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Evaluation of the microbial community of upflow anaerobic sludge blanket reactors used for the removal and degradation of linear alkylbenzene sulfonate by pyrosequencing

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ABSTRACT

The microbial communities from two upflow anaerobic sludge blanket (UASB) reactors treating synthetic wastewater supplemented with linear alkylbenzene sulfonate (LAS) (RS) and laundry wastewater (RL) were analyzed by pyrosequencing of 16S rRNA genes. A higher LAS degradation rate was observed in RL (82 ± 9%) than RS (45 ± 16%). A high proportion of the LAS removal rate (55–90%) was observed in the PS region for both reactors, most likely due to the low concentration of co–substrates and oxygen diffusion in this region. A microbiological analysis of samples taken at 112 days of operation from the sludge blanket (SB) and the phase–separator (PS) region of both reactors confirmed these findings. The distinct microbial communities found in each reactor resulting from the different wastewaters used were related to the LAS degradation rates obtained. The microbial community from reactor RL was more capable of degrading LAS, most likely because of the presence of xenobiotics.

Introduction

Linear alkylbenzene sulfonate (LAS) is widely used for domestic and industrial purposes, which leads to its appearance in wastewater treatment plants at concentrations ranging from 1 to 18 $mg.L^{-1}$ (Mungray and Kumar, 2009). Amounts of 1,040,000 t/year of LAS are produced in the U.S.A., Japan and Western Europe (Scott and Jones, 2000). The presence of LAS in wastewater could be problematic for applying anaerobic technology, as the recalcitrance and inhibition of LAS has been reported in anaerobic systems (Garcia et al., 2005). Additionally, the presence of LAS in aquatic environments can lead to foaming in streams and rivers, dispersing pollution. As a result, many studies have been performed to evaluate the degradation of LAS under anaerobic conditions (Almendariz et al., 2001; Okada et al., 2013).

The LAS degradation pathway involves aromatic ring cleavage, desulfonation, β– and ω–oxidation reactions under aerobic and anaerobic conditions (Lara-Martin et al., 2007). Aerobic, facultative anaerobic and anaerobic organisms have been reported to be involved in the degradation of LAS. Some of the genera frequently reported to harbor species with this capability are Acinetobacter, Aeromonas, Comamonas, Dechloromonas, Desulfovibrio, Geobacter, Holophaga, Parvibaculum, Pseudomonas, Sporomusa, Stenotrophomonas, Variovorax and *Zoogloea* (Jimenez et al., 1991; Cook et al., 1998; Schleheck and Cook, 2005; Abboud et al., 2007; Lara-Martin et al., 2007; Oliveira et al., 2009, 2010; Schleheck et al., 2010; Duarte et al., 2010a,b; Delforno et al., 2012).

To identify the microorganisms involved in LAS degradation, the microbial community of reactors used to treat synthetic wastewater containing LAS has been described using cloning libraries and Sanger sequencing (Duarte et al., 2008, 2010a; Oliveira et al., 2010; Delforno et al., 2012). Nevertheless, it is difficult to detect key players of low abundance by these methods due to their low coverage. Currently, high throughput sequencing methods, such as pyrosequencing, are available to provide a better understanding of microbial communities. Additionally, few studies have evaluated the microbial community found in

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reactors treating wastewater other than synthetic wastewater containing LAS.

In previous work, the effects of the hydraulic retention time (HRT), bioavailability of LAS and co–substrate addition on LAS degradation in UASB reactors fed with synthetic wastewater were studied (Okada et al., 2013). The results showed that the highest degradation rate of LAS (76%) was related to the lowest specific organic loading rate (SOLR) (0.03 g COD.g $TVS^{-1}.d^{-1}$) (Okada et al., 2013). Denaturing gradient gel electrophoresis (DGGE) indicated that the microbial composition of the biomass developed in the phase separator section of the reactors differed from the microbial communities of the sludge blanket samples. This result was in accordance with the fact that most of the degradation of LAS occurs in the phase–separator region of the reactor (Okada et al., 2013). To understand this fact, a deep sequencing method was applied to study the microbial communities involved in LAS degradation, and the microbial communities from two different regions were analyzed: the phase–separator and sludge blanket from a UASB reactor. In addition, the microbial communities developed in reactors fed with real laundry wastewater and synthetic wastewater containing LAS were compared to understand the differences in LAS removal in each reactor. Although the LAS removal rate has previously been observed to be higher in the expanded granular sludge bed (EGSB) than in the UASB reactors when operated at similar conditions (at an HRT of 35 h, the rates were 64–76% and 37–76% in the EGSB and UASB reactors, respectively) (Delforno et al., 2012; Okada et al., 2013), the UASB arrangement was employed in this study because it develops a more distinct microbial community in the different regions of the reactor. In this work, two UASB reactors were operated at an HRT of 35 h; one reactor was fed synthetic wastewater supplemented with LAS, and the other reactor was fed real laundry wastewater. The microbial communities from the two reactors were evaluated using pyrosequencing of samples taken from the sludge blanket and the gas–separation compartments. The structures of the microbial communities were analyzed, and the role of the different microorganisms in LAS degradation is discussed.

Materials and methods

Reactor setup and operation

Two UASB reactors were operated using an HRT of 35 h (Fig. 1). The reactors consisted of borosilicate glass and steel components and had a total volume of 0.650 L.

The two reactors (RL and RS) were fed a synthetic substrate for 15 days. The synthetic feed was consisted of a mineral medium (Angelidaki et al., 1990) with $MgCl_2.6H_2O$ at a concentration of 25 $mg.L^{-1}$, a vitamin solution (Touzel and Albagnac, 1983), sodium bicarbonate ($NaHCO_3$: 400 $mg.L^{-1}$) and the following co–substrates: ethanol (30 mg $COD.L^{-1}$), methanol (30 mg $COD.L^{-1}$) and yeast extract (30 $mg.L^{-1}$). After 15 days, reactor RL was fed laundry wastewater that was diluted (1:25) with sodium bicarbonate (400 $mg.L^{-1}$) to obtain an LAS concentration of 11 ± 3 $mg.L^{-1}$. Reactor RS was continuously fed the described synthetic substrate, supplemented with LAS at a final concentration of 10 ± 3 $mg.L^{-1}$. The sodium bicarbonate concentration was increased to 1000 $mg.L^{-1}$ to maintain a neutral pH (Table 1). The LAS used was synthesized by Aldrich (CAS 25155–30–0: technical grade). Each reactor was fed wastewater containing LAS for 97 days, for a total of 112 days of operation.

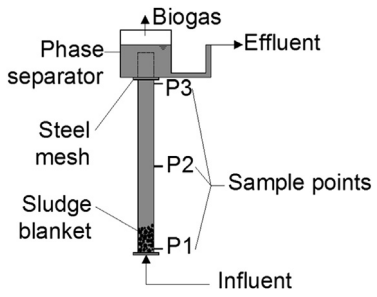

Fig. 1. Setup of the UASB reactor.

The reactors were inoculated with granular sludge taken from a methanogenic UASB reactor from the wastewater treatment system of a poultry slaughterhouse (Avicola Dacar/Tietê, SP, Brazil). The final concentration of biomass was 0.45 ± 0.09 and 0.50 ± 0.09 g $TVS.L^{-1}$ in reactors RL and RS, respectively.

On the 97th day of operation, samples were taken from the influent, effluent and sample points (P1, P2 and P3) to analyze the spatial variations of LAS and volatile fatty acids (VFA) in the reactors. Resazurin (0.1%, Aldrich) was added to the influent as a redox indicator.

Samples from the biomass in the sludge blanket and phase–separator regions were taken on the last day of operation (112^{th}) for microbiological analysis.

Laundry wastewater

The laundry wastewater used in the present study was taken from a commercial laundry facility located in São Carlos/SP, Brazil. The wastewater was collected from the first rinse without softener, which could contain some compounds that are toxic to the anaerobic process, such as quaternary ammonium compounds (Ying, 2006). The wastewater was collected in polyethylene terephthalate (PET) flasks and stored at 4 °C. Detergents and neutralizer–acidulant products were used in this commercial laundry facility. The detergent composition consisted of LAS, nonionic surfactants, builders, bleaching agents, enzymes, fragrance, neutralizers and alkalizing agents (sodium hydroxide). The neutralizer–acidulant consisted of sodium sulfate, sodium metabisulfite and an alkaline vehicle. The chemical composition of the wastewater was determined as described in the chemical analysis section.

Analytical methods

LAS content was determined by high performance liquid chromatography (HPLC) utilizing a *Shimadzu* system (Shimadzu Co., Kyoto, Japan) with a reversed–phase C8 column (*Supelco*), according to the method described by Duarte et al. (2006). LAS adsorbed onto the biomass was extracted in methanol by employing an ultrasonic bath (Duarte et al., 2008) and was analyzed by HPLC (Duarte et al., 2006).

The volatile fatty acids (acetate, butyrate, isobutyrate, caproic, formic, propionate, lactic, malic, succinic, valeric and isovaleric) were quantified by HPLC using a *Shimadzu* system (Shimadzu Co.,

Table 1
Feed composition for reactors RL and RS from the 15th to 112th day of operation.

	RL	RS
Wastewater	Laundry	Synthetic
Ethanol ($mg\ COD.L^{-1}$)	–	30
Methanol ($mg\ COD.L^{-1}$)	–	30
Yeast extract ($mg.L^{-1}$)	–	30
Sodium bicarbonate ($mg.L^{-1}$)	400	1000
LAS ($mg.L^{-1}$)	11[a]	10

[a] LAS was provided by the laundry wastewater.