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# Influence of support material on the immobilization of biomass for the degradation of linear alkylbenzene sulfonate in anaerobic reactors

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ABSTRACT

Two horizontal-flow anaerobic immobilized biomass reactors (HAIB) were used to study the degradation of the LAS surfactant: one filled with charcoal (HAIB1) and the other with a mixed bed of expanded clay and polyurethane foam (HAIB2). The reactors were fed with synthetic substrate supplemented with 14 mg $l^{-1}$of LAS, kept at $30 \pm 2$ °C and operated with a hydraulic retention time (HRT) of 12 h. The surfactant was quantified by HPLC. Spatial variation analyses were done to quantify organic matter and LAS consumption along the reactor length. The presence of the surfactant in the load did not affect the removal of organic matter (COD), which was close to 90% in both reactors for an influent COD of 550 mg $l^{-1}$. The results of a mass balance indicated that 28% of all LAS added to HAIB1 was removed by degradation. HAIB2 presented 27% degradation. Molecular biology techniques revealed microorganisms belonging the uncultured *Holophaga* sp., uncultured *delta Proteobacterium*, uncultured *Verrucomicrobium* sp., *Bacteroides* sp. and uncultured *gamma Proteobacterium* sp. The reactor with biomass immobilized on charcoal presented lower adsorption and a higher kinetic degradation coefficient. So, it was the most suitable support for LAS anaerobic treatment.



## 1. Introduction

Linear alkylbenzene sulfonate (LAS) is a tensoactive agent widely used in the production of detergents and is present in domestic and industrial wastewaters, representing more than 40% of all the surfactant used worldwide. Its worldwide consumption in 2000 was estimated at approximately 2.5 million tons (Sanz et al., 2003). Thus, special attention should be given to its occurrence and destination in the environment.

Commercial LAS is usually composed of a mixture of various homologues (carbon chains of different lengths – $C_{10}$–$C_{14}$). Each homologue contains a sulfonated aromatic ring in the *p*-position, linked to the linear alkyl chain in any position except to the terminal carbons.

This surfactant is usually considered biodegradable under aerobic conditions and non-biodegradable under anaerobic conditions. Its removal from wastewaters has been restricted to conventional biological treatments (Manousaki et al., 2004).

Among the technologies available for the treatment of wastewaters containing organic compounds, biological treatment is one of the most advantageous due to its relatively low cost in comparison to physicochemical treatments. Under biological treatment conditions, LAS can remain in water even, mainly due to the presence of the aromatic ring, which is resistant to degradation. However, high percentages of degradation (above 97%) have been achieved in some wastewater treatment systems using aerobic processes (Brunner et al., 1988). This removal is probably due to the phenomena of degradation and adsorption in biological sludge.

Scott and Jones (2000) suggested that aerobic microorganisms need syntrophic relations to degrade the surfactant. Some microorganisms probably oxidize the alkyl chain while others participate in the mineralization of the aromatic chain.

There is still a paucity of studies on LAS degradation under anaerobic conditions. Among the various bioreactor configurations used in the treatment of effluents, the bench-scale upflow anaerobic sludge blanket (UASB) reactor has been the one most frequently applied in the anaerobic treatment of LAS (Almendariz et al., 2001; Sanz et al., 2003).

In Brazil, however, Duarte et al. (2008, in press) evaluated LAS removal in horizontal-flow anaerobic immobilized biomass reactors (HAIB) fed with synthetic substrate. In that study, the reactors were filed with polyurethane foam as support material to immobilize different biomasses originating from UASB reactors treating domestic sewage and swine culture wastes. Their results

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demonstrated that approximately 35% of all LAS added to the reactors were removed by biological degradation for an influent concentration of 14 mg $l^{-1}$, after 313 days of operation and a hydraulic retention time (HRT) of 12 h.

A wide variety of support materials have been used on both laboratory bench-scale and industrial scale in anaerobic fixed-film reactors.

Modifications can be made to the support medium of anaerobic reactors to evaluate and compare the removal of LAS present in synthetic substrate, as well as to compare modifications in the microbial diversity responsible for this degradation.

Based on this information, this work purported to compare the efficiency of LAS removal in HAIBs filled with charcoal, expanded clay and polyurethane foam inoculated with anaerobic sludge from a UASB reactor used in the treatment of swine culture wastes. Thus, the application of different support materials in the biomass immobilization bed can serve to determine which of them is the most suitable for LAS removal. Moreover, it was intended to evaluate the microbial community present in each different support material.

## 2. Material and methods

### 2.1. Horizontal-flow anaerobic immobilized biomass reactor

The reactors, two horizontal-flow anaerobic immobilized biomass reactors (HAIBs), were made of borosilicate glass, 100 cm in length ($L$) and 5 cm internal diameter ($D$), with a length-diameter ($L/D$) ratio of 20 and a total volume of 2000 ml each (Fig. 1).

The reactors were kept in a controlled temperature chamber ($30 \pm 2$ °C) and operated with a hydraulic retention time (HRT) of 12 h in conditions presented in Table 2. Both reactors were inoculated with sludge from a UASB reactor treating swine culture wastes according Zaiat et al. (1994).

### 2.2. Support material for immobilization of the anaerobic sludge

One of the reactors (HAIB1) was filled with 425 mg charcoal broken up by hand into about 5-mm-side pieces. The bed of the second reactor (HAIB2) was filled with 160 mg expanded clay beads of about 10 mm diameter, occupying about 20% of its volume ($L/D$ of 0 and 4) and 19.6 mg polyurethane foam cut into 5-mm-side cubes (80% of the reactor's volume, $L/D$ of 4–20).

The characteristics of the applied support media are presented in Table 1.

### 2.3. Composition of the synthetic substrate

The surfactant used in this study was sodium dodecylbenzenesulfonate (Sigma), also known as commercial LAS, with a purity of 80%. The reactors were fed with a concentration of 14 mg $l^{-1}$ of surfactant. This concentration was chosen because is similar to that one observed in the sanitary sewage that is treated on the wastewater treatment plant of the University of São Paulo - São Carlos. The influent of the wastewater treatment plant were monitored everyday during 10 days and the mean concentration of LAS observed was 14 mg $l^{-1}$

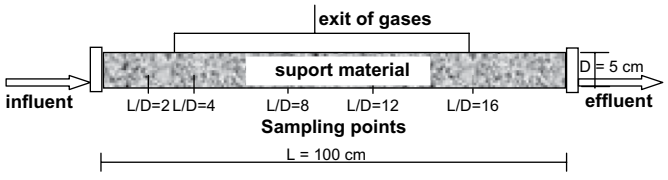


**Fig. 1.** Schematic representation of the HAIB reactor.

**Table 1**
Characteristics of the support material

| | Charcoal | Expanded clay | Polyurethane foam |
|---|---|---|---|
| Shape | *Pellet* irregular | Spherical | Cubic |
| Apparent density (g $ml^{-1}$) | 0.51 | – | 0.023 |
| Equivalent diameter (cm) | 0.5 | – | 0.6 |
| Porosity | 0.43 | – | 0.92 |
| Superficial area ($m^2$ $g^{-1}$) | 3.51 | 1.08 | 43.8 |

Source: CCDM – University Federal of São Carlos – UFSCar, Brazil. Certificate AMP05-000129.

The synthetic substrate was composed of yeast extract (500 mg $l^{-1}$), saccharose (80 mg $l^{-1}$), sodium bicarbonate (400 mg $l^{-1}$), and 0.5 ml of saline 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 $CaCl_2 \cdot 2H_2O$).

Duran flasks with 5.0 L for each reactor were utilized to storage the substrate during the feeding of the reactors. The flasks were kept on refrigeration (4 °C). A rubber balloon was adapted to the feeding flask and filled with $N_2$ (100%). Biomass growth and cloud points in the feeding flasks were not observed during all the operation of the reactors.

### 2.4. Sampling

The operating stages of the reactors were defined by different loading conditions, as indicated in Table 2.

Liquid samples of influent and effluent were analyzed twice a week. Chemical oxygen demand (COD), pH, methane, volatile acids, alkalinity and LAS were analyzed according to methods described in the Section 2.5. During the operation, liquid samples from sampling points were analyzed. The results allowed determining LAS, COD, sulfate, sulfide and organic acids concentrations along the length reactors. These analyzes were made with 31, 157, 266 and 344 days of operation (Table 2).

### 2.5. Chemical and chromatographic analysis

Physicochemical analyses of pH, chemical oxygen demand (COD) – raw and filtered through a 1.2 μm membrane, sulfate and sulfide were determined according to the Standard Methods for Examination of Water and Wastewater (APHA, 1998). Volumetric assessments of the total volatile fatty acids (TVFA) and the bicarbonate alkalinity (BA) were carried out as described by Dillalo and Albertson (1961) and adapted by Ripley et al. (1986). Volatile acids concentrations were determined by Gas Chromatograph HP 6890 (HP Innovax column × 30 m × 0.25 mm with internal diameter × 0.25 mm) according to Moraes et al. (2000).

The biogas composition (methane and carbon dioxide) was measured by Gas Chromatography (Gow-Mac chromatograph with thermal conductivity detector and PorapakQ column-2 m × 1/4 in 80–100 mesh). The injector, oven, and detector temperatures were 50 °C, 50 °C, and 80 °C, respectively. Hydrogen was used as the carrier gas (60 mL/min).

The LAS determination was achieved by HPLC (Shimadzu) using a fluorescence detector, C8 column (Supelco) with eluting gradient using methanol and sodium perchlorate (0.075 M), 0.5 ml × $min^{-1}$

**Table 2**
Operating stages of the reactors

| Stage | Load | Duration (days) | Spatial variation (day) |
|---|---|---|---|
| I | Synthetic substrate | 32 | 31 |
| II | Synthetic substrate + LAS | 276 | 157,266 |
| III | Synthetic substrate without Saccharose + LAS | 7 | – |
| IV | LAS + sodium bicarbonate + saline solution | 30 | 344 |