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Anaerobic degradation of linear alkylbenzene sulfonate (LAS) in fluidized bed reactor by microbial consortia in different support materials

Lorena Lima de Oliveira [a,*], Rachel Biancalana Costa [a], Dagoberto Yukio Okada [a], Daniele Vital Vich [a], Iolanda Cristina Silveira Duarte [b], Edson Luiz Silva [c], Maria Bernadete Amâncio Varesche [a]

[a] Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo, Av. Trabalhador Sãocarlense, 400, 13566-590 São Carlos, SP, Brazil
[b] Federal University of São Carlos, Rod. João Leme dos Santos, Km 110, SP 264, 18052-780 Sorocaba, SP, Brazil
[c] Department of Chemical Engineering, Federal University of São Carlos, Rod. Washington Luiz, Km 235, SP 310, 13565-905 São Carlos, SP, Brazil

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ABSTRACT

Four anaerobic fluidized bed reactors filled with activated carbon (R1), expanded clay (R2), glass beads (R3) and sand (R4) were tested for anaerobic degradation of LAS. All reactors were inoculated with sludge from a UASB reactor treating swine wastewater and were fed with a synthetic substrate supplemented with approximately 20 mg l^{-1} of LAS, on average. To 560 mg l^{-1} COD influent, the maximum COD and LAS removal efficiencies were mean values of 97 ± 2% and 99 ± 2%, respectively, to all reactors demonstrating the potential applicability of this reactor configuration for treating LAS. The reactors were kept at 30 °C and operated with a hydraulic retention time (HRT) of 18 h. The use of glass beads and sand appear attractive because they favor the development of biofilms capable of supporting LAS degradation. Subsequent 16S rRNA gene sequencing and phylogenetic analysis of samples from reactors R3 and R4 revealed that these reactors gave rise to broad microbial diversity, with microorganisms belonging to the phyla Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria, indicating the role of microbial consortia in degrading the surfactant LAS.

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1. Introduction

The motivation for this work is the evaluation of a novel technology for the removal and degradation of the surfactant linear alkylbenzene sulphonate (LAS) using fluidized bed anaerobic reactors. Most previous work in this area has focused on UASB and fixed-bed reactors (Almendariz et al., 2001; Sanz et al., 2003; Duarte et al., 2008; Oliveira et al., 2009).

Due to demand from researches in recent years in the area of anaerobic treatment, high-rate systems have been developed. They are characterized by their ability to retain large amounts of active biomass, even with low hydraulic retention times. Thus, it is possible to maintain high time of solids retention, even with the application of high hydraulic loads on the system. The result is compact reactors with volume below the anaerobic reactor with suspended growth, keeping, however, a high stabilization of sludge (Iza, 1991). The fluidized bed reactor is inserted in the high-rate systems with attached microbial growth.

Several factors contribute to the treatment efficiency in a fluidized bed reactor: (a) maximum contact between the liquid and the support medium, (b) diffusional resistance of the liquid film is minimal due to the particle movement and fluid velocity, (c) problems of preferential channels and packaging, commonly found in fixed bed, are avoided, (d) control and optimization of the biological film thickness, (e) it can be operated with a wide range of organic concentration and the degradation rates are proportional to this concentration, (f) improved stability and efficiency of organic matter removal when compared with upflow anaerobic sludge blanket reactor (UASB) (Buffière et al., 1995).

Duarte et al. (2008) used two horizontal anaerobic immobilized biomass (HAIB) reactors inoculated with sludge from different sources. Both sludges were immobilized in polyurethane foam support material, and the reactors were operated with hydraulic retention times (HRTs) of 12 h. The feed consisted of a synthetic substrate combined with 14 mg l^{-1} of LAS. After 145 days of operation, the organic substrate was removed, leaving LAS as the only carbon source for microorganisms. The results showed that the presence of LAS did not influence the organic matter removal, which was approximately 80% for both reactors. The organisms present were able to degrade LAS in both reactors despite having different origins. Mass balances indicated that the microorganisms present in the reactors degraded 35% of LAS after 313 days of operation. In the absence of other carbon sources such as sucrose, yeast extract and starch, the efficiency of surfactant removal was much

* Corresponding author. Tel.: +55 16 3373 8358; fax: +55 16 3373 9550.
E-mail addresses: lorenaloliveira@yahoo.com.br (L.L. de Oliveira), varesche@sc.usp.br (M.B.A. Varesche).

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higher (91%). These findings rebound the importance of mature biofilms in the degradation of toxic compounds.

Evidence from PCR/DGGE analyses indicated that sulfate-reducing bacteria (SRB) were present in the reactors even at low sulfate concentrations. It seems likely that these microorganisms were involved in the surfactant degradation, using the molecule as both a source of carbon and sulfur to maintain their metabolism. However, in addition to these bacteria, others such as *Clostridium* were also identified.

The anaerobic fluidized bed reactor with an immobilized biomass has been widely used for the biological treatment of wastewater. It achieves high efficiency with relatively low hydraulic retention times (Amorim et al., 2009). The anaerobic fluidized bed reactor may be the most promising configuration for low load wastewater treatment from sources such as domestic sewage since the process favors the retention of active microorganisms and is therefore effective in providing total suspended solids removal.

However, LAS degradation in anaerobic fluidized bed reactors has not yet been studied. Based on work done in this reactor, it was observed that several toxic compounds can be degraded and several support media can be used for biomass immobilization in this reactor configuration (Sem and Demirer, 2003; Yang et al., 2004; García Encina and Hidalgo, 2005).

Thus, this work was focused on the LAS degradation in anaerobic conditions in four fluidized bed reactors. Four different inert supports materials were tested (activated carbon, expanded clay, glass beads and sand). The microbial communities from biomass present in two of the reactors (glass beads and sand) were described by 16S rRNA gene analysis. From the results obtained it was possible to infer which microbial consortium present in biofilm was involved in LAS degradation. A comparison of communities between both reactors and the possible role of selection by the inert support was also observed.

In this work, high values of LAS degradation were obtained in anaerobic conditions suggesting the applicability of the fluidized bed reactor for this surfactant treatment.

2. Methods

2.1. Support material for the immobilization of anaerobic sludge

The following support materials were used for biomass immobilization in anaerobic fluidized bed reactors: activated carbon (R1), expanded clay (R2), glass beads (R3) and sand (R4). The diameters of the applied support media were in the range of 1.4–1.7 mm, except for expanded clay, which had particles that were 2.0–2.8 mm in diameter.

2.2. Fluidized bed reactor

The four fluidized bed reactors were made of borosilicate glass and were 50 cm in height by 3 cm internal diameter, with a total volume of 353 ml each. The reactors were kept in a controlled temperature chamber (30 ± 1 °C) and operated with a hydraulic retention time (HRT) of 18 h under the conditions presented in Table 1. The reactors characteristics of operation were presented in Table 2. All reactors were inoculated with a flocculent sludge from a UASB reactor treating swine wastewater (36 g l^{-1} VSS). Another works also used this flocculent sludge for LAS treatment in this VSS concentration (Duarte et al., 2008; Oliveira et al., 2009).

The reactors were kept in a closed circuit for biomass immobilization and adaptation to synthetic substrate. In this stage, it was prepared 3 l of feed to each reactor. This feed consisted of synthetic substrate (560 mg l^{-1} of COD) and anaerobic sludge (10% v/v).

Table 1
Operating stages of the fluidized bed reactors.

Stage	Reactors	Duration (days)	Conditions
I	R1 and R2	22	Immobilization and adaptation of biomass to synthetic substrate
II	R1 and R2	49	Synthetic substrate
III	R1 and R2	47	Synthetic substrate + LAS
IV	R3 and R4	09	Immobilization and adaptation of biomass to synthetic substrate
V	R3 and R4	27	Synthetic substrate
VI	R3 and R4	43	Synthetic substrate + LAS

Table 2
Operation characteristics of fluidized bed reactors.

Reactor	Support material	Recirculation flow-rate ($l\ h^{-1}$)	Height of the fixed bed (cm)	Height of the fluidized bed (cm)	Working volume (ml)
R1	Activated carbon	16	11	18	78
R2	Expanded clay	20	20	29	141
R3	Glass beads	30	15	18	106
R4	Sand	30	15	18	106

2.3. Composition of the synthetic substrate

The surfactant used in this study was sodium dodecylbenzenesulfonate (Sigma), also known commercially as LAS, with a purity of 80%. The reactors were fed with a concentration of approximately 20 mg l^{-1} of surfactant and 560 mg l^{-1} of COD. This surfactant concentration was chosen because is slightly higher than that one observed in the sanitary sewage that is treated on the treatment plant of the University of São Paulo – São Carlos. The influent of the sanitary sewage treatment plant was monitored every day during 10 days and the mean concentration of LAS observed was 14 mg l^{-1}.

The synthetic substrate was composed of yeast extract (500 mg l^{-1}), sucrose (80 mg l^{-1}), sodium bicarbonate (400 mg l^{-1}), and 5 mg l^{-1} 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 store the substrate during the feeding of the reactors. The substrate solution was replaced every two days. The flasks were kept refrigerated at 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 the operation of the reactors. The apparatus setup is presented in Fig. 1.

2.4. Chemical and chromatographic analysis

Physicochemical analyses of pH, chemical oxygen demand (COD) and sulfate were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Volumetric assessments of the total volatile fatty acids (TVFA) and bicarbonate alkalinity (BA) were carried out as described by Ripley et al. (1986).

LAS determinations were carried out according to methodology developed and validated by Duarte et al. (2006). Thus, it was used HPLC (Shimadzu) as a fluorescence detector, a C8 column (Supelco) with gradient elution using methanol and sodium perchlorate (0.075 M), 0.5 ml/min flux and temperature of 35 °C. The LAS con-

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