Anaerobic treatment of p-acetamidobenzene sulfonyl chloride (p-ASC)-containing wastewater in the presence or absence of ethanol in a UASB reactor

Wei cheng Li ^{a, b}, Chunyan Wang ^a, Zhe Tian ^a, Hong Zhang ^a, Yingxin Gao ^{a, **}, Yu Zhang ^a, Min Yang ^a, Yu-You Li ^{b, *}, Osamu Nishimura ^b

^a State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Science, Post Office Box 2871, Beijing 100085, China

^b Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, 6-6-06 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan

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ABSTRACT

A lab-scale UASB reactor was operated for the treatment of p-acetamidobenzene sulfonyl chloride (p-ASC)-containing wastewater with and without ethanol for 210 days. The influence of the organic loading rate on the performance of reactor by step-decreasing HRT reduction/step-increasing the concentration of p-ASC was evaluated. Almost complete degradation of 50 mg/l p-ASC and COD removal efficiency (79.5%) was noticed with a COD removal of 79.5% at HRT of 0.5 day or longer and an applied p-ASC loading rate below 120 mg/l/day. No inhibition to the performance of this reactor was observed even at a p-ASC concentration up to 1000 mg/l. Of interest that despite the absence of sulfate in the feed, a certain quantity of sulfate and sulfide was simultaneously generated in the effluent. The trial result in the absence of ethanol demonstrated that p-ASC could be used as the sole source of carbon and energy. Clone libraries for the archaeal and bacterial communities were constructed for a biomass sample taken on day 190. The majority of bacterial clones were represented by *Proteobacteria*, followed by *Thermotogae*, *Bacteroidetes* and *Firmicutes*. Bacterial groups within the phyla *Clostridia* might be responsible for the desulfonation of p-ASC.

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Introduction

Aromatic sulfonates include a wide range of chemicals and are extensively applied as important industrial chemicals, especially in the dye and pharmaceutical industries. The presence of a sulfonated group makes these compounds highly water-soluble (Tan et al., 2005), which results in the generation of large volumes of high strength industrial waste streams (Zerbinati et al., 1997). The dominant approach to remove organic matter from high strength industrial wastewater is anaerobic digestion due to the low energy input required, the sludge yield and the production of biogas as clean fuel. Two sulfonated azo dyes (Acid Orange 7 and Direct Red

254) have been observed to be effectively degraded in a methanogenic UASB reactor (Brás et al., 2005). However, it has taken time to understand the anaerobic treatability of linear alkylbenzene sulfonate (LAS), which historically had been considered to severely impede methane production and persist during anaerobic treatment. It was reported by Prats et al. (1997) that LAS can be anaerobically degraded in the presence of various electron donors. Until now, the treatability of the aromatic sulfonates-containing wastewater under anaerobic conditions could only be elucidated through experimental approaches.

p-Acetamidobenzene sulfonyl chloride (p-ASC), is a derivative of sulfonated aromatic amines as shown in Fig. 1. It has been applied as an intermediate in the synthesis of p-aminobenzene sulfonamide (sulfanilamide, SN), one of the most frequently used sulfonamide type antibacterial medicines (Tan et al., 2011). Since the synthesis operations in pharmaceutical manufacturing industries usually generate larger volumes of wastewater (Chelliapan et al.,

* Corresponding author. Tel.: +81 22 7957464; fax: +81 373 7957465.

** Corresponding author. Tel.: +86 10 62943475; fax: +86 10 62923541.

E-mail addresses: gyx@rcees.ac.cn (Y. Gao), yyli@ep11.civil.tohoku.ac.jp (Y.-Y. Li).

Fig. 1. Chemical structure of p-acetamidobenzene sulfonyl chloride (p-ASC).

2011), it is vital that wastewater containing p-ASC and other organic compounds is treated both effectively and economically during the SN production process. The reduction of azo dyes results in the formation of aromatic amines that mostly cannot be metabolized anaerobically. The exception of a few examples bearing hydroxyl and carboxyl groups can be fully degraded under methanogenic conditions (Razo-Flores et al., 1996). The conclusion can be also confirmed in an earlier study, which show that sulfonated aromatic amines are not readily degraded (Lange et al., 1995b). Tan et al. (2005) investigated the biodegradation of ten sulfonated aromatic amines including 2,4-diaminobenzenesulfonic acid, 1-aminonaphthalene-4-sulfonic acid, etc. in both aerobic and anaerobic conditions. None of the sulfonated aromatic amines tested were degraded anaerobically, while aerobic degradation was only observed with the inoculum sources that were historically polluted with sulfonated aromatic amines. To the best of our knowledge, almost no literature has been published so far on anaerobic degradation of p-ASC. In this study, a UASB reactor was established to treat p-ASC-containing wastewater under different conditions for a period of 210 days. The performance of the reactor was evaluated by following the changes of the p-ASC, COD and volatile organic acid concentrations in the effluent and the methane production rate. At the same time, clone libraries for the archaeal and bacterial communities were constructed for a biomass sample taken on day 190 to analyze the microbial population possibly involved in p-ASC degradation. The study could provide useful information for the treatment of wastewater containing p-ASC or similar compounds.

Materials and methods

Experiment set-up

The UASB reactor was made of a plexiglass cylinder with an internal diameter of 100 mm and a working volume of 6 l, as shown in Fig. 2. The reactor was water-jacketed to keep a constant temperature at 37 ± 2 °C. The produced gas was sequentially absorbed by a 3 N NaOH solution and soda lime pellets to remove H_2S and CO_2 , respectively, and then recorded by a wet gas meter.

Reactor operation

The mesophilic UASB reactor was inoculated with 3 l granule sludge partly from a UASB reactor treating pharmaceutical wastewater containing p-ASC for one year and partly from a full-scale UASB reactor treating food manufacturing wastewater. The initial sludge concentration was 46.5 g VSS/l. Synthetic wastewater consisting varying concentrations of p-ASC with and without ethanol was prepared and fed into the reactor at different stages over a period of 200 days, as detailed in Table 1. The initial OLR (organic loading rate) was 1.0 g COD/l/day, corresponding to an initial hydraulic retention time (HRT) of 3.5 days. Then the OLR was elevated to around 24.0 g COD/l/day by shortening HRT stepwise in Stage I. Thereafter, in Period II, the OLR was decreased backward to 7.0 g COD/l/day (HRT 0.5 day) to investigate the recovery ability of the reactor. The impacts of p-ASC to anaerobic treatment were evaluated by increasing p-ASC concentration in Stage III. Finally the reactor was operated without ethanol in Stage IV.

Analytical methods

The chemical oxygen demand (COD), pH, soluble sulfide, volatile fatty acids (VFA) and volatile suspended solids (VSS) were determined according to the standard analytical procedures published by the APHA (1998). Prior to COD measurement, the solution was aerated to oxidize S^{2-} to S deposit in order to prevent the interference by sulfide (Sun and Guan, 2009). The sulfide concentration in the effluent was measured using iodometric method, while the

Fig. 2. Schematic diagram of the UASB set-up.

Table 1
Characterization of the different period of UASB reactors operation.

Period	Days	COD (mg/l)	p-ASC (mg/l)	p-ASC loading rate (mg/l/day)	Ethanol (mg/l)	OLR (g COD/l/day)	HRT (day)
I	1–15	3400	50	14.2	1600	1.0	3.50
	16–25		50	28.4	1600	2.0	1.76
	26–35		50	42.6	1600	3.0	1.20
	36–50		50	57.2	1600	4.0	0.88
	51–65		50	99.4	1600	7.0	0.50
	66–80		50	170.4	1600	12.0	0.30
	81–95		50	224.3	1600	16.0	0.22
	96–110		50	340.2	1600	24.0	0.15
II	111–130	3400	50	99.4	1600	7.0	0.5
III	131–150	3900	400	795.2	1600	7.9	0.50
	151–170	4600	1000	1988	1600	9.15	0.50
	171–190	3900	400	795.2	1600	7.9	0.50
IV	191–210	550	400	795.2	0	1.1	0.50

sulfate concentration was determined on an ion exchange chromatography (ICS-2100, DIONEX). The biogas composition was monitored on a gas chromatography (Agilent 7890A) equipped with a thermal conductivity detector (TCD). The concentrations of short-chain organic acids and ethanol were determined on a gas chromatography (GC 2010, SHIMADZU) equipped with an HP-FFAP capillary column and a flame ionization detector using helium as the carrier gas.

The concentration of p-ASC was determined on a high-performance liquid chromatography (HPLC) (Breeze 1525, Waters) equipped with an HPLC column (250_4.6 mm Zorbax Extend-C18, Agilent) and 2487 Dual λ Absorbance Detector (Waters). The column temperature was maintained at 25 °C. The mobile phase consisting of 85% of solvent A (water) and 15% of solvent B (0.05 M ammonium acetate in water) was passed through the column with a flow rate of 1 ml/min. Calibration curves was obtained at a detection wavelength of 245 nm using a series of standard solutions over the concentration range from 1 to 20 mg/l. Calibration curve was linear over the concentration ranges tested with correlation coefficient of 0.999. The corresponding recovery was 98.9% and the detection limit was approximately 30 μ g/l.

Cloning analysis of 16S rRNA gene

A biomass sample was collected from the reactor on day 190. Cells were harvested by the centrifugation of the sample at $20,000 \times g$ for 10 min, and then used for DNA extraction from the sample with a FastPrep DNA Extraction Kit for Soil in a FastPrep FP120 Cell Disrupter (Qbiogene, USA). 16S rRNA genes were amplified with the universal primers 27F (5'-AGAGTTTGATCATGGC-3') and 1492R (5'-TACCTTGTACGACTT-3') for bacteria and 21F (5'-TTCCGGTTGATCCYGC-CGGA-3') and 958R (5'-YCCGGCGTC-CYGCCGGA-3') for archaea. Thermal cycling of PCR consisted of 60 s denaturing at 94 °C, 30 s of annealing at 55 °C, and extracting at 72 °C for 1.5 min with 30 cycles for bacteria. PCR of archaea was performed followed 23 cycles with 94 °C for 45 s, 55 °C for 45 s, 72 °C for 60 s. The PCR products were firstly purified and ligated into pMD18-T simple vectors (Takara, Japan). Recombinants were selected using X-Gal-IPTG LB indicator plates with 100 μ g/ml ampicillin. White colonies were randomly picked and tested by PCR using vector of M13 primer. The successful ones were used for sequencing.

Phylogenetic analysis

Sequences were searched against the Ribosomal Database Project II (RDP II) release 9.49 and the GenBank database ([http://](http://www.ncbi.nlm.nih.gov/blast/)

www.ncbi.nlm.nih.gov/blast/) by using the BLASTn program to determine the nearest matches. The sequences sharing 97% or great similarity were considered to represent the same operational taxonomic unit (OTU) using software DOTUR (Schloss and Handelsman, 2005).

Results and discussion

Performance of UASB reactor under increasing OLR

As shown in Fig. 3(a, b), the COD removal efficiency was kept in the range of 78.5–86.1% with the increase of OLR from 1.0 to 7.0 g COD/l/day corresponding to an HRT decrease from 3.5 to 0.5 days. In parallel, the methane yield rate increased from 0.2 to 1.4 l/l/day (Fig. 3(c)). Meanwhile, almost no p-ASC was detected in the effluent (Fig. 4(c)), and the percentage of methane in the biogas was between 68% and 72% (Fig. 3(c)). Although sulfated aromatic amines have long been considered not readily biodegradable (Lange et al., 1995), the above results indicate that the UASB reactor was very efficient in treating the p-ASC-containing wastewater without acclimation of operation time. This could be attributed to the use of granule sludge from another UASB reactor previously treating pharmaceutical wastewater containing p-ASC where p-ASC-degrading microorganism was progressively acclimated with the extension of treatment process. Further increase of OLR to around 12.0 g COD/l/day led to a decrease in COD removal. However, even at an OLR as high as around 24.0 g COD/l/day and HRT of 0.15 day, a p-ASC removal efficiency of 93.4% was achieved (Fig. 4(c)) whereas the COD removal efficiency was only 52.5% (Fig. 3(a, b)). The reason for deterioration of the reactor performance under reducing HRT was that the contact time between wastewater and sludge was too short to allow sufficient biodegradation (Hazrati and Shayegan, 2011). The methane content in the biogas was still proximately 60% (Fig. 3(c)). As shown in Fig. 3(d), acetate was the dominant VFA species, varying from approximately 250 to 670 mg/l over the whole period. Isopropanol, isobutyric acid, isovaleric acid and isopentanoic acid were detected in much lower concentrations (data not shown). At the same time, the effluent pH varied between 7.6 and 7.0 (Fig. 3(f)), suggesting that no significant inhibition took place in the reactor.

Variations of sulfate and sulfide in the effluent (Fig. 3(e)) with the change of HRT over time further supported the bioconversion of p-ASC. No sulfate was fed to this reactor during the whole process. Besides, the higher pH observed for effluent can be explained by the generation of alkalinity in the form of bicarbonate during the process of sulfate reduction by sulfate-reducing bacteria (SRB). It is speculated that the sulfonyl group linked to the aromatic group was cleaved and forms a sulfonic group, because sulfonyl group readily hydrolyzes in an aquatic environment (Su and Yang, 2002). Moreover, microorganism also involved the desulfonation process, which will be discussed later.

The above result demonstrated that anaerobic treatment was effective for p-ASC-containing wastewater. Even at an OLR as high as around 24.0 g COD/l/day and HRT of 0.15 day, most of p-ASC was removed. A summary of some of aromatic sulfonates (including sulfonated aromatic amines) removal in anaerobic conditions in some literature reviewed is presented in Table 2. Obviously, sulfonated aromatic amines are difficult to be degraded anaerobically. The pathway of p-ASC degradation in this anaerobic treatment isn't clear. According to the anaerobic degradation of aniline (structurally similar to p-ASC) by sulfate-reducing bacteria (Schnell and Schink, 1991), it can be speculated that the pathway of p-ASC degradation can mainly consist of five steps. The first step is the desulfonation process to cleave the sulfonyl group from the aromatic group, which is achieved by the combination of hydrolysis

Fig. 3. Overall performance of the UASB in continuous operation: (a) influent, effluent COD variation and COD removal efficiency; (b) OLR variation; (c) CH₄ gas production rate variation and CH₄ gas percentage; (d) effluent VFA variation; (e) sulfate and dissolved sulfide in effluent; (f) pH changes.

process and degradation by microorganism (phyla *Clostridia*, mentioned later). Then, the second step seems to be carboxylation to 4-acetamidobenzoate, followed by activation of 4-acetamidobenzoate to 4-acetamidobenzoyl-CoA. The subsequent step will be reductive elimination of the acetamido group and enters the normal benzoate degradation pathway, finally leading to three acetyl-CoA. The pathway of p-ASC degradation under anaerobic condition need be further studied.

Influence of p-ASC concentration on reactor performance

To investigate the influence of p-ASC concentration on reactor performance, the reactor was firstly operated at an OLR of

7.0 g COD/l/day and HRT of 0.65 days from 131 day to 150 day (Stage II) to restore its performance (Stage II). As shown in Fig. 3(a), the COD removal efficiency quickly increased to 79.0% three days after the change of the operational conditions. The quick recovery further indicated that the microbial community was not seriously inhibited even under an OLR of around 24.0 g COD/l/day.

At the beginning of Period III (day 131), the reactor was fed with 400 mg/l of p-ASC and same ethanol concentration as Period II, providing a COD and p-ASC loading rate of around 3900 mg/l and 795.2 mg/l/day at an HRT of 0.5 days, respectively (Figs. 3(a, b), 4(a, b) and Table 1). A slight rise in the methane production rate to 1.55 l/l/day indicated that p-ASC was not removed only by sorption process on granule. Actually, adsorption role was very limited for p-

Fig. 4. (a) The concentration of p-ASC in the influent and p-ASC loading rate (mg/l/day) in the continuous operation. (b) p-ASC loading rate in the continuous operation. (c) The concentration of p-ASC in the effluent and removal efficiency of p-ASC.

Table 2

Anaerobic systems treating some of aromatic sulfonates in literature.

Anaerobic system	Compounds	Influent	Removal efficiency (%)	HRT	References
UASB	Acid Orange 7 ^a	300 mg/l	96	14 h	Carvalho et al., 2008
UASB	Acid Orange 7	300 mg/l	>88	24 h	Brás et al., 2005
UASB	Direct Red 254 ^b	300 mg/l	>88	24 h	Same as above
UASB	LAS	4–5 mg/l	64–85	24 h	Sanz et al., 2003
UASB	LAS	14 mg/l	35 ± 12	12 h	Duarte et al., 2010
UASB	LAS	18.8 ± 4.2 mg/l	99 ± 2	18 h	de Oliveira et al., 2010
Batch	TS ^c	30 µM	100	–	Denger et al., 1996
Batch	SPO ^d	30 µM	100	–	Same as Above
Batch	3-A-4-HBS ^e	100–200 mg/l	0	–	Tan et al., 2005
Batch	1-AN-5-S ^f	100–200 mg/l	0	–	Same as Above
Batch	2-AN-1,5-DS ^g	100–200 mg/l	0	–	Same as Above
Batch	3-AN-2,7-DS ^h	100–200 mg/l	0	–	Same as Above

Trial names not explained in the text: Acid Orange 7^a, sodium 4-[(2E)-2-(2-oxonaphthalen-1-ylidene)hydrazinyl]benzenesulfonate; Direct Red 254^b, (3E)-7-amino-4-oxo-3-[[4-[(4-sulfophenyl)diazenyl]phenyl]hydrazinylidene]-naphthalene-2-sulfonic acid; TS^c, 4-tolysulfonate; SPO^d, 1-(4-sulfophenyl)octane; 3-A-4-HBS^e, 3-amino-4-hydroxybenzenesulfonic acid; 1-AN-5-S^f, 1-aminonaphthalene-5-sulfonic acid; 2-AN-1,5-DS^g, 2-aminonaphthalene-1,5-disulfonic acid; 3-AN-2,7-DS^h, 3-aminonaphthalene-2,7-disulfonic acid.

ASC removal and can almost be neglected. In a two-week continuous column study conducted in our research group, p-ASC adsorption capacity on unadapted granule sludge was only around 0.12 mg p-ASC/g VSS (Data not shown). Starting at day 151, the UASB received more p-ASC, up to 1000 mg/l (the p-ASC loading was as high as 1988 mg/l/day), to evaluate the system performance under concentration shock (Fig. 4(a, b) and Table 1). Generally, a wide range of organic compounds has been reported to be toxic to various microbial groups involved in the methanogenesis process (Chen et al., 2008). According to Sponza and Demirden (2007), the specific methanogenic activity (SMA) of granular sludge was found to decrease by 46% if the sulfamerazine was increased to 90 mg/l. In another previous experiment conducted by Tsuno and Kawamura (2009), almost no methane production was observed upon increasing the terephthalate concentration up to 430 mg/l. In the case of aromatic sulfonates, inhibition by LAS (>10 g/kg dry sludge) on anaerobic process was also observed (Garcia et al., 2005). Clearly, inhibition concentration ranges varied widely for specific toxicants. In this work, with the increase of p-ASC concentration to 400 and 1000 mg/l (Fig. 4(b)), the COD removal decreased from 74.6% to 63.7%, respectively, whereas the p-ASC removal efficiency decreased to 70% and 38%. However, the increase of methane production rate and the relatively low VFA concentrations suggested that inhibition of methanogens due to an increase in the p-ASC concentration did not occur in the system. The decrease in the p-ASC removal efficiency with increasing p-ASC concentration (Fig. 4(b, c)) was perhaps due to the lack of sufficient p-ASC degrading bacteria because of the low growth rates of these bacteria. In a previous study, it was also shown that sulfonated aromatic amine, such as 1-aminonaphthalene-4-sulfonic acid etc., did not exhibit toxicity to the anaerobic microbes even at a concentration up to 1000 mg/l (Tan et al., 2005). The presence of an ionizable sulfonate group will result in the sulfonated aromatic amines highly soluble. Therefore, bacteria are not easily taken up by these compounds (Tan et al., 2005). In textile industries, sulfonated azo dyes are commonly used since they bond more strongly to the fiber and have lower toxicity than their non-sulfonated analog (Gan et al., 2011). Besides, the lower hydrophobicity of aromatic amines leads to less inhibitory among N-substituted aromatics, which are

generally reactive toxicants (Razo-Flores et al., 1997; Chen et al., 2008). Chen (2006) combined chemostat pulse technique (CPT) and a dose–response analysis to quantitatively determine the toxicity ranking of model aromatic amines (MAAs) to *Pseudomonas luteola*. The result suggested the relative toxicity series of MAAs were *ortho* > *meta* > MAA-free control > *para* position (for isomeric aminophenols) through the CPT at 200 mg/l MAAs. Based on the molecular formula of p-ASC (Fig. 1), it can be speculated that p-ASC has lower toxicity.

Treatment of p-ASC-containing wastewater without ethanol

Usually, organic solvents (such as methanol or ethanol, etc.) are employed in large quantities in the pharmaceutical industry. Ethanol, used as a co-substrate in this study, was almost completely degraded throughout the experiment (data not shown). Short-chain alcohols, such as ethanol, can be easily degraded under both aerobic and anaerobic conditions by microbial enzymes associated with central microbial metabolic pathways (Hunt et al., 1997a). In the process of anaerobic digestion of wastewater containing complex organics, the co-substrates are often fundamental for microbial community adaptation and for the maintenance of a diverse microbial community (Okada et al., 2013).

As shown in (Fig. 4(c)), the removal efficiency of p-ASC actually increased from 68.4% to 77.4% when ethanol was removed from the synthetic wastewater, showing that p-ASC could be utilized as the sole carbon and energy source. This result was in accordance with the finding of a previous study (Sanz et al., 2003) showing that the withdrawal of co-substrates resulted in an increase in the degradation efficiency of LAS from 64% to 85% in an UASB reactor.

Microbial composition analysis

An archaeal clone library and a bacterial one were constructed using the sludge sample taken before the end of the experiment (day 190), respectively. Of 100 clones (12 OTUs), the archaeal community could be divided into seven kinds of phylogenetic groups (Table 3). The aceticlastic methanogens including the genus *Methanosaeta* (6 OTUs) and *uncultured archaeon* (2 OTUs) were dominant, accounting for 52% and 32% of total clones, respectively. The founding was in accordance with the fact that acetate was the dominant VFA during the whole experimental period (de Lucena et al., 2011). Three OTUs (36.0% of total clones) were clustered in “Unknown *Methanosaeta* sp.” indicating that the environment favored the growth of some novel aceticlastic methanogens. Two OTUs (12.0% of total clones) were assigned to *Methanosaeta harundinacea*, a mesophilic specie isolated from a UASB reactor treating beer-manufacturing wastewater, and produced methane exclusively from acetate (Ma et al., 2006). The *Methanosarcinales*-like group has been reported to be able to utilize wide range of H₂, CO₂, methanol, acetate and mono-, di-, and trimethylamines (Mah and Kuhn, 1984).

Table 3
Archaea composition of the granular sludge.

Phylogenetic group	OTUs ^a	No. of clones	Percentage (%)
<i>Methanobacterium Beijngensis</i>	1	1	1
<i>Uncultured crenarchaeote</i>	1	1	1
<i>Methanosarcinales archaeon</i>	2	14	14
<i>Methanosaeta harundinacea</i>	2	12	12
<i>Methanosaeta concilii</i>	1	4	4
<i>Uncultured Methanosaeta</i> sp.	3	36	36
<i>Uncultured archaeon</i>	2	32	32

^a OTUs were defined based on 97% sequence similarity (archaea) of 16S rDNA clones.

Table 4
Bacteria composition of the granular sludge.

Phylum	Class	OTUs ^a	No. of clones	Percentage (%)
<i>Firmicutes</i>	<i>Erysipelotrichia</i>	1	2	2.3
	<i>Clostridia</i>	2	6	7.1
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	8	31	36.5
<i>Chlorobi</i>	<i>Chlorobia</i>	1	1	1.1
<i>Chloroflexi</i>	<i>Anaerolineae</i>	1	8	9.4
<i>Acidobacteria</i>	<i>Holophagae</i>	1	1	1.1
<i>Bacteroidetes</i>	<i>Sphingobacteria</i>	1	14	16.5
<i>Thermotogae</i>	<i>Thermotogae</i>	2	15	17.7
<i>Planctomycetes</i>	<i>Planctomycetacia</i>	1	4	4.7
<i>Spirochaetes</i>	<i>Spirochaetes</i>	1	3	3.5

^a OTUs were defined based on 97% sequence similarity (bacteria) of 16S rDNA clones.

Of total 85 bacterial clones (19 OTUs) identified (Table 4), 9 phyla were detected with *Proteobacteria* (8 OTUs, 36.5% of total clones) as the dominant one. As shown in Table 5, the phylum *Proteobacteria* was represented by one class closely related to *Deltaproteobacteria*, mainly composed of the *Desulfovibrio* genus (12.9% of total clones) followed by *Syntrophobacter* (8.2% of total clones), *Desulfobacca* (7.1% of total clones), *Smithella* (4.7% of total clones), *Desulfovira* (2.4% of total clones), and *Syntrophus* (1.2% of total clones). In most of the sulfate-rich environments, the *Deltaproteobacteria* always constitute a big part of the microbial community (Lin et al., 2011). *Desulfovibrio* species can utilize wide range of organic substrates including lactate, pyruvate, and ethanol as electron donors for sulfate reduction and alternately utilize hydrogen as the sole energy (Odom and Peck, 1981). However, *Desulfovibrio* is not able to utilize aromatic compounds (Rabus et al., 1996) or acetate directly as electron donors (Kunapuli et al., 2010). Thus, in this work, *Desulfovibrio* mainly utilized ethanol as electron donor and converted it into acetate during sulfate reduction. Some of *Desulfovira* species, such as *Desulfovira adipica*, can completely oxidize adipate in the presence of electron acceptors, such as sulfate, sulfite, thiosulfate and element sulfur (Tanaka et al., 2000). The presence of *Syntrophobacter* species (8.2% of total clones), which could transfer propionate to acetate and CO₂ in syntrophic co-culture with methanogens (Chen et al., 2005), may explain the low concentration of propionate in the reactor. The abundance of *Syntrophobacter* in this reactor is thought to be closely related to the abundance of *Desulfovibrio* species since the co-culture of their growth groups is favored (Tanaka et al., 2000). Some of *Smithella* species, such as *Smithella propionica*, have been reported to have the ability to degrade aromatics (Liu et al., 1999). Similarly, *Syntrophus*-related species could degrade fatty acids and benzoate in syntrophic association with hydrogen-using microorganisms (Jackson et al., 1999). Thus, the presence of these *Smithella* and *Syntrophus*-related species is believed to closely associate with p-ASC degradation in this study.

Table 5
Genera in class *Deltaproteobacteria*.

Phylum	Class	Genus	OTUs ^a	No. of clones	Percentage (%)
<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Smithella</i>	1	4	4.7
		<i>Syntrophus</i>	1	1	1.2
		<i>Syntrophobacter</i>	2	7	8.2
		<i>Desulfovibrio</i>	2	11	12.9
		<i>Desulfovira</i>	1	2	2.4
		<i>Desulfobacca</i>	1	6	7.1

^a OTUs were defined based on 97% sequence similarity (bacteria) of 16S rDNA clones.

Among the remaining phyla, Phylum *Thermotogae* (2 OTUs, 17.7% of total clones) has been associated with benzene degradation under methanogenic conditions (Chang et al., 2005). *Sphingobacterium* sp. ATM strain belonging to the phylum *Bacteroidetes* (1 OTU, 16.5% of total clones) was found to decolorize the dye and textile wastewater (Tamboli et al., 2010). The class *Clostridia* within *Firmicutes* (3 OTUs, 9.4% of total clones) has been reported in a previous experiment for LAS degradation under anaerobic conditions (Duarte et al., 2010). Cao et al. (2003) found that *Clostridium pasteurianum* strain DSM 12136 could use the sulfonate group of 4-toluenesulfonate as a source of sulfur. *Clostridium beijerinckii* strain EV4 was also observed to desulfonate alkyl- and arylsulfonates (Chien, 2005). Therefore, the *Clostridia*-like clones may also be responsible for the desulfonation of p-ASC. Successful degradation requires a consortium of bacteria that act on different parts of the molecule (Mogensen et al., 2003).

Conclusion

This study demonstrated that the UASB reactor can achieve almost the complete removal of 50 mg/l p-ASC in the presence of ethanol as the co-substrate at an OLR of 7.0 g COD/l/day and HRT of 0.5 days. A concentration of up to 1000 mg/l of p-ASC did not show any inhibition to anaerobic microorganisms. Simultaneous generation of sulfate and sulfide was observed though the reactor received no sulfate in the influent.

The anaerobic degradation of p-ASC was achieved with or without ethanol. The aceticlastic uncultured *Methanosaeta* sp. and uncultured archaeon were the dominant methanogens. Bacterial groups within the phyla *Proteobacteria* and *Thermotogae* might be mainly responsible for the degradation of benzene ring of p-ASC. The phylum *Clostridia* plays the role for the desulfonation of p-ASC.

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References

- APHA, 1998. Standard Methods for Examination of Water and Wastewater, 20th ed. American Public Association, New York.
- Brás, R., Gomes, A., Ferra, M.I.A., Pinheiro, H.M., Gonçalves, I.C., 2005. Monoazo and diazo dye decolourisation studies in a methanogenic UASB reactor. *J. Biotechnol.* 115, 57–66.
- Cao, X., Liu, X., Dong, X., 2003. *Alkaliphilus crotonatoxidans* sp. nov., a strictly anaerobic, crotonate-dismutating bacterium isolated from a methanogenic environment. *Int. J. Syst. Evol. Microbiol.* 53, 971–975.
- Carvalho, M.C., Pereira, C., Gonc-alves, I.C., Pinheiro, H.M., Santos, A.R., Lopes, A., Ferra, M.I., 2008. Assessment of the biodegradability of a monosulfonated azo dye and aromatic amines. *Int. Biodeterior. Biodegradation* 62, 96–103.
- Chang, W., Um, Y.S., Pulliam Holoman, T.R., 2005. Molecular characterization of anaerobic microbial communities from benzene-degrading sediments under methanogenic conditions. *Biotechnol. Prog.* 21, 1789–1794.
- Chellipani, S., Wilby, T., Yuzir, A., Sallis, P.J., 2011. Influence of organic loading on the performance and microbial community structure of an anaerobic stage reactor treating pharmaceutical wastewater. *Desalination* 271, 257–264.
- Chen, B.Y., 2006. Toxicity assessment of aromatic amines to *Pseudomonas luteola*: chemostat pulse technique and dose–response analysis. *Process Biochem.* 41, 1529–1538.
- Chen, S.Y., Liu, X.L., Dong, X., 2005. *Syntrophobacter sulfatireducens* sp. nov., a novel syntrophic, propionate-oxidizing bacterium isolated from UASB reactors. *Int. J. Syst. Evol. Microbiol.* 55, 1319–1324.
- Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. *Bioresour. Technol.* 99, 4044–4064.
- Chien, C.C., 2005. Arylsulfonates as sole source of sulfur for *Clostridium pasteurianum* DSM 12136. *J. Basic Microbiol.* 45, 274–278.
- de Lucena, R.M., Gavazza, S., Florencio, L., Kato, M.T., de Morais, M.A., 2011. Study of the microbial diversity in a full-scale UASB reactor treating domestic wastewater. *World J. Microbiol. Biotechnol.* 27, 2893–2902.
- de Oliveira, L.L., Costa, R.B., Okada, D.Y., Vich, D.V., Duarte, I.C.S., Silva, E.L., Varesche, M.B.A., 2010. Anaerobic degradation of linear alkylbenzene sulfonate (LAS) in fluidized bed reactor by microbial consortia in different support materials. *Bioresour. Technol.* 101, 5112–5122.
- Denger, K., Kertesz, M.A., Vock, E.H., Schon, R., Magli, A., Cook, A.M., 1996. Anaerobic desulfonation of 4-tolylsulfonate and 2-(4-sulphophenyl) butyrate by a *Clostridium* sp. *Appl. Environ. Microbiol.* 62, 1526–1530.
- Duarte, I.C.S., Oliveira, L.L., Saavedra, N.K., Fantinatti-Garboggini, F., Menezes, C.B.A., Oliveira, V.M., Varesche, M.B.A., 2010. Treatment of linear alkylbenzene sulfonate in a horizontal anaerobic immobilized biomass reactor. *Bioresour. Technol.* 101, 606–612.
- Gan, H.M., Shahir, S., Ibrahim, Z., Yahya, A., 2011. Biodegradation of 4-aminobenzenesulfonate by *Ralstonia* sp. PBA and *Hydrogenophaga* sp. PBC isolated from textile wastewater treatment plant. *Chemosphere* 82, 507–513.
- Garcia, M.T., Campos, E., Ribisa, I., Latorre, A., Sanchez-Leal, J., 2005. Anaerobic digestion of linear alkylbenzene sulfonates: biodegradation kinetics and metabolite analysis. *Chemosphere* 60, 1636–1643.
- Hazrati, H., Shayegan, J., 2011. Optimizing OLR and HRT in a UASB reactor for pre-treating high-strength municipal wastewater. *Chem. Eng. Commun.* 24, 1285–1290.
- Hunt, C., Alvarez, P., dos Santos Ferreira, R., Corseuil, H., 1997a. Effect of ethanol on aerobic BTEX degradation. In: Alleman, B.C., Leeson, A.L. (Eds.), *In Situ and Onsite Bioremediation*. Battelle Press, Columbus, OH, pp. 49–54.
- Jackson, B.E., Bhupathiraju, V.K., Tanner, R.S., Woese, C.R., McInerney, M.J., 1999. *Syntrophus aciditrophicus* sp. nov., a new anaerobic bacterium that degrades fatty acids and benzoate in syntrophic association with hydrogen-using microorganisms. *Arch. Microbiol.* 171, 107–114.
- Kunapuli, U., Jahn, M.K., Lueders, T., Geyer, R., Heipieper, H.J., Meckenstock, R.U., 2010. *Desulfotobacterium aromaticivorans* sp. nov. and *Geobacter toluenoxidans* sp. nov., iron-reducing bacteria capable of anaerobic degradation of monoaromatic hydrocarbons. *Int. J. Syst. Evol. Microbiol.* 60, 686–695.
- Lange, F.T., Wenz, M., Brauch, H.J., 1995b. Race-level determination of aromatic sulfonates in water by on-line ion-pair extraction/ion-pair chromatography and their behavior in the aquatic environment. *J. High Resolut. Chromatogr.* 18, 243–252.
- Lin, W.T., Luo, J.F., Guo, Y., 2011. Comparison and characterization of microbial communities in sulfide-rich wastewater with and without propidium mono-azide treatment. *Curr. Microbiol.* 62, 374–381.
- Liu, Y., Balkwill, D.L., Aldrich, H.C., Drake, G.R., Boone, D.R., 1999. Characterization of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov., sp. nov. and *Syntrophobacter wolnii*. *Int. J. Syst. Bacteriol.* 49, 545–556.
- Ma, K., Liu, X.L., Dong, X.Z., 2006. *Methanosaeta harundinacea* sp. nov., a novel acetate-scavenging methanogen isolated from a UASB reactor. *Int. J. Syst. Evol. Microbiol.* 56, 127–131.
- Mah, R.A., Kuhn, D.A., 1984. Transfer of the type species of the genus *Methanococcus* to the genus *Methanosarcina*, naming it *Methanosarcina mazei* (Barker 1936) comb. nov. et emend. and conservation of the genus *Methanococcus* (Approved Lists 1980) with *Methanococcus vannielii* (Approved Lists 1980) as the type species. *Int. J. Syst. Bacteriol.* 34, 263–265.
- Mogensen, A.S., Haagsen, F., Ahring, B.K., 2003. Anaerobic degradation of linear alkylbenzene sulfonate. *Environ. Toxicol. Chem.* 22, 706–711.
- Odom, J.M., Peck Jr., H.D., 1981. Hydrogen cycling as a general mechanism for energy coupling in the sulfate-reducing bacteria. *Desulfovibrio* sp. *FEMS Microbiol. Lett.* 12, 47–50.
- Okada, D.Y., Delforno, T.P., Estenves, A.S., Sakamoto, I.K., Duarte, I.C.S., Varesche, M.B.A., 2013. Optimization of linear alkylbenzene sulfonate (LAS) degradation in UASB reactors by varying bioavailability of LAS, hydraulic retention time and specific organic load rate. *Bioresour. Technol.* 128, 125–133.
- Prats, D., Ruiz, F., Vazquez, B., Rodriguez-Pastor, M., 1997. Removal of anionic and nonionic surfactants in a wastewater treatment plant with anaerobic digestion. A comparative study. *Water Res.* 31, 1925–1930.
- Rabus, R., Fukui, M., Wilkes, H., Widdel, F., 1996. Degradative capacities and 16S rRNA-targeted whole-cell hybridization of sulfate-reducing bacteria in an anaerobic enrichment culture utilizing alkylbenzenes from crude oil. *Appl. Environ. Microbiol.* 62, 3605–3613.
- Razo-Flores, E., Donlon, B., Field, J.A., Lettinga, G., 1996. Biodegradability of N-substituted aromatics and alkylphenols under methanogenic conditions using granular sludge. *Water Sci. Technol.* 33, 47–57.
- Razo-Flores, E., Donlon, B., Lettinga, G., Field, J.A., 1997. Biotransformation and biodegradation of N-substituted aromatics in methanogenic granular sludge. *FEMS Microbiol. Rev.* 20, 525–538.
- Sanz, J.L., Culubret, E., de Ferrer, J., Moreno, A., Berna, J.L., 2003. Anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in upflow anaerobic sludge blanket (UASB) reactors. *Biodegradation* 14, 57–64.
- Schloss, P.D., Handelsman, J., 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl. Environ. Microbiol.* 71, 1501–1506.
- Schnell, S., Schink, B., 1991. Anaerobic aniline degradation via reductive deamination of 4-aminobenzoyl-CoA in *Desulfobacterium anilini*. *Arch. Microbiol.* 155, 183–190.

- Sponza, D.T., Demirden, P., 2007. Treatment of sulfamerazine in sequential upflow anaerobic sludge blanket reactor (UASB)/completely stirred tank reactor (CSTR) processes. *Sep. Sci. Technol.* 56, 108–117.
- Su, Y.X., Yang, J.Q., 2002. Improved technology for synthesis of p-acetanmidobenzenesulfonyl chloride. *J. Hebei Norm. Univ., Nat. Sci. Ed.* 26, 162–164.
- Sun, D.Y., Guan, X.Y., 2009. Elimination of interference on COD measurement by $K_2Cr_2O_7$ method. *Environ. Sci. Manag.* 34, 124–126 (in Chinese).
- Tamboli, D.P., Kagalkar, A.N., Jadhav, M.U., Jadhav, J.P., Govindwar, S.P., 2010. Production of polyhydroxyhexadecanoic acid by using waste biomass of *Sphingobacterium* sp. ATM generated after degradation of textile dye Direct Red 5B. *Bioresour. Technol.* 101, 2421–2427.
- Tan, N.C.G., van Leeuwen, A., van Voorthuizen, E.M., Slenders, P., Prenafeta-Boldú, F.X., Temmink, H., Lettinga, G., Field, J.A., 2005. Fate and biodegradability of sulfonated aromatic amines. *Biodegradation* 16, 527–537.
- Tan, S., Yang, Y., Luo, Z.P., Zhao, S., Huang, D.F., Zhang, J., Dong, L.C., Wang, G., 2011. An alternative synthetic process of p-acetaminobenzenesulfonyl chloride through combined $HCISO_3$ and PCl_5 . *Chem. Pap.* 65, 510–518.
- Tanaka, K., Stackebrandt, E., Tohyama, S., Eguchi, T., 2000. *Desulfovira adipica* gen. nov., sp. nov., an adipate-degrading, gram negative, sulfate reducing bacterium. *Int. J. Syst. Bacteriol.* 50, 639–644.
- Tsuno, H., Kawamura, M., 2009. Development of an expanded-bed GAC reactor for anaerobic treatment of terephthalate-containing wastewater. *Water Res.* 43, 417–422.
- Zerbinati, O., Vincenti, M., Pittavino, S., Gennaro, M.C., 1997. Fate of aromatic sulfonates in fluvial environment. *Chemosphere* 35, 2295–2305.