UASB treatment of chemical synthesis-based pharmaceutical wastewater containing rich organic sulfur compounds and sulfate and associated microbial characteristics

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HIGHLIGHTS

- Successful application of UASB in treatment of rich organic S-bearing wastewater.
- Sulfate in effluent was higher than influent at COD/SO₄²⁻ ratio of 8 and 5.
- Conversely, sulfate in influent was higher than effluent at COD/SO₄²⁻ ratio of 1.5.
- Archaea and bacterial community varied greatly with altered OLR.
- Some species can be responsible for S release from organic sulfur compounds.

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ABSTRACT

The feasibility of treating of chemical synthesis-based pharmaceutical wastewater containing rich organic sulfur compounds and sulfate using an upflow anaerobic sludge blanket (UASB) was investigated. The initial COD/SO₄²⁻ ratio of 8 in the wastewater varied from 5 to 1.5 after adding sulfate. Of interest that under the condition of COD/SO₄²⁻ at 8 and 5, despite the simultaneous generation of sulfide and hydrogen sulfide, the sulfate concentration in the effluent was higher than in the influent. This is due to the sulfur release in the form of sulfate during the degradation of these organic sulfur compounds in this reactor. Conversely, at COD/SO₄²⁻ of 1.5, influent sulfate was higher than effluent sulfate due to reduction of more sulfates in the reactor. At COD/SO₄²⁻ of 8, for practical application, the optimum OLR was found to be 8 kg COD/m³/d, where a nearly 70% COD reduction occurred with biogas containing 63% methane. In this stage, the distribution of the archaea and bacterial community varied greatly with altered OLR (accompanied with prolonged operation time). Some species, such as *Lysinibacillus sphaericus*, *Clostridium cellulovorans* were expected to be partly responsible for S release from some organic sulfur compounds in the reactor. By increasing the sulfate loading at a COD/SO₄²⁻ ratio up to 1.5 resulted in a light inhibition of methanogenesis due to the high sulfide concentration (1212 SO₄²⁻ – S mg/L) with no obvious suppression of sulfidogenesis.

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1. Introduction

Most pharmaceutical compounds are prepared by chemical synthesis, which involves a complex series of chemical reactions. During the production processes, a wide range of organic and inorganic constituents including raw materials, solvents, reaction

intermediates and products will be released into wastewater at high concentrations. The high chemical oxygen demand (COD) characteristics of the chemical synthesis-based pharmaceutical wastewaters make them potential candidates for anaerobic treatment. However, it is well known that many chemicals, especially aromatic pollutants originated from chemical synthesis such as sulfanilic acid and its derivatives are hardly anaerobically degraded [1]. In particular, some of these compounds may be inhibitory to activities of wastewater treatment microbes. For example, almost complete inhibition of methanogenesis occurred upon an

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anaerobic reactor receiving high concentration of terephthalate up to 430 mg/L [2]. At the same time, high concentrations of ammonium or sulfate may exist in wastewater, which could inhibit the growth of methanogenic microbes [3,4].

Under the presence of high concentrations of sulfate, the growth of sulfate reducing bacteria (SRB) could be stimulated to out-compete methane producing bacteria (MPB) for substrates (H_2 and acetate) [5]. Moreover, the SRB can convert sulfate into sulfide which could be toxic to MPB and decrease methane production [6]. Methanogenesis inhibition by 50% has been observed when H_2S , the free soluble form of sulfide, was present in water with a concentration being over 50 mg/L [7,8]. It has been concluded that a COD/S ratio higher than 10 is desirable to prevent the inhibition of methane production [9,10].

On the other hand, for the production of some pharmaceuticals, such as sulfonylureas, sulfur organic compounds like sulfonamides are also extensively used as raw materials, and thus end up in wastewater [11]. Up to now, most previous studies have focused on the degradation ability of these organic sulfur compounds under anaerobic conditions since many of them (such as xenobiotics) were regarded as resistance to anaerobic degradation [12]. For instance, a recent work demonstrated that sulfamerazine (an antibacterial agent) in pharmaceutical wastewater was found to be biodegradable and the threshold concentration of inhibition to methanogenesis was found to be as high as 90 mg/L in a UASB reactor [13]. Another example, linear alkylbenzene sulfonate (LAS) has historically been considered inaccessible to biodegradation under anaerobic condition [14]. However, there is growing evidence of the success of degradation of LAS in the presence of various electron donors recently [15]. Once degraded under anaerobic digestion, sulfur in these organic sulfur compounds will be readily released as sulfate and sulfide into water [16]. To date, many literatures just demonstrated that the sulfur discharged in the form of sulfate and sulfide from organic sulfur compounds was only used as a sulfur source during the anaerobic process [17]. However, little is known regarding the impacts of the release of sulfur from the organic sulfur compounds in wastewater on anaerobic digestion performance. The purpose of this work was to investigate the possibility of treating real pharmaceutical

wastewater rich in organic sulfur compounds and sulfate using a UASB and to characterize the variation of archaeal and bacterial microbial community corresponding to process performance.

2. Materials and methods

2.1. Experimental setup

A schematic diagram of the experimental set up is illustrated in Fig. 1. The UASB reactor was made of plexiglass cylinder with an internal diameter of 100 mm and a working volume of 6 L. The reactor has several sampling points at different heights and was water-jacketed to maintain a constant temperature at $37 \pm 1^\circ C$. On top of the reactor was a gas–liquid–solid separator with an internal diameter of 150 mm and a height of 295 mm. The effluent was collected in a container. The produced biogas was connected to a wet gas flow meter via a simple liquid displacement unit.

2.2. Pharmaceutical wastewater characteristics

Wastewater was provided by Fukang Pharmaceutical Company, Shandong Province, China. As shown in Table 1, this wastewater contained diverse chemicals including 1,3,5-tribromobenzene, 1-bromo-3-nitrobenzene, bromopropane, 3-aminophenol, 1-bromobutane and two organic sulfur compounds (p-acetylsulfanilyl chloride and para-ester) (Sulfonated aromatic) and some unknown compounds, and was characterized with high concentrations of sulfate (7995 mg/L) and total sulfur (TS) ($1.70 \times 10^4 SO_4^{2-} - S$ mg/L). Prior to this experiment, raw wastewater was diluted by tap water to give a COD of approximately 10,000 mg/L. The COD/ SO_4^{2-} of raw wastewater was around 8. To provide enough alkalinity, 15,000 mg/L $NaHCO_3$ was added to the influent of the UASB reactor.

2.3. Experimental design

The reactor was inoculated with 3 L mesophilic granular sludge from a full-scale UASB reactor treating food manufacturing wastewater in Shijiazhuang City, Hebei Province, China. The initial

Fig. 1. Schematic diagram of the UASB set-up.

Table 1
Characteristics of the pharmaceutical industry wastewater.

Name	Unit	Range	Average
1,3,5-Tribromobenzene	mg/L	1398–1508	1453
1-Bromo-3-nitrobenzene	mg/L	1664–1836	1750
3-Aminophenol	mg/L	2217–2433	2325
1-Bromopropane	mg/L	2268–2490	2379
1-Bromobutane	mg/L	2385–2590	2488
p-Acetamidobenzene sulfonyl chloride	mg/L	483–529	502
Para-ester	mg/L	558–577	568
pH		1.9–2.1	2.0
COD	mg/L	5.6×10^4 – 6.4×10^4	6.0×10^4
Conductivity	$\mu\text{S}/\text{cm}$	76–80	78
NH_4^+-N	mg/L	80–116	98
SO_4^{2-}	mg S/L	2615–2715	2665
	mg $\text{SO}_4^{2-}/\text{L}$	7845–8145	7995
TS	mg S/L	5515–5815	9561
	mg $\text{SO}_4^{2-}/\text{L}$	1.65×10^4 – 1.75×10^4	1.70×10^4
Organic S	mg S/L	2900–3100	3000
	mg $\text{SO}_4^{2-}/\text{L}$	8700–9300	9000

sludge concentration of the reactor was 45 g VSS/L. Table 2 summarizes the operating conditions applied to the UASB in different experimental periods (I–V) over a period of nearly 200 days. The COD and pH of the feed was kept at around 10,000 mg/L and 6.8 throughout the operation period. The organic loading rate (OLR) was increased from 1 to 24 kg COD/m³/day in a stepwise fashion by reducing the HRT (hydraulic residence time) (Phase I). In Phase II, the OLR was returned to 8 kg COD/m³/day to evaluate the recovering ability in treatment efficiency. After this, the COD/SO₄^{2−} was changed to 5 and 1.5 respectively via adding Na₂SO₄ in order to test the COD/SO₄^{2−} effect on the performance of the reactor (Phases III and IV). Finally, the COD/SO₄^{2−} was reduced again to 8 in Phase V, which lasted for 26 days.

2.4. Analytical methods

The chemical oxygen demand (COD), pH, soluble sulfide, volatile fatty acids (VFA) and volatile suspended solids (VSS) were routinely determined during the operation using standard analytical procedures published by the American Public Health Association [18]. The sulfide concentration in the effluent was measured by the standard iodometric method, and sulfate was determined by ion chromatography (DIONEX, ICS-2100). TS (total sulfur) was determined by Elemental Analyzer (ELEMENTAR, VARIO ELIII).

Table 2
Operational conditions of the UASB reactor.

Period	Days	COD/SO ₄ ^{2−}	OLR (kg COD/m ³ /d)	HRT (d)
I	1–20	8	1	10.7
	21–30		2	5.3
	31–40		3	3.5
	41–50		4	2.6
	51–60		6	1.7
	61–70		8	1.3
	71–86		12	0.89
	87–100		16	0.67
	101–120		24	0.45
II	121–140	8	8	1.3
III	141–154	5	8	1.3
IV	155–170	1.5	8	1.3
V	171–196	8	8	1.3

The organic S in the feed and effluent was given by subtracting all of the inorganic sulfur species from the TS. The methane content was monitored by gas chromatography (GC 2010, SHIMADZU) equipped with a thermal conductivity detector (TCD) after biogas was washed with a 3 N NaOH solution and passed through a column filled with soda lime pellets to remove H₂S and CO₂. Aqueous H₂S was calculated based on the following equation [19]: H₂S fraction = $1/(1 + (K_1/10^{-\text{pH}}))$, where K_1 is the first ionization constant of H₂S. H₂S in the biogas was measured with hydrogen sulfide detecting tubes (Gastec, No. 4H). All the analytical estimates were made in duplicate and the average figures are presented.

2.5. Cloning analysis of 16S rDNA gene

Biomass samples were collected on days 60 and 120 from the UASB for the analysis of microbial community structures. Biomass was harvested by centrifuging of the samples at 20,000×g for 10 min, and the genomic DNA was extracted from the samples with an Ultra Clean Soil DNA Isolation Kit (MO-BIO). The amplification of 16S rDNA was performed with the primers EUB 8F [20] and Univ1500R [21] for bacteria and A109F [22] and 1059R [23] for archaea. Thermal cycling of PCR consisted of 30 s denaturing at 94 °C, 40 s of annealing at 50 °C and extracting at 72 °C for 1 min with 30 cycles for archaea and 23 cycles for bacteria. The PCR products were purified with Micro Spin™ S-400 HR (Amersham Pharmacia GE, USA). The purified DNA was cloned with the TOPO TA Cloning® Kit (Invitrogen, USA) and transformed into *Escherichia coli* DH5_a competent cells. Cloned DNA fragments were obtained and spread on plates. After an incubation period of 24 h at 37 °C, the white ones were randomly picked out and transferred to LB with another 6 h of continuous incubation. An insert check was performed using a vector of an M13 primer. The successful ones were used for sequencing. Similarity searches for the assembled sequences were performed using the NCBI Blast search program within the GenBank database (<http://www.ncbi.nlm.nih.gov/blast/>).

3. Results

3.1. Effect of OLR on treatment performance (Phases I and II)

The treatment performance of the UASB is shown in Fig. 2. With an OLR below 8.0 kg COD/m³/d and an HRT above 1.3 day, the COD removal efficiency was around 69–78%. Starting from day 71, the COD removal efficiency dropped sharply to 58% at OLR of 12.0 kg COD/m³/d at an HRT of 0.83 day and decreased further to 36% at around 24.0 kg COD/m³/d on day 120 at an HRT of 0.45 day (Fig. 2a and b). In parallel, an obvious increase of VFA in the effluent was accompanied with HRT reduction and OLR increase (Fig. 2c). The VFA level was approximately 220 mg/L at an OLR of 8 kg COD/m³/d, in comparison to 450 mg/L at 12 kg COD/L/d and 850 mg/L at the highest OLR. The increase of VFA was accompanied by a decrease in pH (from 8.5 to 7.1, Fig. 2d). Similarly, accompanying the increase in the VFA level during HRT reduction, methane content in the biogas tended to decrease (Fig. 2f). The methane content was 66% or higher at an OLD below 16 kg COD/L/d, but dropped to 48% at an OLD of 24 kg COD/L/d, showing that the reactor was in serious acidification condition, which was in accordance with the sharp increase of VFA and the changes in methane yield rate. After resetting the OLR to 8 kg COD/m³/d by increasing the HRT to 1.3 days (Phase II), the COD removal efficiency quickly recovered to over 60%, together with recoveries in pH, VFA and methane content, showing the resilience of the UASB system. Thus, in terms of practical application, an OLR of 8 kg COD/m³/d will be suitable.

Fig. 2. Overall performance of the UASB during continuous operation: (a) influent and effluent COD; (b) changes of OLR; (c) effluent VFA; (d) pH changes; (e) CH₄ gas production rate and (f) gas compositions.

3.2. Effect of OLR on variations of sulfur related species (Phases I and II)

As shown in Fig. 3a and b, the effluent sulfate concentration was higher than the influent sulfate and increased significantly in spite of the generation of sulfide. This can be clearly attributed to the release of sulfate from organic sulfur compounds since the concentration of organic S decreased significantly from approximately 1500 mg SO₄²⁻ – S/L to 300 mg SO₄²⁻ – S/L during the treatment (Fig. 3d). It should be noted that the increase of OLR from 12 to 24 COD/m³/d did not affect the release of sulfate, but affected the reduction of sulfate, suggesting that sulfur release could occur

under acidified conditions. On the other hand, the variations of free and dissolved sulfide should be related with the changes of pH.

3.3. Effect of COD/SO₄²⁻ on treatment performance (Periods III and IV)

From day 140 to day 170, the COD/SO₄²⁻ ratio was decreased from 8 to 5 and then 1.5 by increasing the sulfate concentration from 1.25 to 2.0 and then 6.5 g/L. As shown in Fig. 2a, c and e, the treatment performance in terms of COD removal, VFA formation and methane production was not perceptibly affected by the decrease of the COD/SO₄²⁻ ratio from 8 to 5. At the same time,

Fig. 3. Sulfate, sulfide, TS and organic S variation during continuous operation: (a) influent and effluent sulfate; (b) dissolved and free sulfide in effluent; (c) TS in influent and effluent and (d) organic S in influent and effluent.

formation of sulfide was not affected, either, as shown in Fig. 3b. However, when the $\text{COD}/\text{SO}_4^{2-}$ was further decreased to 1.5, a decrease in COD removal (from 64% to 54%) and methane content (from over 60% to around 50%), and an increase of VFA production (from 300 to 680 mg/L) were observed, together with a drop of methane production rate from 1.5 L/L/d to 1.2 L/L/d (Fig. 2a, c and e). At the same time, significant increases in sulfide (dissolved and free sulfide, over 1200 $\text{SO}_4^{2-} - \text{S}$ and 240 $\text{SO}_4^{2-} - \text{S}$ mg/L, respectively, in comparison with 600 $\text{SO}_4^{2-} - \text{S}$ and 40 $\text{SO}_4^{2-} - \text{S}$ mg/L) and H_2S gas (3% in comparison with 0.26%) were observed (Figs. 2f and 3b), indicating the enhanced sulfate reduction activity. However, it is interesting that the release of sulfur from organic sulfur compounds was almost unaffected by the sudden increase in sulfur concentration. Thus it was concluded that the decomposition of the organic S compounds was not inhibited by the enhanced sulfate reduction activities.

When the $\text{COD}/\text{SO}_4^{2-}$ was set back to 8 on day 171, however, the reactor exhibited a quick recovery, as shown in Figs. 2 and 3. The treatment performance in terms of COD removal and methane production almost returned to the original state (Phase II) within 15 days of operation.

3.4. Microbial community structure analysis

Two archaeal clone libraries and two bacterial ones were established for the biological samples taken on days 60 and 120,

respectively. In total, 48 archaeal clones were acquired in each of the two samples containing 26 and 29 operational taxonomic units (OTUs) respectively. As shown in Fig. 4, *Methanobacterium* sp., a hydrogenotrophic methanogen, was dominant, accounting for 54% of total clones of day 60 (OLR, 6 kg COD/m³/d) followed by acetate-clastic methanogens (38%) including *Methanosaeta concilii* GP6 (23%), *Methanosaeta harundinacea* (9%) and *Methanosarcina mazei* (6%). For the day 120 clone library (OLR, 24 kg COD/m³/d), on the other hand, the acetate-clastic methanogens including *M. concilii* GP6 (31%), *M. harundinacea* (2%) and *M. mazei* (21%) accounted for 54% of the total clones, seconded with the hydrogenotrophic methanogen *Methanobacterium* sp (27%) (Fig. 4).

At total of 111 and 117 bacterial clones were acquired in for the two samples, with 44 and 58 OTUs in each respectively. As shown in Table 3, the bacterial clone library of day 60 was dominated by the clones affiliated with the phylum *Firmicutes* (67.5%) followed by the phylum *Proteobacteria* (10.8%) and phylum *Chloroflexi* (9%). The *Lysinibacillus sphaericus*-like OTU was the single largest group (36%) followed by the *Ignavibacterium album* one (13.5%, though with a low similarity of 85%). For the library of day 120 presented in Table 4, however, the phyla *Firmicutes* (24.8%) and *Proteobacteria* (23.2%) became the two largest groups, followed by the phyla *Chloroflexi* (7.7%), *Bacteroidetes* (11.9%) and *Candidate division* (12%) and *Thermotogae* (11.2%). The distribution of the clones became much more even in comparison with that in the clone library of day 60.

Fig. 4. Archaeal on days 60 and 120.

4. Discussion

4.1. Effect of high concentrations of inorganic and organic sulfur on UASB performance

It has long been known that the presence of high concentration of sulfate could stimulate the growth of sulfate reducing bacteria (SRB) to out-compete methane producing bacteria (MPB) for substrates, and even to inhibit the growth of MPB through the production of sulfide [24,25]. So great efforts have been devoted to the development of strategies to control the substrate competition inhibition and the sulfide toxicity, such as sulfide precipitation using iron salts [19,26], gas stripping [27] and oxygenation [28]. In recent 10 years, some novel processes have been developed to alleviate the toxicity of sulfide to anaerobic treatment. Molybdate was investigated because molybdate is both a SRB inhibitor and a nutrient for methanogens [29]. Zero-valent iron (ZVI) serving can

sever as an electron donor and be successfully used to alleviate the competition for substrates between SRB and MPB [30]. Microbial Fuel Cells (MFC) using graphite rods as electrodes provided a new approach for simultaneous anaerobic sulfide and nitrate removal coupled with electricity generation [31].

Though effective to some extent, all of these measures would increase the investment, and in some cases, may lead to other new problems. As a matter of fact, a chemical synthesis-based pharmaceutical production process generally generates many different waste streams, which may allow us to select suitable waste streams for anaerobic treatment. So the most important thing is to understand the conditions causing methanogenic inhibitions. COD/SO₄²⁻ (S) ratio in influent is an important parameter affecting the competition between SRB and the other anaerobic bacteria and consequently significantly influences the performance of the anaerobic system [32]. A COD/S ratio higher than 10 (COD/SO₄²⁻ > 3.3) is a threshold to prevent the sulfide from poisoning

Table 3
Bacterial composition of granular sludge on day 60.

Pylum	Phylogenetic group	OTUs	No. of clones	Percentage (%)	Similarity (%)
Firmicutes	<i>Lysinibacillus sphaericus</i>	2	36	32.4	96
	<i>Bacillus megaterium</i> (WSH)	2	2	1.8	87
	<i>Bacillus megaterium</i> (DSM)	2	2	1.8	95
	<i>Solibacillus silvestris</i>	1	1	0.9	91
	<i>Clostridium cellulovorans</i>	1	4	3.6	94
	<i>Clostridium clariflavum</i>	1	1	0.9	87
	<i>Eubacterium limosum</i>	2	3	2.7	92
	<i>Acetobacterium woodii</i>	2	4	3.6	99
	<i>Pelotomaculum thermopropionicum</i>	1	1	0.9	87
	<i>Ignavibacterium album</i>	1	15	13.5	85
	<i>Syntrophothermus lipocalidus</i>	1	1	0.9	87
	<i>Syntrophomonas wolfei</i> subsp.	2	2	1.8	93
	<i>Moorella thermoacetica</i>	3	3	2.7	88
Proteobacteria	<i>Desulfobacca acetoxidans</i>	1	2	1.8	95
	<i>Syntrophus aciditrophicus</i>	2	3	2.7	98
	<i>Syntrophobacter fumaroxidans</i>	1	3	2.7	95
	<i>Geobacter uraniireducens</i>	1	1	0.9	97
	<i>Lawsonia intracellularis</i>	1	1	0.9	83
	<i>Aminobacterium colombiense</i>	1	1	0.9	91
	<i>Acinetobacter baumannii</i>	1	1	0.9	98
Caldiserica	<i>Solitalea canadensis</i>	1	1	0.9	81
	<i>Owenweeksia hongkongensis</i>	1	1	0.9	82
Chloroflexi	<i>Anaerolinea thermophila</i>	4	7	6.3	88
	<i>Caldilinea aerophila</i>	1	3	2.7	85
Actinobacteria	<i>Conexibacter woesei</i>	1	2	1.8	89
	<i>Rubrobacter xylanophilus</i>	1	2	1.8	84
Bacteroidetes	<i>Owenweeksia hongkongensis</i>	3	3	2.7	87
Thermotogae	<i>Mesotoga prima</i>	1	3	2.7	98
Synergistetes	<i>Moorella thermoacetica</i>	1	1	0.9	89
Spirochaetes	<i>Spirochaeta caldaria</i>	1	1	0.9	89
		44	111	100	

methanogens and avoids the failure of the anaerobic process [9,10]. However, an exception was reported by a recent study that though a COD/SO₄²⁻ ratio was as high as 5, anaerobic reactor in digesting high strength and sulfate rich vinasse eventually failed due to the toxicity of H₂S (free form of sulfide) on methanogens and SRBs [33]. Another exception was given in another recent study that though COD/SO₄²⁻ ratio was as below as 1 (COD/S = 3), a UASB can remove 80% of COD and 30% of sulfate with HRT above 6 h and OLR below 12.3 g COD/L/d in treating sulfate-rich wastewater containing ethanol and acetate [34]. Thus, the inhibition to various trophic groups by sulfide generated must be complex due to the variations in wastewater, seed sludge and operating conditions. However, to date, little has been done to investigate the impacts of the released sulfate from the organic sulfur compounds on methanogenic activities.

In this study, we have demonstrated that the methanogenic activities would not be affected by the presence of sulfate under a COD/SO₄²⁻ of 5, or a COD/S of 15, even when abundant organic sulfur compounds existed. Under a COD/SO₄²⁻ of 8, the methane production rate increased linearly from 0.2 L/L/day to 2.7 L/L/day with the increase of the OLR from 1 to 16 kg COD/m³/d (Fig. 2b and e), though the COD removal rate decreased from 78% to approximately 50% (Fig. 2a). However, no obvious change in methane yield rate was found when the OLR was further adjusted to 24 kg COD/m³/d. This can be further explained by COD balance as the function of OLRs represented (Data not shown) that at OLR of 16 kg COD/m³/d, the proportion for COD consumed for methane yield was round 41.5% whereas the proportion sharply reduced to 31.5% at OLR of 24 kg COD/m³/d (Fig. 2b and e). In parallel, the COD removal rate reduced sharply from approximately 50% to 35%, reflecting the decrease of COD rate efficiency was mainly due to the accumulation of VFA (Fig. 2c). Moreover, it should be noted

that sulfate release from the organic S compounds was not affected perceptibly even when the reactor was under the acidification state at the OLR of 24 kg COD/m³/day.

A further decrease of the COD/SO₄²⁻ to 1.5 (or to 4.5 as COD/S) resulted in a decreases in COD removal (from 64% to 54%) and methane production rate (from 1.5 L/L/d to 1.2 L/L/d), which should be caused by the competition of sulfate reduction activities since a significant increase in sulfide production (up to 1212 SO₄²⁻ – S mg/L) was observed (Figs. 2a, b, e and 3a and b). Even under such a condition, however, sulfate release from organic sulfur compounds was not affected, either. Considering many arguments regarding to threshold of sulfide concentration and free sulfide which can result in inhibition to various trophic groups in anaerobic digestion processes, it is difficult to compare this work to the data obtained in earlier research. Additionally, it should be noted that the inhibition of methanogenic activity was reversible, and the reactor performance could be recovered easily once the COD/SO₄²⁻ was returned to the normal condition.

4.2. Characteristics of microbial community structures

As described above, the increase of OLR from 6 kg (day 60) to 24 kg COD/m³/d (day 120) resulted in significant changes in both the bacterial and archaeal community structures. The hydrogenotrophic *Methanobacterium*-like methanogen (54%) dominated the archaeal clone library of day 60, followed by the aceticlastic *M. concilii* GP6 (23%), *M. harundinacea* (9%) and *M. mazei* (6%), while the aceticlastic *M. concilii* GP6 (31%), *M. harundinacea* (2%) and *M. mazei* (21%) dominated the day 120 clone library (Fig. 4). Generally, *Methanosaeta*-related species are more competitive in systems with high acetate concentration [19]. Actually, the acetate concentration did increase from 138 mg/L to 610 mg/L when the

Table 4

Bacterial composition of granular sludge on day 120.

Pylum	Phylogenetic group	OTUs	No. of clones	Percentage (%)	Similarity (%)
Firmicutes	<i>Eubacterium limosum</i>	3	9	7.7	92
	<i>Eubacterium rectale</i>	2	2	1.7	95
	<i>Acetobacterium woodii</i>	3	6	5.1	99
	<i>Desulfobacterium dichloroeliminans</i>	1	1	0.9	88
	<i>Thermincola potens</i>	1	1	0.9	92
	<i>Filifactor alocis</i>	1	2	1.7	87
	<i>Clostridium difficile</i>	2	2	1.7	90
	<i>Syntrophomonas wolfei</i> subsp.	1	2	1.7	91
	<i>Clostridium acidurici</i>	1	2	1.7	94
	<i>Moorella thermoacetica</i>	1	2	1.7	88
Proteobacteria	<i>Desulfovibrio vulgaris</i> str.	1	1	0.9	84
	<i>Desulfohalobium retbaense</i>	1	1	0.9	78
	<i>Desulfobacca acetoxidans</i>	1	1	0.9	85
	<i>Syntrophus aciditrophicus</i>	4	5	4.3	92
	<i>Syntrophobacter fumaroxidans</i>	3	10	8.5	95
	<i>Pseudomonas mendocina</i>	1	1	0.9	99
	<i>Acinetobacter</i> sp.	1	2	1.7	97
	<i>Nautilia profundicola</i>	1	2	1.7	78
	<i>Lawsonia intracellularis</i>	1	4	3.4	84
Bacteroidetes	<i>Solitalea canadensis</i>	2	10	8.5	87
	<i>Owenweeksia hongkongensis</i>	3	4	3.4	88
Chloroflexi	<i>Anaerolinea thermophila</i>	6	8	6.8	88
	<i>Caldilinea aerophila</i>	1	1	0.9	85
Caldiserica	<i>Caldisericum exile</i>	2	2	1.7	93
Candidate division	<i>Candidatus Cloacamonas acidaminovorans</i>	1	2	1.7	93
	<i>Candidatus Methyloirabilis oxyfera</i>	1	1	0.9	86
Synergistetes	<i>Thermanaerovibrio acidaminovorans</i>	2	4	3.4	89
	<i>Aminobacterium colombiense</i>	2	3	2.6	93
Ignavibacteria	<i>Ignavibacterium album</i>	1	6	5.1	85
Thermotogae	<i>Mesotoga prima</i>	3	12	10.3	98
	<i>Moorella thermoacetica</i>	1	1	0.9	85
Actinobacteria	<i>Rubrobacter xylanophilus</i>	1	2	1.7	84
	<i>Conexibacter woesei</i>	2	5	4.3	89
		58	117	100	

OLR was increased from 6 kg to 24 kg COD/m³/d (Data not shown). Thus the shift from the hydrogenotrophic methanogens to the acetoclastic ones may be attributed to the changes in acetate concentrations. Of course, other factors, such as the concentrations of nutrients, micronutrients and the hydraulic load, may also play a role in the selection of *Methanosaeta* and *Methanosarcina* [35].

In comparison with the archaeal community structures, the changes in the bacterial community structures were more significant. As shown in Table 3, the bacterial clone library of day 60 was highly dominated with the clones affiliated with the phylum *Firmicutes* (67.5%), with the *L. sphaericus*-like OTU as the largest single group (36%). A recent work [36] revealed that a novel strain *L. sphaericus* DMT-7 could serve as model system for the efficient biodesulfurization of diesel and petrol. Another dichloromethane (DCM)-degrading organism *L. sphaericus* strain wh22 which can be isolated from pharmaceutical wastewater sludge was reported by Wu et al. [37]. Given the good performance in COD removal and sulfur release from the organic sulfur compounds at the OLR of 6 kg COD/m³/d, it is reasonable to speculate that this *L. sphaericus*-like organism may have played an important role in organic matter degradation, particularly the decomposition of organic sulfur compounds. It is interesting that the presence of normal SRBs, such as *Desulfobacca acetoxidans*, was not very impressive (1.8% of total clones, as presented in Table 3), which might explain why the sulfate reduction did not proceed very well, as indicated by the relatively low sulfide concentrations.

For the library of day 120, as described in Table 4, however, the high dominance of phylum *Firmicutes* (24.8%) disappeared, with the increased presence of other groups including *Proteobacteria*

(23.2%), *Bacteroidetes* (11.9%) and *Candidate division* (12%) and *Thermotogae* (11.2%). It should be noted that the important *L. sphaericus*-like organism was not found in this clone library. As described previously, the sulfur release performance of the reactor did not change perceptibly, so the reason for the disappearance of this microbe was not clear. On the other hand, an OUT sharing 95% sequence similarity with *Syntrophobacter fumaroxidans* accounted for 8.5% of the clones in library day 120. *Syntrophobacter* species, such as *S. fumaroxidans*, *Syntrophobacter pfennigii* and *Syntrophobacter wolnii* have been described as syntrophic propionate – oxidizing bacterial species, and are closely related to the mesophilic sulfate reducers for propionate oxidation and some other organic compounds as well as hydrogen [38]. The appearance of this microbe might be related to the increase of the propionate concentration from 12 mg/L on day 60 to 98 mg/L on day 120. *Mesotoga*, which shared a 98% sequence similarity with *prima* accounted for 10.3% of the clones on day 120, almost three times as many as were noted on day 60 (Tables 3 and 4). Recent molecular studies have revealed the presence of “*Mesotoga*” in microbial communities degrading aromatic pollutants under anaerobic conditions [39]. Similarly, the appearance of *Desulfovibrio*-like SRB (84% sequences similarity to *vulgaris*) after day 60 resulted from more hydrogen or sulfur being generated along with a simultaneous increase of OLR and SLR in the reactor (Table 4). The increased VFA concentration did not stimulate the growth rate of *Desulfovibrio* species because most *Desulfovibrio* species have been shown not to grow in the presence of VFA [40]. As such, the appearance of *Desulfovibrio* species is most likely attributed to the biodegradation of organic pollutants. The appearances of *Clostridium cellulovorans*, *Clostridium*

acidurici, etc were identified during the sampling period (Tables 3 and 4). As described by Chien [41], *Clostridium beijerinckii* strain EV4 was observed to desulfonate alkyl- and arylsulfonate. Therefore, it can be postulated that some species from *Clostridium* genus played certain role in the release of sulfur from the p-acetylsulfanilyl chloride and para-ester and other unknown sulfonated aromatics in the reactor.

5. Conclusion

A UASB operating under a continuous mode achieved satisfactory organic matter removal efficiency at a higher OLR of up to 8 kg COD/m³/d with the HRT of 1.3 day in treating high strength pharmaceutical wastewater in the presence of rich organic sulfur compounds and sulfate. The sulfate generated by the degradation of organic sulfur compounds had the undesirable effect of increasing the sulfate loading rate of the digester. A significant variation in the bacterial and archaeal community composition of sludge was observed when the reactor was fed from the initial OLR of 1 kg COD/m³/d up to 24 kg COD/m³/d (day 120). The COD/SO₄²⁻ ratio appears to have influenced the syntrophic and competitive relationships between sulfate-reducing and methane-producing organisms. No toxicity effects on sulfate-reducing bacteria were found, even at sulfide concentration as high as 1212 SO₄²⁻ – S mg/L. However, the methane-producing microorganisms may have been slightly inhibited at this sulfide concentration. The system can be 95% recovered by resetting the COD/SO₄²⁻ ratio to 8 again within 15 days of operation.

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