stage of the reactor.

ability to resist shock load, and the EGSB reactor would be reliable as a pre-treatment unit for antibiotic wastewater treating that contained AMX.

4. Conclusions

EGSB reactor consistently achieved typical COD reduction of 85% and AMX reduction of 80% in this research. Furthermore, it was confirmed that AMX around 200 mg L^{-1} was unlikely to create serious problems in treating AMX-contained wastewater with EGSB reactor. And EGSB reactor would be reliable as a pre-treatment unit for treating AMX-contained wastewater. Research on the impact of VLR of the reactor discovered that despite high VLR (HRT is 8 h) will make trouble to the reactor, AMX and COD removal efficiencies can quickly recover to previous levels when VLR switch to adaptive value (HRT is 14 h). Therefore, it is important to control adaptive VLR in running EGSB reactor for treating this kind of wastewater.

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