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Microbial characterization and degradation of linear alkylbenzene sulfonate in an anaerobic reactor treating wastewater containing soap powder

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HIGHLIGHTS

- The anaerobic degradation of liner alkylbenzene sulfonate (LAS) was evaluated.
- The fluidized bed reactor was operated with a hydraulic retention time of 15 h.
- LAS degradation was 48% and the VFA increased with its addition.
- *Dechloromonas* sp. and *Geobacter* sp. were identified in LAS degradation.
- The compounds of soap powder affected the LAS removal and volatile fatty acids.

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ABSTRACT

The aim of this study was to evaluate the removal of linear alkylbenzene sulfonate (LAS) in an anaerobic fluidized bed reactor (AFBR) treating wastewater containing soap powder as LAS source. At Stage I, the AFBR was fed with a synthetic substrate containing yeast extract and ethanol as carbon sources, and without LAS; at Stage II, soap powder was added to this synthetic substrate obtaining an LAS concentration of 14 ± 3 mg L^{-1}. The compounds of soap powder probably inhibited some groups of microorganisms, increasing the concentration of volatile fatty acids (VFA) from 91 to 143 mg HAc L^{-1}. Consequently, the LAS removal rate was 48 ± 10% after the 156 days of operation. By sequencing, 16S rRNA clones belonging to the phyla Proteobacteria and Synergistetes were identified in the samples taken at the end of the experiment, with a remarkable presence of *Dechloromonas* sp. and *Geobacter* sp.

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1. Introduction

Surfactants are widely used for domestic and industrial cleaning, emulsifying, and wetting agents. Linear alkylbenzene sulfonate (LAS) is the most frequently used anionic surfactant in the world, and its application in laundry and household cleaning represents approximately 80% of its market use (HERA, 2013). The concentration of this surfactant in wastewater treatment plants (WWTP) can range from 1.2 to 9.2 mg L^{-1} (Camacho-Muñoz et al., 2014). Because of its extensive use, LAS affects WWTP since the foam layers hinder the processes of the aeration tanks and carry many pollutants and bacteria over long distances. Furthermore, the recalcitrance of LAS has been related to the inhibition in anaerobic process (Garcia et al., 2005). Thus, LAS degradation has been studied in various anaerobic arrangements such as horizontal-flow anaerobic immobilized biomass (HAIB) (Duarte et al., 2008), stirred sequencing batch (Duarte et al., 2010a) , expanded granular sludge bed (EGSB) (Delforno et al., 2012), upflow anaerobic sludge blanket (UASB) (Lobner et al., 2005; Okada et al., 2013) and fluidized bed (Oliveira et al., 2010b) reactors.

The anaerobic fluidized bed reactor (AFBR) packed with sand has presented high rates of degradation (>90%) of anionic and non-ionic surfactants (Motteran et al., 2014; Oliveira et al., 2010b). The high efficiency of AFBR in LAS degradation is caused by the recirculation, which provides initial dilution of the effluent

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and increase the mass transfer. The dilution contributes to a decrease in the toxic effect, favoring the biological treatment of recalcitrant compounds (Garibay-Orijel et al., 2005).

The molecule of LAS consists of an aromatic ring sulfonated attached to a linear alkyl chain containing 10–14 carbon atoms. Under aerobic conditions, the degradation pathway involves the transformation into sulfophenyl carboxylic acids (SPC) by β- and ω-oxidation reactions, desulfonation reactions and cleavage of the aromatic ring. An anaerobic pathway for LAS degradation has been proposed by Lara-Martin et al. (2010), which consist of the transformation of LAS molecule via fumarate addition into SPC, which was degraded by successive β-oxidation reactions. Moreover, benzenesulfonic acid, benzaldehyde, toluene and benzene have been suggested as intermediates of LAS degradation under anaerobic condition (Duarte et al., 2008; Mogensen and Ahring, 2002).

Because of the complexity of LAS molecule and the reactions involved in its degradation, studies have been reported a higher efficiency of a microbial consortium to achieve LAS degradation (Abboud et al., 2007; Khleifat, 2006). Evidence from 16S rRNA gene sequencing indicated a broad microbial diversity related to arrangements treating wastewater containing LAS. Microorganisms belonging to the phyla Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Synergistetes have been found in anaerobic reactors employed for LAS removal (Delforno et al., 2012; Duarte et al., 2008; Oliveira et al., 2010b).

Until now, the wastewater treatment has been studied in anaerobic reactors with commercial mixture of LAS homologues (C_{10}–C_{13}) (Delforno et al., 2012; Oliveira et al., 2010b). There are few studies evaluating the LAS degradation using household cleaning products as surfactant source (Duarte et al., 2010a). Despite the significant contribution of soap powder to the LAS concentrations in sewage, the degradation of LAS provided by this product has not been studied. The soap powder contains other compounds in addition to LAS, such as builders, enzymes, optical brighteners, fillers, and dyes, which can make the biological removal of LAS quite difficult. Although the biological removal can be impaired by the soap powder compounds, LAS degradation can be achieved in the AFBR due to the dilution, which decreases the toxic effect of these compounds. Furthermore, the biological treatment requires lower input costs than the advanced oxidation processes, which makes the biological treatment a profitable alternative.

Thus, the present study aimed to evaluate the biodegradation of LAS using soap powder as surfactant source. The influence of soap powder compounds on LAS degradation was evaluated in an AFBR using sand as material support, at hydraulic retention time (HRT) of 15 h. The present study characterized the microbial community of the AFBR and compared the samples taken from the support material and biomass of the upper portion of the reactor.

2. Methods

2.1. Reactor setup and operation

The conditions employed in this study were similar to the applied by Oliveira et al. (2010b), who also treated wastewater containing LAS in an RALF using sand as support material. The AFBR was filled with 308 g of sand for the biomass immobilization. The applied support media was 1.4–1.7 mm in diameter with 2.3 g cm^{-3} density. The AFBR was made in acrylic, with 102 cm in length and 3.5 cm in internal diameter, totalizing a volume of 981 mL. The reactor was operated with an HRT of 15 ± 2 h, in a controlled temperature chamber (30 ± 2 °C). The recirculation flow-rate was 74 L h^{-1}. The apparatus setup is presented in Fig. 1.

At the first 15 days, the AFBR was kept in a closed circuit (recycling feeding without discharging) for biomass immobilization and adaptation to the synthetic substrate. Three liters of feed were

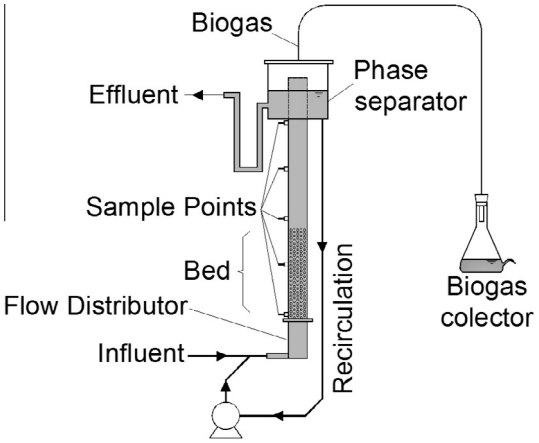

Fig. 1. Schematic representation of the AFBR.

prepared and consisted of a synthetic substrate with an anaerobic sludge (10% v/v). This anaerobic sludge was obtained from a full-scale UASB reactor treating poultry slaughterhouse wastewater (Avícola Dacar, Tietê, SP, Brazil). The seed sludge contained 45 g L^{-1} of volatile solids (VS). After 15 days, the system was opened, and the AFBR was operated for 156 days. The reactor operation was performed in two stages as follows: at Stage I, the AFBR was fed with a synthetic substrate without LAS; at Stage II, soap powder was added to the synthetic substrate, resulting in an LAS concentration of 14 ± 3 mg L^{-1}.

Duran® flasks of 5.0 L were utilized to store the substrate during the feeding of the reactor. The flasks were kept refrigerated at 4 °C. A rubber balloon was adapted to the feeding flask and filled with N_2 (100%).

2.2. Composition of the synthetic substrate

The synthetic substrate consisted of 400 mg L^{-1} of yeast extract, 103 mg L^{-1} of ethanol, 480 mg L^{-1} of sodium bicarbonate, and 5.0 ml L^{-1} of salt solution (50.0 g L^{-1} of NaCl, 1.4 g L^{-1} of $MgCl_2 \cdot 6H_2O$ and 0.9 g L^{-1} of $CaCl_2 \cdot 2H_2O$). LAS was provided by soap powder, which consisted of adjuncts, synergist, buffering, optical brightener, dyes, enzymes, charge, fragrance, polarzyme, lipex, in addition to anionic surfactant (LAS).

2.3. Chemical and chromatographic analysis

The physicochemical analyses of the pH and chemical oxygen demand (COD) of the influent and effluent samples were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA et al., 2005). The volatile fatty acids (VFA) were quantified using a high performance liquid chromatograph (HPLC) (Shimadzu Co., Kyoto, Japan) with an Aminex HPX-87H column (Biorad) (Lazaro et al., 2012).

The LAS determinations were conducted according to the methodology developed by (Duarte et al., 2006) using an HPLC (Shimadzu Co., Kyoto, Japan) with a fluorescence detector and a C8 column (Supelco).

2.4. Extraction of LAS adsorbed

At the end of the experiments, the samples of the solids (sludge, total effluent solids and media support with biofilm) were submitted to consecutive washing with methanol in ultrasound for 30 min to extract the adsorbed LAS based on protocol described by (Duarte et al., 2008). The extracted material was quantified by HPLC (Duarte et al., 2006).

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