Comparison of three types of anaerobic granular sludge for treating pharmaceutical wastewater

Yibo Wang, Minquan Feng, Yonghong Liu, Yaozhong Li and Bofei Zhang

ABSTRACT

Three types of anaerobic granular sludge for treating chemical synthesis-based pharmaceutical wastewater were compared: (1) an up-flow anaerobic sludge blanket (UASB) filled with polyvinyl alcohol (PVA) gel beads (UASB-PVA); (2) a UASB filled with traditional anaerobic granular sludge; and (3) a UASB filled with traditional anaerobic granular sludge and granular active carbon (UASB-GAC). The domestication times for the UASB-PVA, UASB, and UASB-GAC reactors were 30, 47, and 47 days, respectively. When the organic loading rate (OLR) was up to 7 kg chemical oxygen demand (COD)/(m^3·d), the COD efficiency of the UASB-PVA, UASB, and UASB-GAC stayed in the range of 69% to 75% (deviation 1.8%), 46% to 69% (deviation 8.6%), and 61% to 73% (deviation 4.0%), respectively. The highest OLRs reached for the UASB-PVA, UASB, and UASB-GAC were 12, 7, and 8 kg COD/(m^3·d), respectively. The performance of the UASB-PVA was the best of the three, the UASB-GAC was second, and the UASB was the worst. High-throughput pyrosequencing analysis showed that *Levilinea*, *Syntrophorhabdus*, *Desulfovibrio* and *Acetobacterium* were the dominant bacteria in the UASB-PVA, UASB, and UASB-GAC reactors' granular sludge. The abundance and diversity of the microbial community in the UASB-PVA sludge were higher than for the UASB and UASB-GAC granular sludge.

Key words | GAC, high-throughput sequencing, pharmaceutical wastewater PVA-gel bead, UASB reactor

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INTRODUCTION

Pharmaceutical wastewater has a complex composition with a high salt content and high concentrations of many types of organic matter. It is highly toxic and has poor biodegradability, so it poses a great threat to large bodies of water and human health. Pharmaceutical wastewater is also considered to be difficult to treat. The anaerobic biological process for treatment process of wastewater with high concentrations of organic waste has several advantages, such as low cost, low sludge production, and high OLR rate (Shi *et al.* 2014), so this process is widely used in pharmaceutical wastewater treatment (Li *et al.* 2015).

According to previous studies, the formation and the maintenance of granular sludge are key factors for the stable operation of up-flow anaerobic sludge blanket (UASB) reactors, expanded granular sludge bed (EGSB) reactors, internal circulation reactors, and other similar reactors. The sludge granulation process is controlled by many factors, and the process is very slow (Abbasi & Abbasi 2012), especially for pharmaceutical wastewater with biological toxicity and inhibition, so it is more difficult to obtain mature granular sludge. Therefore, anaerobic reactors are difficult to successfully start-up and the time of start-up is too long. It is import to solve these problems.

doi: 10.2166/wrd.2017.040

Yu *et al.* (1999) added 0.4 mm granular carbon (GAC) to a UASB reactor. Their results showed that the granulation time was reduced by 35 days, and 10% of the granules were larger than 4.0 mm at the end of the process. Wang *et al.* (2011) added GAC (0.4–0.5 mm) into a UASB reactor used to treat brewery wastewater, and their results showed that the granulation time was reduced to 64 days. When the OLR reached 15.0 kg chemical oxygen demand (COD)/(m^3·d), the COD removal efficiency was up to 90%. Xu *et al.* (2015) found that GAC can enhance biogas production and methane content, shorten the start-up time, and enhance the ability of microbes to resist organic loading shock. Lee *et al.* (2016) found that UASB supplemented with GAC (1%) showed a 1.8 times higher methane production rate than without GAC, and *Geobacter, Methanospirllum*, and *Methanolinea* were enriched on the surface of the GAC. This shows that adding GAC improves sludge granulation.

Recently, some researchers have explored adding polyvinyl alcohol (PVA) gel beads to bioreactors as granular sludge, and they have achieved excellent results. For example, Hoa *et al.* (2006) treated synthetic wastewater with a fluidized-bed reactor with PVA-gel beads, and the nitrogen removal efficiency was up to 83%. Rouse *et al.* (2007) treated municipal wastewater with a moving-bed biofilm reactor filled with PVA-gel beads, and the COD and 5-day biochemical oxygen demand (BOD_5) removal efficiencies were greater than 90%. Zhang *et al.* (2009) treated high-concentration corn steep liquor with an anaerobic fluidized-bed reactor filled with PVA-gel beads, and a COD removal efficiency of 90% was achieved. Khanh *et al.* (2011) treated aquaculture wastewater with an EGSB reactor, and COD and total suspended solids (TSS) removal efficiencies were greater than 92%.

Researchers have done a great deal of research work with UASB-PVA, UASB, and UASB-GAC granular sludge, but the relative advantages and disadvantages of the three types of granular sludge are not clear for the treatment of pharmaceutical wastewater. Therefore, in order to provide a reference for related technology and engineering applications, we tested the three types of granular sludge needed to start-up and UASB reactors. We compared the advantages and disadvantages of the three types of granular sludge with respect to microorganisms and performance.

MATERIALS AND METHODS

Materials

Inoculation granular sludge was taken from a starch factory in Shaanxi Province, China. The granular sludge had a density of 1.01 g/cm^3, a volatile suspended solids to total suspended solids (VSS/TSS) ratio of 89.27%, and a mixed liquor suspended solids concentration of 12.7 g/L.

The PVA-gel beads were provided by Kuraray Co., Ltd, Japan. Each bead was spherical with a network of 10 to 20 micron pores that allow cultivation of bacteria in a sheltered environment and thus reduce sloughing of the biomass. The beads had a diameter of 2 to 3 mm and a density of 1.03 g/cm^3. As the beads are only slightly more dense than water, they are easily fluidized (Hoa *et al.* 2006; Zhang *et al.* 2009; Gani *et al.* 2016).

At the reactor start-up stage, glucose was used as the carbon source in the simulated wastewater. Ammonium chloride (NH_4Cl) was the nitrogen source, potassium dihydrogen phosphate (KH_2PO_4) was the phosphorus source, and sodium hydrogen carbonate ($NaHCO_3$) was added to maintain alkalinity at about 2,000 mg $CaCO_3$/L. The ratio of COD:N:P was approximately 200:5:1, and 1 mL nutrition solution was added to 1 L of wastewater, the nutrition solution containing beef extract 0.6 g/L, yeast extract, tryptone 1.8 g/L, KH_2PO_4 7.54 g/L, and $MgSO_4$ 0.22 g/L (Xu *et al.* 2017).

Actual pharmaceutical wastewater was collected from an outlet of an iron carbon micro-electrolysis device in a chemical synthesis pharmaceutical factory (Shaanxi, China). The wastewater contained a variety of substances, including toluene, methanol, formic acid, acetone, and sodium chloride. In the factory, the wastewater was treated by oil separation, sedimentation, regulation tank, iron carbon micro-electrolysis, air flotation, secondary sedimentation, anaerobic-aerobic-flocculation precipitation, and oxidation disinfection.

Table 1 shows the characteristics of the wastewater.

Table 1 | The characteristics of the wastewater

pH	COD (mg/L)	NH_4^+-N (mg/L)	BOD_5 (mg/L)	SO_4^{2-} (mg/L)
4.8–10	1,400–6,200	10–80	800–2,400	9–12

Experimental device and method

Three identical UASB reactors with an effective volume of 2.5 L, a height of 67.3 cm, and an inner diameter of 8 cm were used in this study. The temperature in each UASB reactor was kept at 37 ± 2°C. The wastewater entered the reactor at the bottom of each UASB reactor and overflowed at the top.

Following the work of Zhang *et al.* (2007) on UASB-PVA reactors with inoculated PVA-gel beads and inoculation granular sludge, 1 L of PVA-gel beads was placed in the UASB-PVA reactor and thoroughly mixed to prepare an inoculation granular sludge concentration of 1 L. Simulated wastewater containing glucose was intermittently pumped into the reactor, and the effluent was recycled. After 17 training days, embryonic PVA granules sludge was obtained, the train process was stopped, and all of the inoculation granular sludge was poured out, leaving only embryonic PVA granules sludge in the UASB-PVA reactor. Most of the PVA-gel beads changed from the original white to gray. A UASB reactor inoculated 1 L of inoculation granular sludge, and the procedure was the same as UASB-PCA. The same procedure was used for a UASB-GAC reactor that contained 100 mL GAC (0.4 mm) that inoculated 1 L of inoculation granular sludge. In the early start-up phase for all of these reactors, the OLR was 1 kg COD/(m^3·d).

The harmful components in the pharmaceutical wastewater can inhibit microbial activity, so in order to reduce the inhibitory effect, in the early stage of the process, the influent flow to the UASB reactors was a mixed liquid (simulated wastewater containing glucose and actual pharmaceutical wastewater) that was pumped into the UASB reactor. The proportion of pharmaceutical wastewater in the mixed liquid was gradually increased from 10% to 20% to 40% to 60% to 80% to 100% pharmaceutical wastewater. The influent flow pH was adjusted to between 7 and 8, and the nutrition solution was added into the influent.

Analytical methods

Wastewater quality and reactor operation parameters

COD was measured by the potassium dichromate method, NH_4^+-N concentration was determined by Nessler's reagent spectrophotometry, and alkalinity levels were determined by titration, density of the sludge, the VSS (volatile suspended solids), TSS, and suspended solids were measured by standard methods (State Environmental Protection Administration of China 2002). Volatile fatty acids (VFAs) were determined by high performance liquid chromatography using a Merck-Hitachi chromatographer equipped with a UV (ultraviolet) detector and an Aminex HPX-87H pre-column and a column from BioRad (USA). Sedimentation velocity of the granular sludge was measured by the gravity sedimentation method, the particle size distribution of the granular sludge was measured by wet sieving method (Laguna *et al.* 1999), and PVA gel size was estimated by vernier caliper. Microbial morphology was observed with a VEGA II XMU scanning electron microscopic (TESCAN Europe). In order to evaluate the quality of the PVA granular sludge, the biomass attachment to the PVA gel needed to be measured. This parameter was determined by the wet weight difference from an average of 30 pairs of new (unused) and granulated PVA-gel beads (Zhang *et al.* 2011).

High-throughput sequencing

DNA was extracted from sludge samples and purified using an E.Z.N.A.® Soil DNA Kit (D5625-01, Omega, USA). PCR (polymerase chain reaction) primer was 341F/805R (Jia *et al.* 2015), (CCTACACGACGCTCTTCCGATCTN (barcode) CCTACGGGNGGCWGCAG) and (GACTGGAGTT CCTTGGCACCCGAGAATTCCAGACTACHVGGGTATC TAATCC).

DNA samples were sent to Tiny Gene Bio-Tech (Shanghai) Co. Ltd, who carried out the high-throughput sequencing (Roche 454 GS FLX sequencer) of the 16S rRNA V4 region. After comparing the optimization sequence with the ribosome sequence of aligned (16S/18S) small sub-units in the SILVA 119 database, an operational taxonomic unit cluster was determined. The bacteria abundance index (chao 1, ACE) and the diversity index (Shannon index) were computed using mothur software (http://mothur.org/).

RESULTS AND DISCUSSION

Performance comparison of the three UASB reactors

The three UASB reactors ran for 73 days, and there were three phases: the sludge domesticated phase, the OLR increase phase, and the stable operation phase. During the start-up period, a necessary condition for the load rate increase is that the COD removal efficiency was greater than 70% for 2 days, after which the load rate was increased. Figure 1 shows the performance of the three UASB reactors.

The running conditions for the UASB-PVA reactor

Figure 1 shows that from the first day to the 7th day, the COD removal efficiency of the UASB-PVA reactor was in the range of 36% to 70%. On the 10th day, pharmaceutical wastewater accounted for 10% in the influent flow, the OLR was 1.2 kg COD/(m^3·d), the hydraulic retention time (HRT) was 19 h, the influent COD was 1,600 mg/L, and the effluent COD declined to below 200 mg/L. From the 10th day to the 30th day, as the pharmaceutical wastewater proportion and OLR increased, the COD removal efficiency fluctuated slightly, but the efficiency stayed above 80%. On the 31st day, when the influent flow was 100% pharmaceutical wastewater, the domestication of the granular sludge was complete. From the 31st day to the 43rd day, the OLR gradually increased from 5 kg COD/(m^3·d) to 7 kg COD/(m^3·d), the influent COD ranged from 1,402 mg/L to 6,012 mg/L, and the HRT stayed in the range of 6 h to 23 h. From the 43rd day to the 52nd day, the OLR was stable at around 7 kg COD/(m^3·d), the HRT was in the range of 8.5 h to 17 h, the influent COD ranged from 2,538 mg/L to 5,210 mg/L, and the effluent COD was basically stable in the range of 888 mg/L to 1,386 mg/L. The COD removal efficiency remained in the range of 64% to 77%, but it fluctuated slightly as the influent flow quality changed. From the 53rd day to the 60th day, the ability to resist the loading shock was evaluated. The OLR fluctuated significantly in the range of 5–12 kg COD/(m^3·d), and the COD removal efficiency was around 70%, so the UASB-PVA reactor exhibited a strong ability to resist loading shock. From the 60th day to the 73rd day, OLR was set at around 7 kg COD/(m^3·d), the HRT ranged from 12 h to 15 h, and the COD removal efficiency was stable at around 70%.

The running conditions for the UASB reactor

The start-up methods for the three UASB reactors were similar. As shown in Figure 1, the COD removal efficiency of the UASB reactor reached above 80% during the early start-up phase. On the 47th day, the influent flow was 100% pharmaceutical wastewater, the OLR was 7 kg COD/(m^3·d), and the domestication of the granular sludge was completed. On the 47th day, the COD removal efficiency declined to 45%, because the proportion of the pharmaceutical wastewater in the mixed liquid had increased from 80% to 100%. On the 48th day, the influent flow was reduced, and the OLR decreased to 6 kg COD/(m^3·d). From the 48th day to the 51st day, the OLR remained at 6 kg COD/(m^3·d), and the COD removal efficiency remained in the range of 61% to 75%. From the 52nd day to the 55th day, the OLR increased to 6.5 kg COD/(m^3·d), and the COD removal efficiency remained in the range of 53% to 70%. From the 56th day to the 59th day, the OLR increased to 7 kg COD/(m^3·d), and the COD removal efficiency was around 70%. From the 60th day to the 65th day, the OLR increased to 7.5 kg COD/(m^3·d), and the COD removal efficiency remained in the range of 27% to 64%. There were large fluctuations during this time period, therefore the highest OLR of the UASB reactor was 7 kg COD/(m^3·d). From the 66th day to the 73th day, the OLR declined to 7 kg COD/(m^3·d), and the COD removal efficiency remained in the range of 46% to 69%. The UASB reactor could be stably run at this OLR, the influent COD ranged from 3,038 mg/L to 3,540 mg/L, and the effluent COD declined to 1,002 mg/L to 1,742 mg/L.

Oktem *et al.* (2008) studied the UASB reactor treatment of chemical synthesis pharmaceutical wastewater. The UASB reactor was seeded with a granular sludge taken from an alcohol factory. The highest OLR was 8 kg COD/(m^3·d), and the COD removal efficiency was up to 72%. Our running conditions of the UASB reactor were similar to this previous study.

The running conditions for the UASB-GAC reactor

Figure 1 shows that the COD removal efficiency of the UASB-GAC reactor reached above 80% from the 1st to the 33rd day.

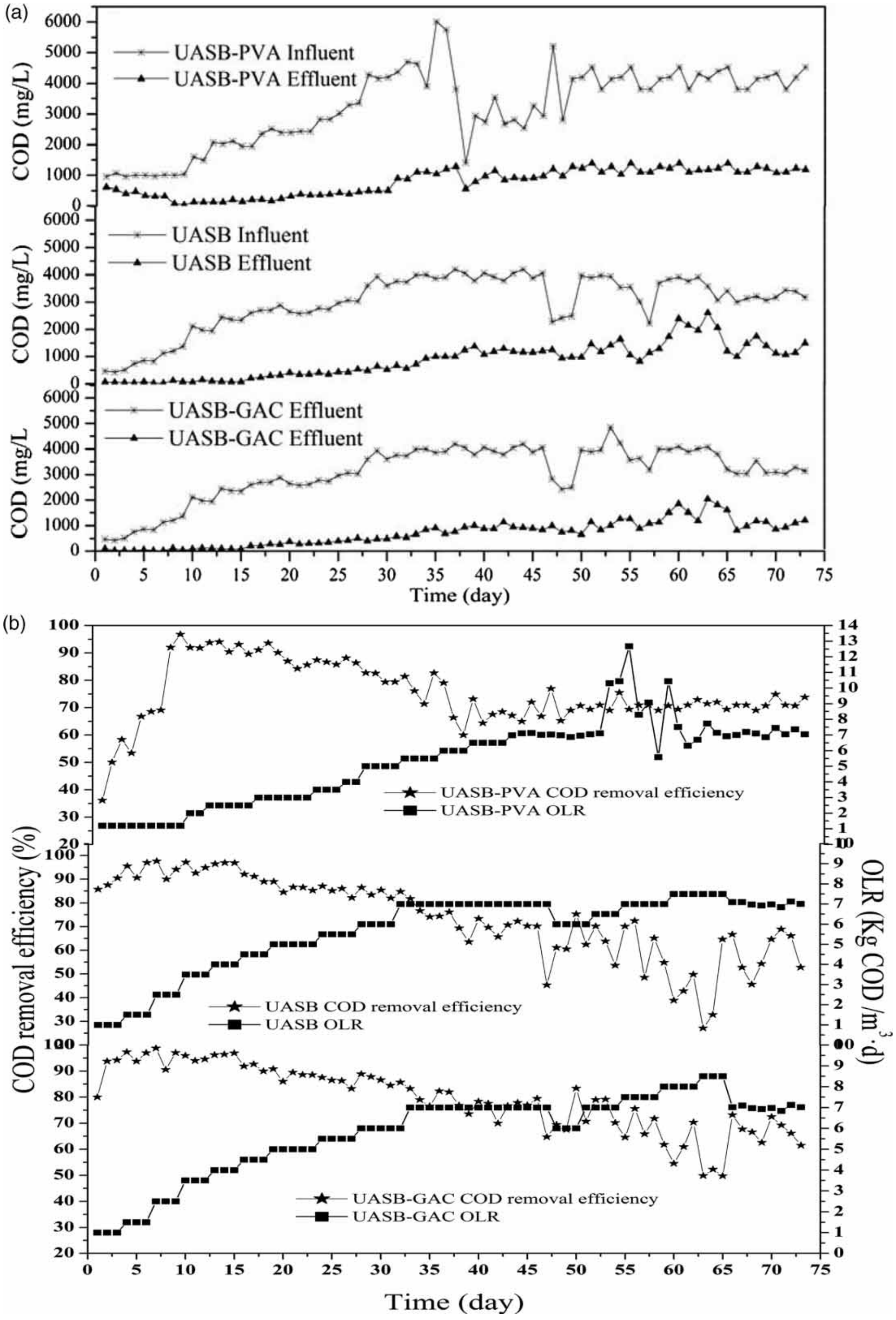

Figure 1 | Performance comparison of the three UASB reactors: and (a) influent and effluent COD and (b) COD removal efficiency and OLR.

From the 34th day to the 48th day, the COD removal efficiency was above 70%. On the 47th day, the proportion of pharmaceutical wastewater in the mixed liquid was increased from 80% to 100%, and the COD removal efficiency decreased to 65% with an OLR of 7 kg COD/(m^3·d). From the 48th day to the 52nd day, the OLR remained at 6 kg COD/(m^3·d), and the COD removal efficiency was in the range of 69% to 83%. From the 54th day to the 65th day, the ability to resist loading shock was evaluated. The OLR fluctuated significantly in the range of 7 to 8.5 kg COD/(m^3·d), and the COD removal efficiency remained in the range of 50% to 75%. The UASB-GAC reactor exhibited an ability to resist loading shock. It was found that the highest OLR of the UASB-GAC was 8 kg COD/(m^3·d). From the 66th day to the 73rd day, the OLR remained at 7 kg COD/(m^3·d), the influent COD ranged from 3,038 mg/L to 3,540 mg/L, the effluent COD decreased to between 814 mg/L and 1,209 mg/L, and the COD removal efficiency remained in the range of 61% to 73%.

Comparison of the three UASB reactors

Table 2 shows the comparison of the three UASB reactors.

In the early start-up phase, the COD removal efficiency of the UASB-PVA reactor is lower than for the UASB and UASB-GAC reactors. Sludge domestication of the UASB-PVA reactor needed 30 days, and the UASB and UASB-GAC reactors needed 48 days. During the stable operation phase, the OLR remained at 7 kg COD/(m^3·d).

The COD removal efficiency of the UASB-PVA, UASB, and UASB-GAC reactors remained in the range of 69% to 75%, 46% to 69%, and 61% to 73%, respectively, and the removal efficiency deviations of the UASB-PVA, UASB, and UASB-GAC reactors were 1.8%, 8.6%, and 4.0%, respectively. Therefore, the fluctuation of COD removal efficiencies of the three UASB reactors ranked from high to low in the following order: UASB > UASB-GAC > UASB-PVA. To sum up, in several respects, such as removal efficiency, the speed of the sludge domestication, the stability of the operation, and the highest OLR, the performance of the UASB-PVA reactor was the best of the three, the UASB-GAC reactor was second, and the UASB was the worst.

Table 2 | Comparison of the three UASB reactors

Reactors	Domestication time (day)	Highest OLR kg COD/(m^3·d)	66th–73th day COD removal efficiency (%)/deviation (%)	Ability to resist loading shock
UASB-PVA	30	12	69–75/1.8	High
UASB	47	7	46–69/8.6	Low
UASB-GAC	47	8	61–73/4.0	Middle

The changes in effluent VFAs for the three UASB reactors

During the running process of the reactors, VFAs are an important control indicator. Figure 2 shows the changes of the effluent VFAs of the three UASB reactors.

In order to keep the pH in the range of 6.5 to 7.8, sodium hydrogen carbonate, $NaHCO_3$, and hydrochloric acid, HCl, were used to adjust the pH of the influent flows, allowing the anaerobic microorganisms for different phases to grow and reproduce under the proper pH conditions. From the 1st day to the 47th day, VFA levels for the three USAB (up-flow anaerobic sludge bed) reactors increased slowly with the increase of the OLR. From the 47th day to the 73th day, VFA levels of the three USAB reactors remained stable. The VFA levels of the UASB-PVA, UASB, and UASB-GAC kept in the range of 2.46–3.30 m mol/L, 2.35–3.48 m mol/L, and 2.56–3.41 m mol/L, respectively. Keeping the reactor pH at around 7 ensured that the concentration of undissociated VFAs remained well below the inhibitory thresholds of a total VFA level of 3.42 m mol/L (Oktem *et al.* 2008). During the running process of the UASB reactor, before a further increase in OLR, care was taken to ensure that the effluent VFA level was less than 500 mg/L as acetic acid.

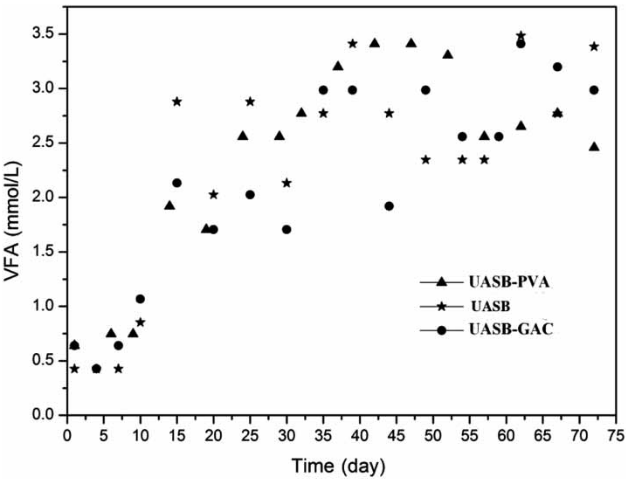

Figure 2 | The changes in effluent VFAs for the three UASB reactors.

Physical properties and microbial characteristics of the granular sludge

Observation of the PVA-gel beads

Figure 3 shows the images of the PVA-gel beads.

The color of PVA-gel bead surfaces changed from the initial white to black after 73 days of operation. Biomass attachment to the PVA-gel beads was up to 0.568 g VSS/g PVA-gel bead after 73 days, indicating that the PVA-gel beads performed well for adsorption of microorganisms.

The microorganism characteristics of the granular sludge

Figure 4 shows the SEM (scanning electron microscope) micrographs of the granular sludge.

Figure 4(a) shows that there were a large number of *Sphaerita, Filamentous fungi* and *Bacillus* attached to the inoculation granular sludge. Figure 4(b) shows that many of the microorganisms were adsorbed to the PVA granular sludge of UASB-PVA. There were many *Sphaerita* and *Bacillus*, there was less *Filamentous fungi* than for the inoculation granular sludge, and there was more *Bacillus* than for the inoculation granular sludge. Figure 4(c) shows that there were many *Sphaerita* and *Bacillus* in the UASB granular sludge, the *Filamentous fungi* clearly decreased, and there was more *Bacillus* than for the inoculation granular sludge. Figure 4(d) shows that the levels of microorganisms for the UASB-GAC granular sludge were similar to the UASB.

The *Filamentous fungi* decreased in all three UASB reactors. The reason may be that the pharmaceutical wastewater inhibits growth of the *Filamentous fungi*.

Settling properties of the granular sludge

With a stable operation under high OLR, good settling properties can help to maintain a high level of microorganism biomass. Table 3 shows the settling properties of the granular sludge with different particle size.

Table 3 shows that the settling velocity of the UASB-PVA granular sludge was between 120 m/h and 130 m/h. A small quantity of the UASB-PVA sludge's settling velocity was up to 130–140 m/h, and 23% of the sludge settling velocity was over 140 m/h. The settling velocity of the traditional anaerobic granular sludge was between 18 m/h and 100 m/h, so the settling velocity of the PVA granular sludge was higher than for the traditional anaerobic granular sludge. Therefore, not only can the UASB-PVA reactor stably run with high OLR and short HRT, but it has more efficient settling.

Table 3 shows that, the settling velocities of the small particles of the UASB and UASB-GAC reactors were slow, but the settling velocities of the medium and large particles were fast. Therefore, the settling velocity of large particle size sludge and the small size sludge were measured individually. The settling velocity of the inoculation sludge (>2 mm) was 75.54 m/h, UASB sludge (>2 mm) decreased to 70.81 m/h without GAC, and the UASB-GAC sludge

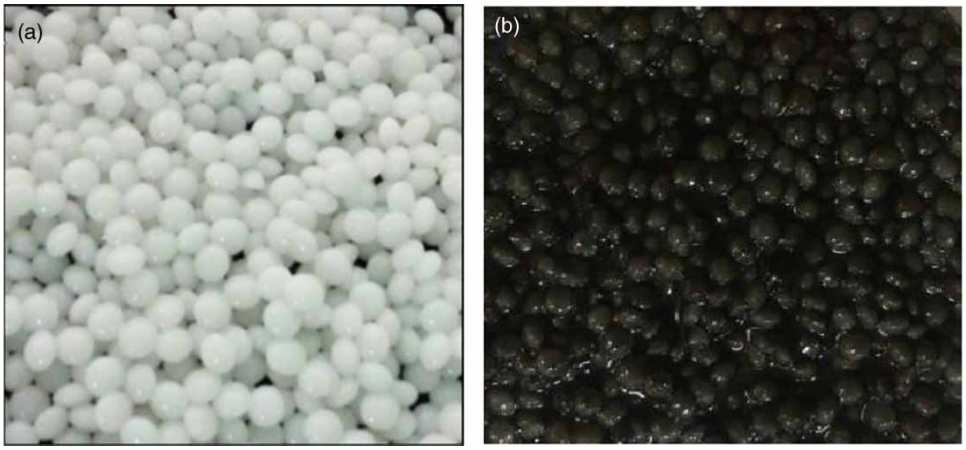

Figure 3 | Images of the PVA-gel beads: (a) surface on day 1 and (b) after 73 days.

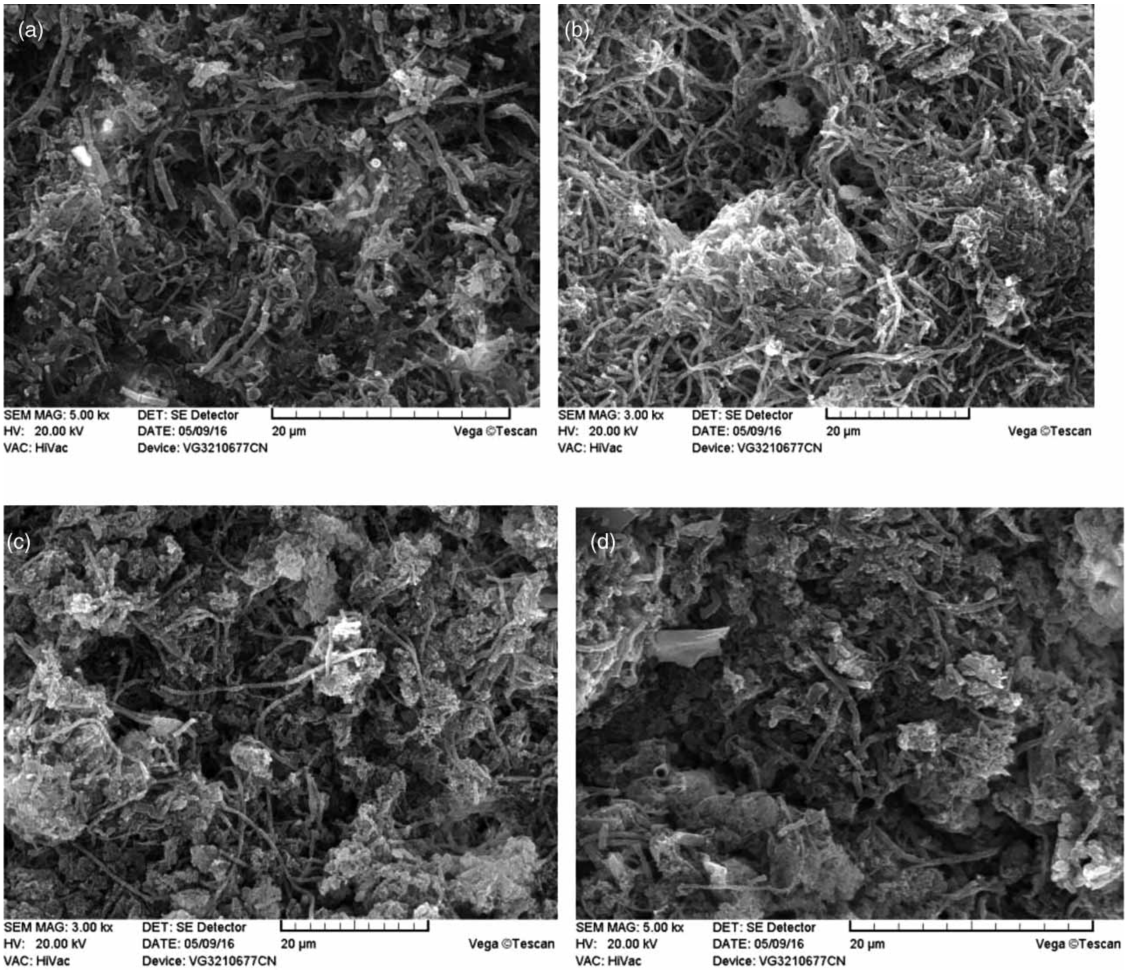

Figure 4 | SEM micrographs of granular sludge: (a) inoculation granular sludge, (b) UASB-PVA granular sludge, (c) UASB granular sludge, and (d) UASB-GAC granular sludge (magnification ×5,000).

Table 3 | Settling velocity of granular sludge with different particle size

Particle size	Inoculation V (m/h)	UASB-PVA V (m/h)	UASB V (m/h)	UASB-GAC V (m/h)
2.0–4.0 mm	75.54	120–160	70.81	84.46
0.9–2.0 mm	45.40	–	45.96	46.19
0.6–0.9 mm	30.37	–	34.00	36.90

(>2 mm) settling velocity increased to 84.46 m/h due to the added GAC. The settling velocity of UASB-GAC (0.9–2 mm) increased slightly after 73 days. The method of adding GAC was not only beneficial for the granulation of sludge, but it was also beneficial for raising the settling velocity. Adding GAC to the sludge slowed the movement of the sludge out of the reactor, allowing the microbial biomass to be more effectively maintained in the reactor.

Particle size distribution of the granular sludge

The particle size distribution in the granular sludge in reactors can intuitively reflect the impact on granular sludge by the reaction system's conditions, so it is an important indicator for the efficiency of the running process. The particle size distribution of sludge at the end of the experiment was determined by the wet sieving method, and Table 4 shows the results.

Table 4 shows that the UASB-PVA reactor had no significant change in the particle size, which ranged from

Table 4 | Particle size distribution of granular sludge

Particle size (mm)	Inoculation (TSS %)	UASB-PVA (TSS %)	UASB (TSS %)	UASB-GAC (TSS %)
>2.0	17.99	100	18.58	17.84
0.9–2.0	27.82	–	25.66	27.80
0.6–0.9	15.37	–	12.30	13.04
0.45–0.6	4.29	–	5.18	3.82
0.3–0.45	13.41	–	9.00	8.04
0.2–0.3	1.75	–	1.00	2.17
0.125–0.2	3.44	–	3.55	5.42
<0.125	15.93	–	24.72	21.86

2 mm to 3 mm. For the sludge particle size distribution of UASB and UASB-GAC changes at the end of the experiment, the large particles (>0.9 mm) did not change significantly, the proportion of medium particles (0.3–0.9 mm) declined, and the proportion of small particles (<0.3 mm) increased. This suggests that pharmaceutical wastewater has a destructive effect on medium sized granular sludge particles, but the larger granular sludge particles were hardly affected. The UASB-GAC particle size distribution is closer to that of the inoculation sludge than that of the UASB, suggesting that adding GAC can enhance the stability of granular sludge.

Microbial community structure analysis

Microbial diversity studies of sludge can help us further analyze the mechanism of degradation of pollutants. Table 5 shows the biological diversity of the different sludges.

Table 5 shows that the coverage values of the four samples were more than 0.99, suggesting that almost all of the samples were measured and that the results can represent the real situation for the samples. The inoculation sludge's series were the least of the four samples. The OUT (operation taxon unit) numbers for the four samples were ranked from high to low in the following order: UASB-PVA > inoculation > UASB-GAC > UASB; Shannon index: inoculation >UASB-PVA > UASB > UASB-GAC; ACE index: UASB-PVA > inoculation > UASB > UASB-GAC; Simpson index: UASB-GAC > UASB > UASB-PVA > inoculation. This suggests that the species diversity and total number of species for the UASB-PVA reactor were higher than for the UASB and UASB-GAC reactors. The species diversities and the UASB and UASB-GAC reactors were lower than for the inoculation sludge. There was some toxic matter in the wastewater that may reduce or eliminate microbial activity.

Analysis of microbial community structure

The ribosomal database project classifier was used to classify species for the highest abundance sequences of each OUT. The proportion of corresponding species was analyzed at genus level, and species with a proportion of less than 1% were represented within the category of 'other'. The species abundance is shown in Figure 5.

Figure 5 shows that the proportions of *Levilinea* in the inoculated sludge, UASB-PVA, UASB, and UASB-GAC were 11.03%, 17.84%, 10.11%, and 21.97%, respectively. There were higher values for the UASB-PVA and UASB-GAC reactors, but a slight decrease in the UASB reactor. The proportions of *Longilinea* in the inoculation sludge, UASB-PVA, UASB, and UASB-GAC accounted for 3.22%, 3.98%, 2.32%, and 2.43%, respectively. *Levilinea* and *Longilinea* belong to the class *Anaerolinea* of the *Chloroflexi* phylum. They are able to metabolize a variety of carbohydrates, and they are common microorganisms in anaerobic sludge (Yamada *et al.* 2007). Wang *et al.* (2014) treated dye wastewater with a UASB reactor, and they found that *Levilinea* and *Longilinea* were the dominant

Table 5 | Biological diversity of index

Sludge	Filtered number	OUT number	Shannon index	ACE index	Chao1 index	Coverage	Simpson index
Inoculation	28,022	508	4.192	914.049	901.825	0.993648	0.034194
UASB-PVA	35,646	538	4.019	993.137	874.018	0.994642	0.045078
UASB	30,622	443	3.962	778.824	680.272	0.995265	0.050968
UASB-GAC	32,009	425	3.931	720.172	666.578	0.995751	0.058279

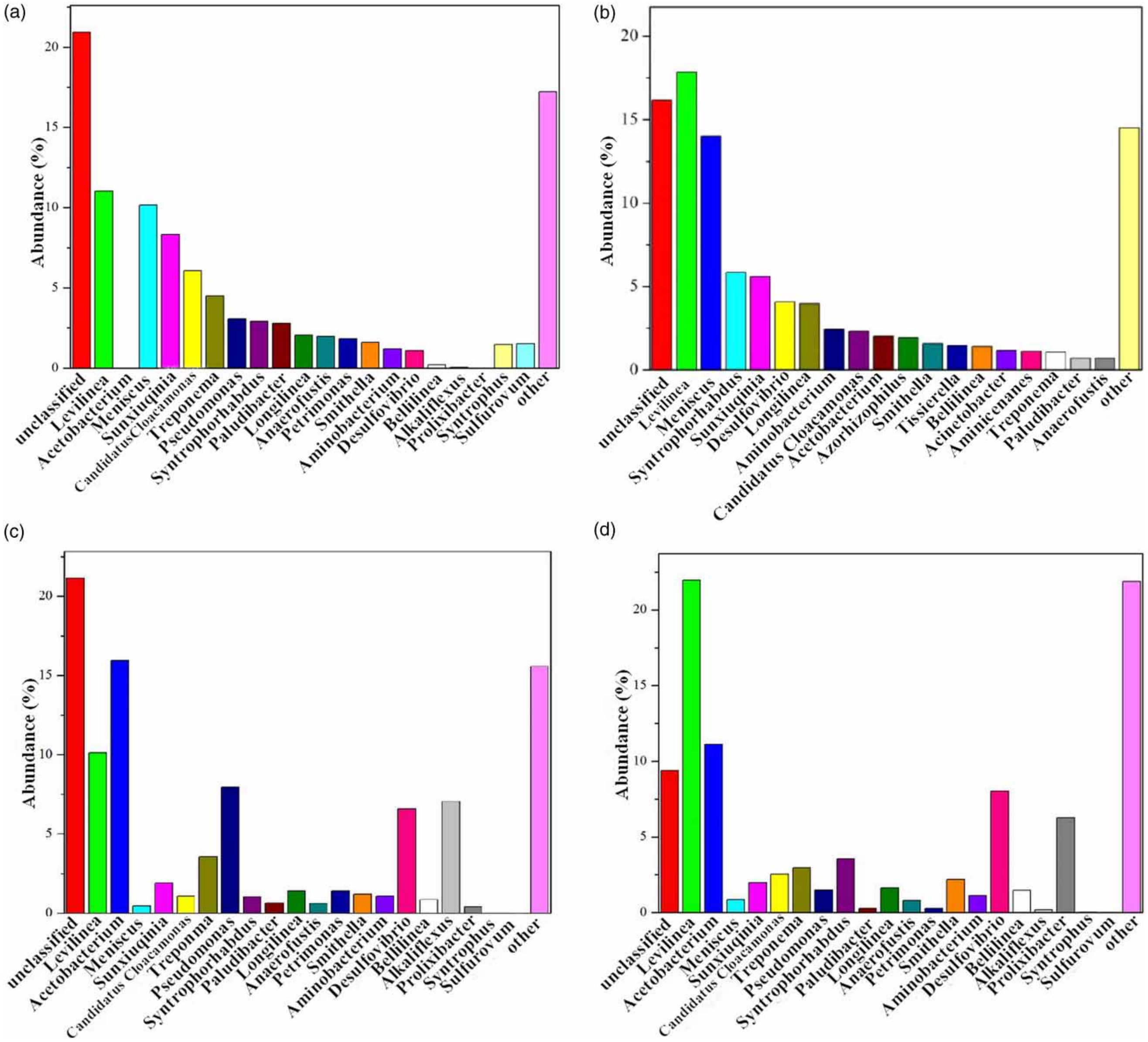

Figure 5 | Species abundance: (a) inoculation, (b) UASB-PVA, (c) UASB, and (d) UASB-GAC.

bacteria strains. The information in Figure 5 shows that *Levilinea* and *Longilinea* are capable of resisting toxic substances in industrial wastewater and can degrade macromolecular organic matter.

There was no *Acetobacterium* in the inoculation sludge. However, the proportions of *Acetobacterium* in UASB-PVA, UASB, and UASB-GAC sludges were 2.02%, 15.94%, and 11.12%, respectively. *Acetobacterium* is an anaerobic functional bacteria that can oxidize sugars, sugar alcohols and alcohols, producing sugar alcohols, ketones, and organic acids (Matsushita *et al.* 2005). The proportions of *Syntrophorhabdus* in the inoculation, UASB-PVA, UASB, and UASB-GAC sludges were 3.21%, 1.26% 3.12%, and 5.86%, respectively. *Syntrophorhabdus* can promote the transformation between hydrogen and acetate (Hadi *et al.* 2015). The proportions of *Desulfovibrio* in the inoculation, UASB-PVA, UASB, and UASB-GAC sludges were 1.1%, 4.07%, 6.58%, and 8.03%, respectively. There was an increase in

UASB-PVA, UASB, and UASB-GAC sludges, and *Desulfovibrio* can use lactic acid, ethanol, and some fatty acids as carbon and energy sources, and it reduced sulfate to hydrogen sulfide.

The proportions of *Sunxiuqinia* and *Anaerofustis* in UASB-PVA, UASB, and UASB-GAC sludges were significantly reduced, and the proportions of *Meniscus* had a significant decrease in the UASB and UASB-GAC sludges and an increase in the UASB-PVA sludge. The proportions of *Sunxiuqinia*, which is widely found in marine sediments (Liu *et al.* 2014), in the inoculation sludge, UASB-PVA, UASB, and UASB-GAC sludges were 7.83%, 5.61%, 2.36%, and 2.38%, respectively. The proportions in the three UASB-PVA, UASB, and UASB-GAC sludges decreased by different degrees.

The proportions of *Aminobacterium* in the inoculation sludge, UASB-PVA, UASB, and UASB-GAC were almost identical. *Aminobacterium* can participate in the metabolism of amino acids, which has been observed in the fluid of straw fermentation. The proportions of *Smithella* in inoculation, UASB-PVA, and UASB sludges were basically the same, but the proportions in UASB-GAC sludge increased slightly. *Smithella* can convert propionic acid into acetic acid and CO_2. The proportions of *Candidatus* Cloacamonas in the inoculation, UASB-PVA, UASB, and UASB-GAC sludges were 6.07%, 2.54%, 1.09%, and 2.53%, respectively, suggesting that *Candidatus* Cloacamonas was inhibited by the pharmaceutical wastewater. *Candidatus* Cloacamonas is a hydrogen-producing bacterium. *Syntrophus* and *Sulfurovum* were observed in the inoculation, but hardly observed in UASB-PVA, UASB, and UASB-GAC sludges, indicating that *Syntrophus* and *Sulfurovum* could not adapt to the pharmaceutical wastewater. *Tissierella* (1.46%) was only observed in UASB-PVA sludge. It can use organic metabolism to produce acetic acid, butyric acid, ammonia, and CO_2 (Harms *et al.* 1998). *Acinetobacter* (1.17%) was also only observed in UASB-PVA sludge. It can degrade caprolactam, herbicides, organophosphorus pesticides, and a variety of hydrocarbon components of oil.

In conclusion, the dominant bacteria in UASB-PVA, UASB, and UASB-GAC granular sludges are *Levilinea*, *Syntrophorhabdus*, *Desulfovibrio*, and *Acetobacterium*. Compared to the inoculation sludge, the strain types for UASB-PVA, UASB, and UASB-GAC changed, and the abundance and diversity of the microbial community decreased. The abundance and the diversity of the microbial community in the UASB-PVA sludge were higher than for the UASB and UASB-GAC granular sludges.

CONCLUSIONS

Considering various factors, such as removal efficiency, the running stability, and the speed of domestication, the performance of the three UASB reactors were ranked from high to low in the following order: UASB-PVA > UASB-GAC > UASB. UASB-PVA can be stably operated under higher OLR, indicating that adding PVA-gel beads to UASB can provide a viable way for upgrading the UASB process without major new construction, and adding PVA-gel beads in UASB can enhance the performance of the treatment. Adding GAC to UASB is not only beneficial for the granulation of sludge, but it also increases the settling velocity, minimizing sludge loss with higher OLR.

The main dominant bacteria of the UASB-PVA, UASB, and UASB-GAC granular sludges are *Levilinea*, *Syntrophorhabdus*, *Desulfovibrio* and *Acetobacterium*. Compared to the inoculation sludge, the diversity of the microbial community of UASB-PVA, UASB, and UASB-GAC sludges showed obvious changes. Some bacteria disappeared due to the toxicity of pharmaceutical wastewater, and some new bacteria that were better adapted to the pharmaceutical wastewater appeared. The abundance and diversity of the microbial community of the UASB-PVA were higher than for the UASB and UASB-GAC granular sludges.

ACKNOWLEDGEMENTS

Funding for this research was provided by the Key Laboratory Research project of Education Department of Shaanxi Province (Project No. 17JS102). The authors wish to thank the National Natural Science Foundation of China (Grant Nos. 51679191 and 21176197) and the Project of Shaanxi Provincial Department of Water resources (Project No. 2017slkj-9).

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First received 1 August 2017; accepted in revised form 9 December 2017. Available online 28 December 2017