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Anaerobic biodegradation of wastewater discharged from a chemical synthesis-based pharmaceutical company

Y. Zhang, J.D. Liang*, S. Gao, Y.P. Wang, J.X. Wang

School of Energy and Power Engineering, Xi'an Jiaotong University, Xi'an, 710049, China, Tel. +86 29 82668045, email: zhangyu2011@xjtu.edu.cn (Y. Zhang), Tel. +86 29 82668572, email: jidongl@xjtu.edu.cn (J.D. Liang), 1172236269@qq.com (S. Gao), 393859491@qq.com (Y.P. Wang), 398156453@qq.com (J.X. Wang)

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ABSTRACT

Anaerobic biodegradability of wastewater discharged from a chemical synthesis-based pharmaceutical company was evaluated using two up-flow anaerobic sludge blanket reactors with or without activated carbon addition to the seeding sludge. The wastewater had an anaerobic biodegradability of 86.1% and biogas yield of 52.9%. The addition of activated carbon improved the quality of the granular sludge, resulting in a strong anti-strike performance and high chemical oxygen demand (COD) removal efficiency of around 75% at the organic loading rate of 7 kg COD·m^{-3}·d^{-1}. MiSeq PE300 analysis showed both reactors were dominated by Bacteroidetes, Chloroflexi, Firmicutes, and Proteobacteria. Microbial diversity was reduced in the pharmaceutical wastewater compared to the seeding sludge. The abundance of dominant Levilinea, Desulfovibrio, and Methanosaeta, which are the functional microorganism in treating this chemical synthesis-based pharmaceutical wastewater, significantly increased with the addition of activated carbon.

Keywords: Pharmaceutical wastewater; Up-flow anaerobic sludge blanket; Biodegradability; Bacterial community

1. Introduction

In general, chemical-synthesis-based pharmaceutical wastewater has high chemical oxygen demand (COD), salts and toxicants [1]. Highly efficient wastewater treatment technique is necessary for the treatment of the pharmaceutical wastewater because of the increasingly stringent discharge standards [2,3]. Up-flow anaerobic sludge blanket (UASB) is a potential technique for this purpose since it consumes less energy, needs small space, produces biogas, and removes pathogen [4,5]. For example, a UASB used for diluted pharmaceutical fermentation wastewater treatment increased the organic load rate (OLR) from 2.7 to 7.2 kg COD·m^{-3}·d^{-1} and COD removal efficiency from 83% to 91% after 140 d of continuous operation [6]. In addition, cephalosporin antibiotics wastewater was treated by UASB, COD removal rate was kept around 85% after stable running [7]. UASB could effectively remove 86.2%–91.6% of herbal compounds from pharmaceutical wastewater [8]. Thus, UASB has been widely used in the treatment of wastewater from different pharmaceutical industries.

However, anaerobic degradation of some pharmaceuticals and their derivatives originated from chemical synthesis is difficult [9], since these chemicals can inhibit the microbial activities in wastewater. For instance, phenylamine, the poisonous benzene derivative, sulfur organic compounds (e.g. chlorobenzene imidazole), and sulfur organic compounds (e.g. sulfonamides) are resistant to anaerobic degradation [10,11]. Meanwhile, the existence of abundant ammonium in wastewater could inhibit the growth of methanogenic microbes. The complexity of some pharmaceutical wastewater can lead to ineffective anaerobic degradation. Thus, it is critical to understand anaerobic biodegradation in wastewater and sludge evolution during anaerobic digestion to successfully apply this technology.

*Corresponding author.

The objectives of this study were to evaluate anaerobic digestion of wastewater discharged from a chemical synthesis-based pharmaceutical company. The study first investigated the anaerobic biodegradation of the wastewater using batch tests, and then its continuous treatment by running two UASB reactors with different seeding sludge conditions. Then, physical characteristics of and microbial community present in the granular sludge were analyzed to comprehensively understand the granular evolution.

2. Materials and methods

2.1. Wastewater, device and seed sludge

Effluent from chemical synthesis and equipment cleaning processes was collected from a pharmaceutical chemical synthesis company in Hanzhong city of Shaanxi province, China in October 2016. The wastewater was rich in various organic and inorganic substances such as solvents, reactants, catalysts, and intermediates. The total COD and dissolved COD concentrations were 2772 ± 20 and 2733 ± 12 mg·L^{-1} respectively, indicating that the wastewater COD was mainly composed of dissolved matter. The NH_3-N concentration was 15±1.5 mg·L^{-1}, while the alkalinity was 1250±30 mg·L^{-1}. The initial pH was 4.77±0.3 and was adjusted to neutral before experiments. Before anaerobic reaction, the wastewater was spiked with 1 g $NaHCO_3$/gCOD_{BD} to reduce the adverse effect of organic acid accumulation.

The device for biodegradation experiments is shown in Fig. 1a. The seeding sludge was obtained from a UASB reactor in Guowei Starch Company in Xi'an, China. Before inoculation, the sludge was meshed to size 40 and washed with deionized water. A vertical and cylindrical UASB reactor with an internal diameter of 80 mm, a height of 673 mm and an effective volume of 2.5 L, and made up of polyvinyl chloride was used for the experiments (Fig. 1b). The influent was pumped to the bottom of the reactor using a peristaltic pump. A gas-liquid-solid separator was installed at the top of the reactor.

2.2. Experimental setup and operation

2.2.1. Biodegradability experiments

Experiments were set up in three groups with the substrate of distilled water (blank), acetate acid solution (control) and the wastewater. In each experiment, 1.5±0.1 g VSS·L^{-1} stabilized anaerobic granular sludge and 400 mL substrate were added to a 600 mL serum bottle. Trace elements were added to provide a balanced nutrient supply to ensure the most efficient bacterial growth [12]. The bottle was adjusted to pH 7.0 using sodium bicarbonate, and was sealed with a butyl rubber stopper and an aluminum crimp cap followed by N_2 purging to the culture medium and the headspace for 5 min in order to create an anaerobic condition. Then, the bottle was shaken at 120 rpm using a shaker in a water bath at 35±1°C. During the incubation, biogas was collected in a gas stockpile bottle after passing through a water lock with 3% NaOH solution for the removal of H_2S and CO_2 [13]. The biogas production was measured at a certain interval during the 30 days of incubation [14,15]. Biodegradability (BD %) was calculated as follows:

$$BD\% = 1 - \frac{COD_1 - COD_{VFA}}{COD_0} \quad (1)$$

where COD_0 is the total COD of the original wastewater, while COD_1 and COD_{VFA} are the total COD and the COD of volatile fatty acids (VFA) in the wastewater after the biodegradability experiment, respectively.

2.2.2. Wastewater treatment by UASB

Two UASB reactors (1# and 2#) were used in this study with Reactor 1# containing 1 L the granular sludge, while Reactor 2# containing with 0.94 L granular sludge and 24 g granular activated carbon. Total volatile solids (TVS) concentrations in Reactors 1# and 2# were 35.6 and 33.8 g·L^{-1}, respectively. The two reactors were started with an initial feed of glucose solution. The organic loading rate (OLR) increased gradually from 0.88 to 4.37 kg·m^{-3}·d^{-1} after 15 d. Then, the pharmaceutical wastewater was gradually

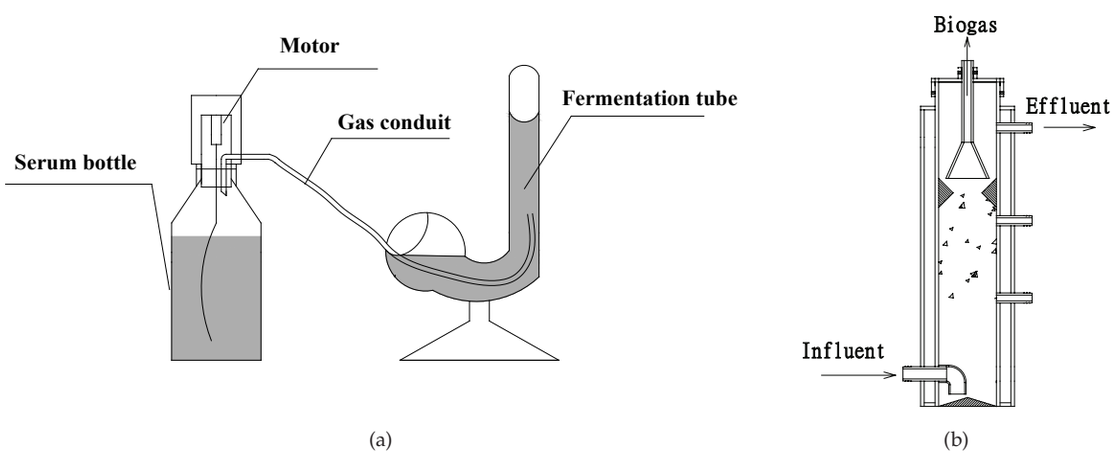


Fig. 1. Schematic diagram of batch experiment and the UASB reactor.

replaced with glucose with the proportion increasing from 10% to 100% in 30 days. During this period, OLR increased from 4.37 to 7.57 kg·m^{-3}·d^{-1}. Thereafter, the reactors were run with only pharmaceutical wastewater for 25 days. During the experimental period, COD and pH were measured every day, whereas alkalinity and VFA were determined every two days, and every few days, respectively.

2.3 Analytical methods

COD_{Cr}, NH_3-N, pH, alkalinity, VFA, total solids (TS) and TVS were analyzed in accordance with the Standard Methods [16]. Settling velocity of anaerobic granular sludge was tested using the gravity sedimentation, while the granular size distribution was measured using a wet sieving method [17]. The surface of granular sludge was observed under a JEOL 7800F scanning electron microscope (SEM).

Seeding sludge and sludge samples were collected on 76 d from two UASB reactors for the analysis of microbial community structures. For DNA extraction, 0.5 g wet sludge pellet was collected and DNA was extracted using the E.Z.N.A each time using D5625-01soil DNA kits (Omega, USA). Genomic DNA was amplified by PCR using primer set 341F and 805R for the V3–V4 region of the 16S rRNAs. Thereafter, the amplicons were pooled and purified with AMPure XP (Beckman Coulter, USA). Finally, a library was constructed and run on a MiSeqIllumina platform (Sangon Biotech, Co., Ltd., Shanghai, China). All the raw reads were archived at NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/blast/).

3. Results and discussion

3.1 Biodegradability and biogas production

According to the biodegradability (BD%) values shown in Table 1, BD% of the control is 98%, suggesting a good bioactivity of the inoculated sludge. BD% of the wastewater is 86.1%, indicating that the wastewater is suitable for anaerobic biodegradation. However, biogas accumulative production for wastewater as substrate was reduced by 28.4% compared with that for acetate acid as substrate (Fig. 2), indicating that the microbial activity was inhibited by some toxicants in the pharmaceutical wastewater. Combination of wastewater toxicants and anaerobic digestion intermediates is toxic to luminescent bacteria [14]. Nevertheless, the biogas yield in this study is in agreement with that in a study on the up-flow anaerobic filter for treatment of chemical synthesis-based pharmaceutical wastewater [18].

Table 1
Biodegradability of pharmaceutical wastewater

Parameter	Acetate acid as substrate (control)	Wastewater as substrate
COD_0 (mg·L^{-1})	2862±30	2733±12
COD_1 (mg·L^{-1})	126±10	779±21
COD_{VFA}	70±15	398±25
BD%	98.0	86.1
Biogas yield (%)	81.3	52.9

3.2. Wastewater treatment by UASB

More than 95% of COD was removed in Reactor 1# in the first 15 days when glucose was influent (Fig. 3a). When the glucose was gradually replaced with pharmaceutical wastewater, OLR gradually increased to 7 kg COD·m^{-3}·d^{-1}, which explains the reason for COD removal efficiency reducing from 95% to 50%.

Under the same conditions as Reactor 1#, over 70% of COD was removed for 100% (w/v) pharmaceutical wastewater as the influent, indicating that activated carbon can improve the resistance of the anaerobic granular sludge (Fig. 3b). Reactor 2# was fed with 100% pharmaceutical wastewater up to an OLR of 9 kg COD m^{-3}·d^{-1} to determine the maximum loading capacity. At the OLR above 8.5 kg COD·m^{-3}·d^{-1}, the COD removal efficiency sharply decreased from 80% to 50%, suggesting that the loading was maximized. Thereafter, OLR returned to 7 kg COD·m^{-3}·d^{-1} and the final COD removal efficiency was maintained around 75%. These findings agree with another study, in which 72% of COD from the chemical synthetic-based pharmaceutical wastewater was removed at OLR of 8 kg COD·m^{-3}·d^{-1} by a UASB reactor [19].

For 100% (w/v) pharmaceutical wastewater, COD removal efficiency varied in both reactors, resulting influctuation of the inflow. In addition, the reactor volume was not adequate to buffer the change in the influent quality.

Fig. 4 shows a steady increase from 7 to 8.5 in pH, from 2200 to 3300 mg·L^{-1} in alkalinity, and from 25 to 210 mg·L^{-1} in VFA as the OLR increases from 1 to 7 kg COD·m^{-3}·d^{-1}. The maximum VFA concentration is about 210 mg·L^{-1} in both reactors, indicating a satisfactory balance between hydrolyzed bacteria, acidogens and methanogens. Yalcin confirmed that the safe VFA concentration in UASB reactors is under 386 mg/L [19].

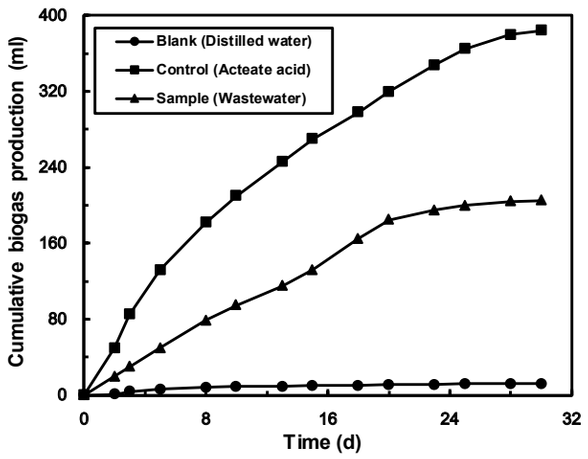
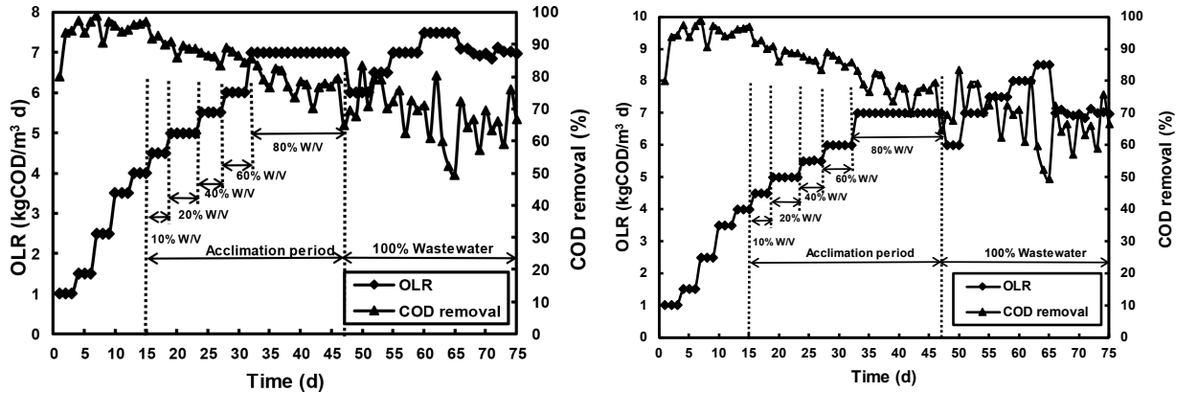
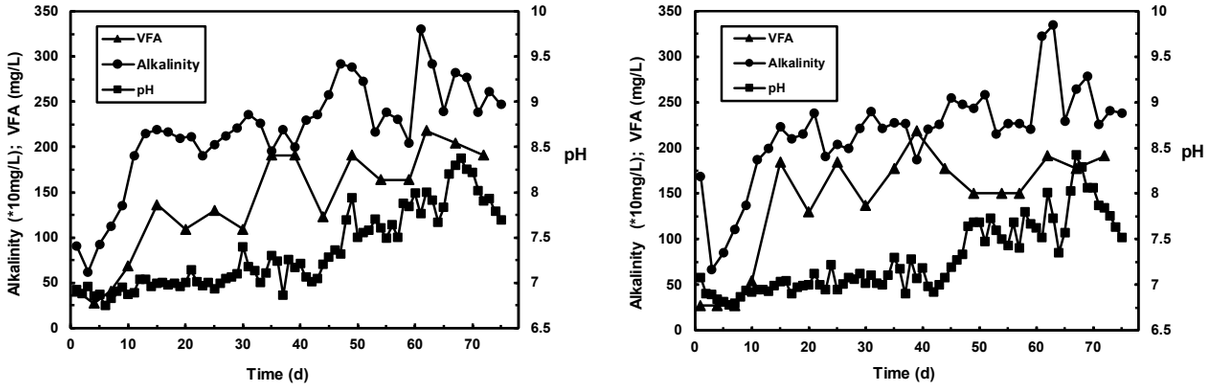


Fig. 2. Cumulative biogas production through the reactor operation.



(a) Reactor 1# (b) Reactor 2#

Fig. 3. COD removal efficiency and OLR for the UASB.



(a) Reactor 1# (b) Reactor 2#

Fig. 4. Variation in alkalinity, VFA and pH in UASB.

3.3 Evaluation of anaerobic granular sludge

Properties of the anaerobic granular sludge before and after incubation in UASB are shown in Table 2. VS/TS was reduced slightly, because the sludge was partially broken down and partially washed out with the effluent when OLR increased [20]. The VS/TS in Reactor 1# was reduced significantly when compared with that in Reactor 2#, indicating that the addition of activated carbon prevented the loss of granular sludge [21]. Despite the decrease in sludge biomass, VS/TS was still above 70%.

Particle size distribution changed slightly in both reactors. The proportion of small particles (<0.9 mm) increased slightly, confirming the breakage of large-size particles into smaller ones. Nevertheless, addition of activated carbon enhanced the resistance against sludge destruction. Reactor 2# contained more large-grain particles (>0.9 mm) with higher settling velocity than Reactor 1#. The settling velocity of granular sludge (>2 mm) in Reactor 2# was up to 84.46 $m \cdot h^{-1}$, suggesting that the granular sludge became denser with closer cells, resulting in good settling performance and effective retention of biomass in the reactor [17].

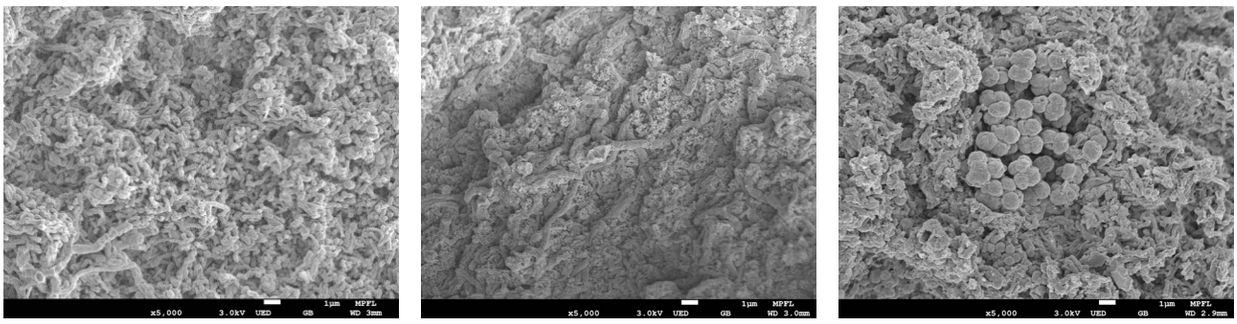
SEM images in Fig. 5 suggest that the bacteria were dispersed as loose aggregates in seeding sludge with diverse species. The rod-shaped bacteria were the dominant group surrounded by a small amount of spherical and filamentous groups. However, the spherical and filamentous species, presumed as methanosarcina and methanothrixsoehngen, gradually became dominant after incubation. Similar results were reported in past studies [22–24]. In particular, the spherical microbial community was apparent in Reactor 2#, indicating that activated carbon assisted the growth of methanosarcina. Furthermore, the granular sludge was densely packed through the interaction among granules, microbes and their induced extracellular polymeric substances [25].

3.4 Community composition in sludge

Miseq PE300 sequencing was used to analyze the bacteria community. A total of 28022–32009 high-quality readings were obtained for these samples. Furthermore, the number of OTUs, the Good's coverage, the Shannon, Chao1, ACE, and Simpson indices for these samples were calculated at cutoff of 3% (Table 3). The Good's coverage of the three samples was above 99%, indicating that the sequence libraries constructed on current studies could mostly cover the microbial communities.

Table 2
Performance of granular sludge

Parameter		Seeding sludge	Reactor 1#	Reactor 2#
VS/TS (%)		89.27	73.52	77.57
Particle size distribution(%)	>2 mm	17.99	18.58	17.84
	0.9~2 mm	27.82	25.66	27.80
	<0.9 mm	54.19	55.76	54.36
Settling velocity ($m \cdot h^{-1}$)	>2 mm	75.54±20	70.81±18	84.46±20
	0.9~2 mm	45.40±14	45.96±13	46.19±15



(a) ×5000 (b) ×5000 (c) ×5000

Fig. 5.SEM images of (a) seeding sludge and (b,c) sludge incubated in Reactors 1# and 2#, respectively.

Table 3
Sequencing data for the three samples

Sample	Seq-num	OTUs	Shannon	ACE	Chao1	Coverage	Simpson
Seeding sludge	28022	508	4.19	914	902	0.9936	0.034
Reactor1#	30622	443	3.96	779	680	0.9953	0.051
Reactor2#	32009	425	3.93	720	667	0.9958	0.058

Rarefaction curves were commonly used to compare species diversity in the ecosystem [26]. From rarefaction analysis, it was hypothesized that new bacterial phylotypes continued to emerge after 28000 sequences in the seeding sludge. Similarly, new bacterial phylotypes also emerged when sequences exceeded 32000 in Reactor 2#, suggesting that the sequencing depths for bacteria were enough to cover the whole diversity. Table 3 shows the change in biodiversity in the sludge after inoculation. Compared to the seeding sludge, the biodiversity in pharmaceutical wastewater was decreased, indicating that some microorganisms were inhibited, even disappeared.

A total of 22 bacterial phyla and one archaeal phylum were identified in the samples (Fig. 6), while Chloroflexi, Proteobacteria, Bacteroidetes and Firmicutes were identified as the four major phyla, accounting for 77–86% of the total effective bacterial sequences. However, the abundance distribution of these dominant phyla shifted after wastewater treatment.

In the seeding sludge, Bacteroidetes (31.09%) was the most abundant phylum, though other phyla including Proteobacteria (16.94%), Chloroflexi (15.96%) and Firmicutes (13.1%) were also abundant. Compared to the seeding sludge, Firmicutes (26.02%) and Bacteroidetes (25.57%) were dominant in Reactor 1#, which agrees with the past studies, where Firmicutes and Bacteroidetes were predominant in the anaerobic treatment of pharmaceutical wastewater. Bacteroidetes were also reported as important heterotrophs involved in the cycling of organic carbon, and proteinaceous substances [27]. Proteobacteria percentage (20.94%) increased in Reactor 1#, while Chloroflexi (13.35%) and Bacteroidetes (25.57%) decreased. Some phyla such as aminicenantes, and ignacibacteriae were weak and eliminated during the treatment of pharmaceutical wastewater, while the microbial species were relatively homogeneous, resulting in biodiversity reduction. This result confirms that some microbial activity was inhibited by toxicants in the pharmaceutical wastewater, leading to 28.4% reduction of biogas accumulation compared with acetate acid as substrate (Fig. 2).

In Reactor 2#, when OLR increased to 7.0 $kg \cdot m^{-3} \cdot d^{-1}$, the abundance of Chloroflexi and Firmicutes increased to 26.27% and 22.32%, respectively. Firmicutes, which are known as extremely resistant microorganisms and produces

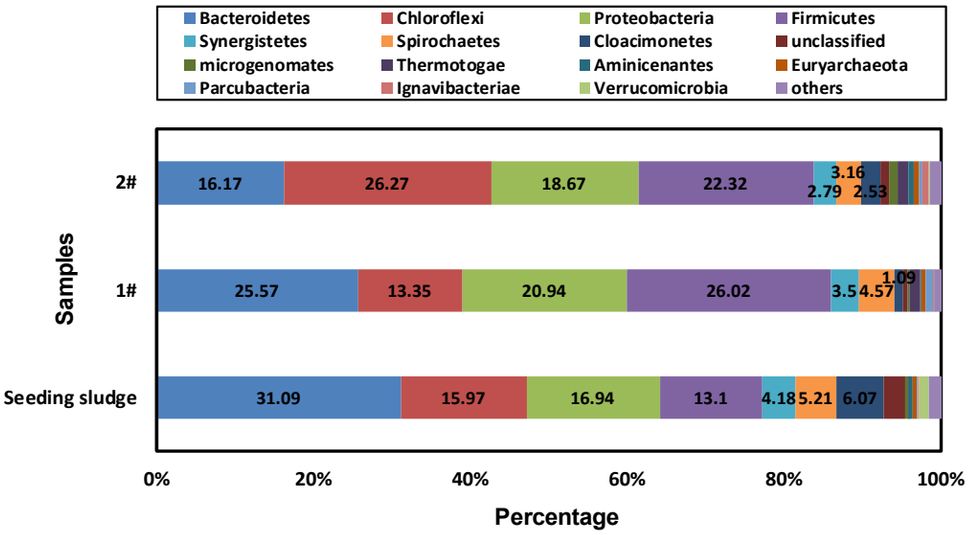


Fig. 6. Bacterial community compositions at the phylum level.

methanogenic precursors, were predominant in both reactors. Previous studies found these microorganisms could degrade organic compounds, for example, it can oxidize ethanol to acetic acid and further to CO_2 and H_2O [28–30]. Hence, Firmicutes could have well adapted to the higher OLR and contributed to the better removal of COD. Compared to Reactor 1#, Chloroflexi became the major phylum in Reactor 2#, and it was an important microorganism in the anaerobic sludge [31]. Chloroflexi was found relatively more abundant in this study compared to previous studies [32,33], which could be because the seeding sludge that was added with activated carbon supported the growth of Chloroflexi populations.

The abundance of Proteobacteria remained almost unchanged, despite being considerably higher. Proteobacteria was also reported as abundant in the active sludge in municipal water treatment plant [34]. The abundance of Cloacimonetes decreased significantly in both reactors, indicating that Cloacimonetes was sensitive to the inhibition of pharmaceutical wastewater. Although Euryarchaeota only accounted for 0.52% in Reactor 1# and 0.71% in Reactor 2#, its abundance increased to 39% in Reactor 2# though not in Reactor 1#, compared to seeding sludge. This result agrees with SEM images of sludge in Reactor 2#, since activated carbon assisted the growth of methanosarcina (e.g. Methanosaeta). This is similar to the domino effect, and low abundance of Euryarchaeota could be a key functional microorganism in anaerobic digestion of complicated contaminants [35].

Therefore, it can be concluded that Bacteroidetes, Chloroflexi, Firmicute, Proteobacteria and Euryarchaeota played important roles in anaerobic degradation of pharmaceutical pollutants. However, the higher distribution of Chloroflexi and Euryarchaeota contributed to the higher COD removal efficiency of around 75% in Reactor 2# than in Reactor 1# (around 50%), when OLR was 7 kg COD·m^{-3}·d^{-1} for the feed of 100% (w/v) pharmaceutical wastewater (Fig. 4). Activated carbon added in Reactor 2# provided a good habitat to these functional microbes, contributing to the generation and stabilization of granular sludge. Therefore, Reactor 2# exhibited a better performance in treating the pharmaceutical wastewater.

Fig. 7 shows the genus-level distribution of bacteria that can explain the performances of UASB reactors. Acetobacterium was the functional bacteria in the anaerobic process, as its abundance significantly increased by 15.94% in Reactor 1# and 11.12% in Reactor 2#, which was not detected in seeding sludge. In conclusion, Acetobacterium is a good indicator of the active role of Firmicutes in UASB reactors.

Anaerolineae of Chloroflexi is 'semi-syntrophic', degraded carbohydrate cooperatively with hydrogenotrophic methanogens. Anaerolinea (e.g. Levilinea) could metabolize primary substrates such as carbohydrates and organochlorine compounds in pharmaceutical wastewater. Some of them may be heterotrophs decomposing organic matter from cells such as amino acids [36].

In addition, the abundances of Meniscus in reactor 1# (0.47%) and reactor 2# (0.86%) apparently decreased from the original 10.16%, indicating their inhibition by the toxicant in pharmaceutical wastewater. Moreover, Prolixibacter and Robinsoniella were highly represented in reactor 2# (6.28% and 1.86%), but almost undetected in the seeding sludge. Thus, the addition of activated carbon can promote the growth of some functional bacteria, which led to a higher COD removal efficiency in Reactor 2#. Alkaliflexus was more abundant in Reactor 1# than Reactor 2#, because Alkaliflexus was in competition with other microbes, reducing them in counts.

4.Conclusions

The addition of activated carbon effectively improved the performance of UASB in the treatment of chemical synthesis-based pharmaceutical wastewater. A COD removal efficiency of 75%was achieved for the organic loading rate of 7 kg COD·m^{-3}·d^{-1}. Additionally, activated carbon improved the anti-strike performance of the granular sludge, effec-

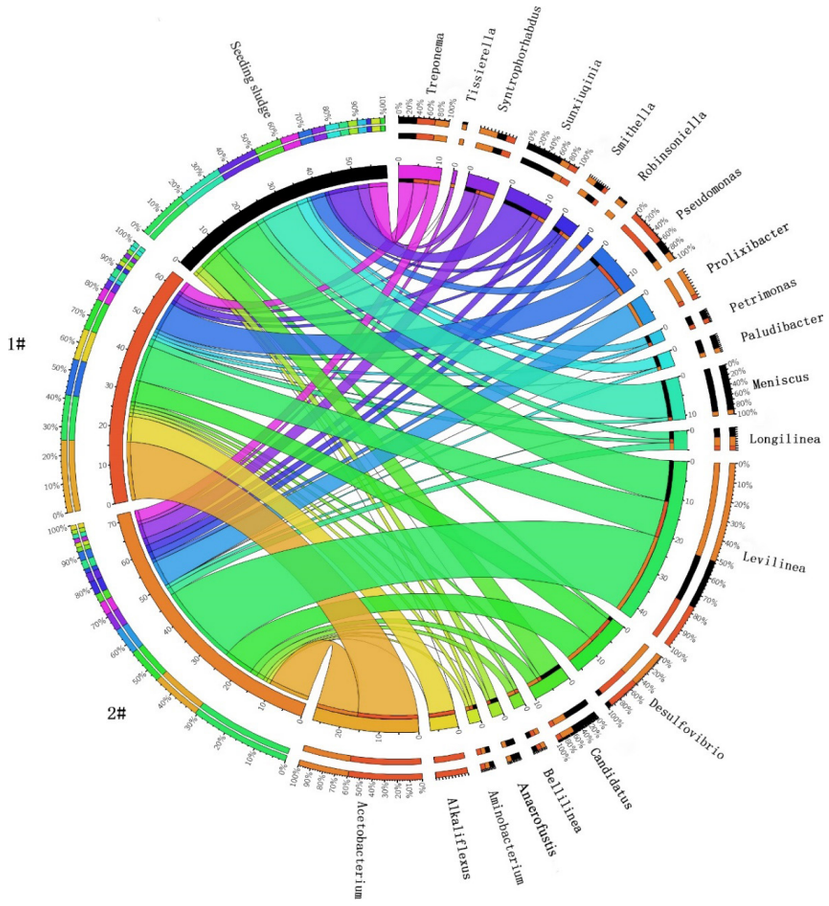


Fig. 7. Genus distribution in three samples based on taxonomy annotation. The thickness of each ribbon represents the abundance of each taxon. The absolute tick above the inner segment and relative tick above the outer segment stand for the read abundance and relative abundance of each taxon, respectively.

tively retained the biomass, and improved COD removal efficiency. Compared to the seeding sludge, the microbes became less diverse and even disappeared in pharmaceutical wastewater. At the phylum level, the four major groups in the sludge were Bacteroidetes, Chloroflexi, Firmicutes, and Proteobacteria. The differences in diversity could be attributed to different cultivation processes. Firmicutes and Bacteroidetes were more suitable for growth in pharmaceutical wastewater, while Chloroflexi and Euryarchaeota could be favored with the addition of activated carbon. Genus level analysis showed Acetobacterium, levilinea and pseudomonas were dominant in the sludge cultivated from pharmaceutical wastewater. Levilinea, Desulfovibrio, and Methanosaeta,as the main functional microorganisms for pharmaceutical wastewater degradation, are likely promoted by the addition of activated carbon.

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