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# Optimization of linear alkylbenzene sulfonate (LAS) degradation in UASB reactors by varying bioavailability of LAS, hydraulic retention time and specific organic load rate

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## HIGHLIGHTS

- The degradation of linear alkylbenzene sulfonate (LAS) was evaluated in seven UASB reactors.
- The bioavailability of LAS, hydraulic retention time (HRT) and co-substrates concentration were varied.
- At a low concentration of co-substrates, the LAS degradation rate was the highest.
- At a HRT of 80 h, the degradation of LAS was limited by a lowered LAS loading rate.
- Increasing the LAS bioavailability resulted in discrete change in the degradation rate.

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## ABSTRACT

Degradation of linear alkylbenzene sulfonate (LAS) in UASB reactors was optimized by varying the bioavailability of LAS based on the concentration of biomass in the system (1.3–16 g TS/L), the hydraulic retention time (HRT), which was operated at 6, 35 or 80 h, and the concentration of co-substrates as specific organic loading rates (SOLR) ranging from 0.03–0.18 g COD/g TVS.d. The highest degradation rate of LAS (76%) was related to the lowest SOLR (0.03 g COD/g TVS.d). Variation of the HRT between 6 and 80 h resulted in degradation rates of LAS ranging from 18% to 55%. Variation in the bioavailability of LAS resulted in discrete changes in the degradation rates (ranging from 37–53%). According to the DGGE profiles, the *archaeal* communities exhibited greater changes than the *bacterial* communities, especially in biomass samples that were obtained from the phase separator. The parameters that exhibited more influence on LAS degradation were the SOLR followed by the HRT.



## 1. Introduction

Linear alkylbenzene sulfonate (LAS) is an anionic surfactant containing an alkyl chain of approximately 10–14 carbon atoms that are bonded to a sulfonated aromatic ring. LAS is widely used for domestic and industrial purposes, which leads to its appearance in wastewater treatment plants at concentrations ranging from 1–18 mg/L (Morita and Santana, 2005; Mungray and Kumar, 2009).

LAS is known to inhibit anaerobic processes (Garcia et al., 2006; Mosche and Meyer, 2002), but this inhibition decreases at LAS concentrations that are less than 25 mg/L (Garcia et al., 2006). Furthermore, the recalcitrance of LAS has been reported in anaerobic sludge systems (Federle and Schwab, 1992; Garcia et al., 2005). Therefore, many studies have evaluated the degradation of LAS in anaerobic arrangements by employing a UASB reactor (Almendariz et al., 2001; Lobner et al., 2005; Sanz et al., 2003). In those studies, the degradation rates in the UASB reactors varied from 13–85% at hydraulic retention times (HRT) ranging from 6–48 h, and a common operating HRT was approximately 12–24 h due to the recalcitrance of LAS; however, the optimum HRT for the degradation of LAS has not been well defined (Almendariz et al., 2001; Mogensen and Ahring, 2002; Mogensen et al., 2003; Sanz et al., 2003).

In UASB reactors, the withdrawal of co-substrates (acetate, propionate, butyrate, lactate, methanol, ethanol and sucrose) resulted in a remarkable increase in the degradation rate of LAS from 64% to 85% (Sanz et al., 2003). However, the high degradation rate was accompanied by a loss of biomass (11%), which indicated that co-substrates had to be added to maintain the structure of the

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microbial community; in particular, the addition of nitrogen and carbon sources was required for the degradation of LAS in an anaerobic facultative consortium (Abboud et al., 2007; Khleifat, 2006). Prior to the introduction of LAS, the UASB reactor operated by Sanz et al. (2003) had been fed with a mixture of co-substrates such as acetate, propionate, butyrate, lactate, methanol, ethanol and sucrose. In another UASB reactor that was only fed with LAS and no co-substrates the degradation rate was only 27% (Mogensen et al., 2003). Therefore, co-substrates are required for microbial adaptation and to maintain the microbial community structure, while the withdrawal of the co-substrates from the system is necessary to increase the LAS degradation rate. However, there is little data available on the quantity of co-substrates required to balance the needs of the microbial community while maintaining a high LAS degradation rate.

The adsorption of LAS to organic matter removes a significant quantity of LAS from wastewater (Jensen, 1999; Temmink and Klapwijk, 2004) and thus, influences the bioavailability of LAS. However, the bioavailability of LAS did not significantly influence degradation rates, i.e. only small differences were observed (5–8%) (Haggensen et al., 2002; Mogensen et al., 2003). In a continuous stirred tank reactor (CSTR), the degradation rate of LAS increased from 20% to 25–28% when the total solids content was reduced from 20 to 11 g/L (Haggensen et al., 2002; Mogensen et al., 2003). The reduction of the total solids content increased the bioavailability of the LAS to the microbial community by reducing the number of available sorption sites. The insignificant differences between the LAS degradation rate raise doubts regarding the impact of LAS bioavailability on the degradation of LAS.

In the present study, the influence of bioavailability, HRT and co-substrates on the degradation of LAS were evaluated in seven UASB reactors at a constant LAS feed concentration. The bioavailability of LAS varied as a function of biomass concentration at a constant HRT. The HRT was varied from 6 to 80 h and evaluated and the concentration of the co-substrates (as the specific organic loading rate – SOLR) was varied within a range of 0.03 to 0.18 g COD/g TVS.d. The polymerase chain reaction – denaturing gradient gel electrophoresis (PCR-DGGE) technique was applied to compare the *archaeal* and *bacterial* communities obtained from the biomass in the UASB reactors.

## 2. Methods

### 2.1. Experimental setup and operation

Seven UASB reactors were maintained at mesophilic conditions (30 ± 1 °C) for 117–122 days. The reactors were constructed with borosilicate glass and steel parts with a volume capacity of 650 mL. The inoculum was a granular sludge from a UASB reactor that had been used to treat effluent from a poultry slaughterhouse (Dacar Poultry, Tietê-SP, Brazil). All reactors were fed with an LAS concentration of approximately 12–13 mg/L.

The influence of bioavailability was evaluated in reactors $R_{35A}$, $R_{35B}$, $R_{35C}$ and $R_{35D}$ at an HRT of 35 h. In these reactors, the biomass concentration was varied from 1.3 to 16 g TS/L to alter the bioavailability of LAS (Table 1).

Reactors $R_6$ and $R_{80}$ were operated at HRTs of 6 and 80 h respectively, to compare the degradation rate of LAS at different HRTs. A low concentration of co-substrates (methanol, ethanol and yeast extract) was fed into reactor $R_{LCS}$ operating at an HRT of 35 h at the lowest SOLR of 0.03 g COD/g TVS.d, while the SOLR was approximately 0.05–0.18 g COD/g TVS.d in the other reactors (Table 1).

The reactors were fed a mineral medium (Angelidaki et al., 1990) with a concentration of $MgCl_2.6H_2O$ adjusted to 25 mg/L, a vitamin solution (Touzel and Albagnac, 1983), sodium bicarbonate ($NaHCO_3$) and co-substrates (ethanol, methanol and yeast extract). The sodium bicarbonate concentration varied from 400 to 900 mg/L to maintain a pH of approximately 7 (Table 1). Ethanol, methanol and yeast extract were added at different concentrations that constituted an SOLR of 0.03–0.18 g COD/g TVS.d (Table 1). The SOLR was based on the HRT, the influent COD concentration and the final concentration of biomass. Therefore, the concentration of co-substrates varied from 19 to 1,152 mg COD/L (totalizing 57–3,458 mg/L in the influent) to maintain a SOLR around 0.03–0.18 g COD/g TVS.d, since the concentration of biomass and the HRT varied in each reactor. The LAS (Aldrich, CAS No. 25155-30-0, technical grade) consisted of a mixture of $C_{10}$–$C_{13}$ homologues and was included at concentrations of 12–13 mg/L in the feed starting with day 31.

At the end of the assay (approximately the 110–118th day of operation), samples were collected from the influent, effluent and sample points P1, P2, and P3, respectively (Fig. 1), to evaluate the spatial variation of volatile fatty acids (VFA) and LAS in the reactor. During the assay, resazurin was added to the influent as a redox indicator. Biomass samples were collected from the sludge blankets and phase separators from reactors $R_{35B}$, $R_{35C}$, $R_{35D}$, $R_6$, $R_{80}$ and $R_{LCS}$ at the end of the assay for analysis by PCR-DGGE.

### 2.2. Analytical methods

LAS concentrations were determined by high performance liquid chromatography (HPLC) utilizing a Shimadzu system ($SCL10A_{VP}$, $LC\text{-}10AD_{VP}$, CTO-10A and RF-10AXL) containing a reversed-phase C8 column from Supelco (Duarte et al., 2006). LAS adsorbed on biomass was extracted by sonication with methanol (Duarte et al., 2008) and determined by HPLC (Duarte et al., 2006).

Acetic, butyric, isobutyric, caproic, formic, propionic, lactic, malic, succinic, valeric and isovaleric acids were quantified by HPLC using a Shimadzu system ($SCL10A_{VP}$, $LC\text{-}10AD_{VP}$, CTO-20A and $SCL10A_{VP}$) equipped with an Aminex HPX-87H column from Biorad (Lazaro et al., 2008).

Chemical oxygen demand (COD), pH, total suspended solids (TSS) and total solids content were determined according to the Standard Methods of Wastewater Examination (APHA et al., 2005).

### 2.3. Analysis of relationships between LAS bioavailability, HRT and co-substrate concentrations

A Pearson correlation matrix was generated using data obtained from all reactors to evaluate the interactions between LAS bioavailability, HRT, co-substrate concentrations and LAS degradation rates. The bioavailability of LAS was expressed as a function of biomass concentration (in terms of total solids), and the co-substrate concentrations were expressed as specific organic loading rates.

### 2.4. PCR-DGGE analysis

Granule samples from the sludge blankets and suspended biomass samples from the phase separators were washed in a phosphate-buffer (using a vortex) and centrifuged at 6000 rpm for 10 min. The pellets were stored at −20 °C.

DNA was extracted from the pellets with a phenol and chloroform mixture buffered with Tris–HCl as described by Griffiths et al. (2000). PCR amplification of 16S rRNA gene fragments employed primers 968F (with a clamp GC) and 1392R for the *Bacteria* domain (Nielsen et al., 1999), while primers 1100F (with a clamp GC) and 1400R were used for the *Archaea* domain (Kudo et al., 1997). The PCR reaction was carried out in an Eppendorf-Mastercycler thermocycler (Eppendorf AG-22331 Hamburg).

The PCR products were loaded onto an 8% (w/v) polyacrylamide gel in 0.5 X TAE (Tris acetate EDTA) with a linear gradient of 7 M to urea and 45% to 65% formamide. Electrophoresis was performed at

**Table 1**
Operating conditions of the UASB reactors used for evaluate the influence of biomass concentration, hydraulic retention time (HRT) and specific organic load rate (SOLR) on LAS degradation.

| | $R_{35A}$ | $R_{35B}$ | $R_{35C}$ | $R_{35D}$ | $R_6$ | $R_{80}$ | $R_{LCS}$ |
|---|---|---|---|---|---|---|---|
| Specific organic loading rate (g COD/g TVS.d) | 0.18 ± 0.06 | 0.11 ± 0.04 | 0.07 ± 0.04 | 0.10 ± 0.02 | 0.18 ± 0.06 | 0.05 ± 0.02 | 0.03 ± 0.01 |
| Final biomass (g/L) | | | | | | | |
| Total solids (TS) | 16 ± 1 | 6.0 ± 0.3 | 2.3 ± 0.1 | 1.3 ± 0.1 | 5.7 ± 0.5 | 1.7 ± 0.3 | 1.6 ± 0.1 |
| Total volatile solids (TVS) | 13 ± 1 | 4.5 ± 0.2 | 1.5 ± 0.1 | 0.7 ± 0.1 | 5.0 ± 0.4 | 1.1 ± 0.1 | 1.3 ± 0.1 |
| HRT (h) | 35 | 35 | 35 | 35 | 6 | 80 | 35 |
| Ethanol (mgDQO/L) | 1,152 | 158 | 77 | 35 | 74 | 57 | 19 |
| Methanol (mgDQO/L) | 1,152 | 158 | 77 | 35 | 74 | 57 | 19 |
| Yeast extract (mgDQO/L) | 1,152 | 158 | 77 | 35 | 74 | 57 | 19 |
| Influent COD (mg/L) | 3,458 ± 143 | 474 ± 19 | 231 ± 9 | 106 ± 4 | 222 ± 13 | 171 ± 11 | 57 ± 4 |
| $NaHCO_3$ (mg/L) | 900 | 400 | 400 | 600 | 600 | 400 | 700 |

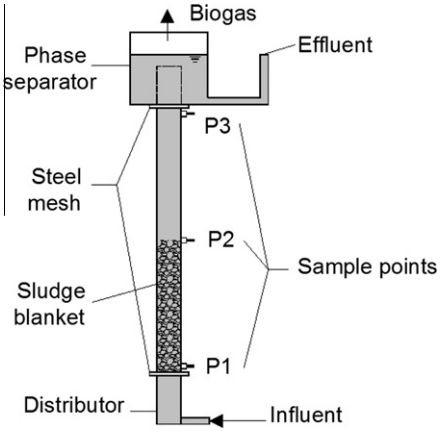


**Fig. 1.** Set up of the UASB reactor.

a constant voltage of 75 V and a constant temperature of 60 °C for a period of 16 h. The image of the DGGE profile was acquired by an Eagle Eye II instrument (Stratagene, La Jolla, CA, USA) equipped with UV illumination after staining the gel with Vistra Green solution (1:10,000).

The DGGE profile was then analyzed with software from Bionumerics. The similarity coefficients were determined according to the Dice coefficient and the dendrogram was determined by an unweighted pair group method with an arithmetic average (UPGMA) algorithm.

## 3. Results and discussion

### 3.1. Performance of the UASB reactors

The pH of the effluent was between 7.0 and 7.3 in all reactors (Table 2), except for reactor $R_{35A}$, which had a pH of 7.6 because a larger quantity of $NaHCO_3$ had been added to the reactor (Table 1). Reactors $R_{35A}$, $R_{35B}$ and $R_{35C}$ exhibited greater biomass losses than the other reactors, with effluent TSS concentrations of more than 33 mg/L; the other reactors exhibited effluent TSS concentrations of 7–14 mg TSS/L (Table 2). The greater loss of TSS in reactors $R_{35A}$, $R_{35B}$ and $R_{35C}$ was related to the biomass concentration of the reactors, which was greater than 2.3 g TS/L in all three (Table 1).

The mean COD removal varied from 63% to 96%; a lower removal efficiency (ranging from 63–71%) could be attributed to a low COD influent of approximately 57–171 mg/L (Tables 1 and 2). However, the mean effluent COD concentrations were approximately 20–60 mg/L, except in reactor $R_{35A}$, where the mean concentration was 123 mg/L because of a high influent COD concentration of 3458 mg/L. The mean VFA content in the effluent was less than 50 mg HAc/L in all reactors (Table 2).

The mean LAS effluent increased from 3.5 to 5.7 mg/L in reactors evaluating the influence of bioavailability on degradation of LAS ($R_{35A}$, $R_{35B}$, $R_{35C}$ and $R_{35D}$) (Table 2). The highest mean LAS effluent concentration was 9 mg/L, coming from reactor $R_6$, while the lowest mean effluent concentrations of approximately 2 mg/L came from reactors $R_{80}$ and $R_{LCS}$.

The degradation rate of LAS varied from 37% to 53% in reactors $R_{35A}$, $R_{35B}$, $R_{35C}$ and $R_{35D}$ according to the mass balance (Table 3). The degradation rate of LAS was only 18% at an HRT of 6 h in reactor $R_6$, while it was 55% at an HRT of 80 h in reactor $R_{80}$. The highest degradation of LAS was observed in reactor $R_{LCS}$ (76%), and reactor $R_{LCS}$ was the reactor that was fed with the lowest co-substrate concentration.

### 3.2. Influence of bioavailability on degradation of LAS

The relationship between the bioavailability of LAS and adsorption can be observed by monitoring the LAS concentrations in the effluent. The time required to increase the LAS concentration in the effluent increased when the biomass concentration increased from 1.3 to 16 g TS/L (Fig. 2a–d). The initial period of reactor operation indicated a remarkable influence of adsorption on the

**Table 2**
Means and standard deviations of the COD, LAS, pH, total suspended solids (TSS) and volatile fatty acids (VFA) analyzed in the UASB reactors.

| | $R_{35A}$ | $R_{35B}$ | $R_{35C}$ | $R_{35D}$ | $R_6$ | $R_{80}$ | $R_{LCS}$ |
|---|---|---|---|---|---|---|---|
| COD | | | | | | | |
| Effluent (mg/L) | 134 ± 25 | 60 ± 21 | 56 ± 20 | 29 ± 14 | 44 ± 10 | 52 ± 41 | 22 ± 13 |
| Removal (%) | 96 ± 1 | 87 ± 6 | 76 ± 9 | 73 ± 12 | 80 ± 7 | 71 ± 23 | 63 ± 19 |
| LAS | | | | | | | |
| Influent (mg/L) | 12.3 ± 2.4 | 12.8 ± 2.5 | 13.0 ± 2.4 | 12.4 ± 1.8 | 12.2 ± 2.4 | 11.7 ± 1.5 | 12.5 ± 2.0 |
| Effluent (mg/L) | 3.5 ± 1.8 | 4.0 ± 1.7 | 4.3 ± 2.5 | 5.7 ± 1.2 | 9.0 ± 2.4 | 2.4 ± 1.2 | 2.0 ± 1.0 |
| Effluent pH | 7.6 ± 0.2 | 7.2 ± 0.1 | 7.3 ± 0.2 | 7.1 ± 0.1 | 7.3 ± 0.2 | 7.0 ± 0.3 | 7.0 ± 0.3 |
| Effluent TSS (mg/L) | 64 ± 33 | 47 ± 42 | 33 ± 26 | 13 ± 8 | 12 ± 10 | 14 ± 10 | 7 ± 5 |
| Effluent VFA (mg HAc/L) | 39 ± 24 | 42 ± 31 | 25 ± 24 | 37 ± 20 | 49 ± 23 | 18 ± 10 | 32 ± 14 |

**Table 3**
Mass balance of linear alkylbenze sulfonate (LAS) in reactors to analyze the fate of LAS.

| | $R_{35A}$ | | $R_{35B}$ | | $R_{35C}$ | | $R_{35D}$ | | $R_6$ | | $R_{80}$ | | $R_{LCS}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (mg) | (%) | (mg) | (%) | (mg) | (%) | (mg) | (%) | (mg) | (%) | (mg) | (%) | (mg) | (%) |
| Mass Added | 497 | 100 | 526 | 100 | 517 | 100 | 487 | 100 | 3027 | 100 | 238 | 100 | 502 | 100 |
| Mass in Effluent | 141 | 28 | 167 | 32 | 175 | 34 | 229 | 47 | 2370 | 78 | 52 | 22 | 81 | 16 |
| Mass Adsorbed | 170 | 33 | 153 | 29 | 69 | 13 | 27 | 6 | 103 | 4 | 54 | 23 | 38 | 8 |
| Mass Degraded | 186 | 37 | 206 | 39 | 273 | 53 | 231 | 47 | 555 | 18 | 132 | 55 | 383 | 76 |
| Period with LAS (d) | 90 | | 89 | | 89 | | 89 | | 92 | | 87 | | 92 | |

increase in the LAS concentration in the effluent. The influence of adsorption decreased as the variation in the LAS concentration found in the effluent decreased. In fact, the adsorption of LAS in granular sludge becomes more difficult as sorption sites are saturated by LAS molecules, according to a Freundlich adsorption isotherm (Okada et al., 2009). Therefore, a reduction in biomass concentration in the reactor decreased the sorption sites available, which also reduced the quantity of LAS adsorbed onto biomass from 33% to 6% (Table 3), consequently resulting in sorption sites reaching a saturation equilibrium more quickly. The LAS concentration in the effluent was fitted to a Boltzmann sigmoidal curve ($y = A2+(A1-A2)/(1 + \exp((x-x0)/dx))$) as a function of time. The steepness of the curve ($dx$) decreased from 8.8 to 1.3, which proved that sorption sites were more quickly saturated as the biomass concentration decreased (Fig. 2a–d).

Table 3 shows that the degradation rate of LAS was approximately 40% when the biomass concentration was greater than 6 g TS/L (in reactors $R_{35A}$ and $R_{35B}$), while the degradation rate was approximately 50% when the biomass concentration was less than 3 g TS/L (in reactors $R_{35C}$ and $R_{35D}$). The difference between the lowest and the highest degradation rates of LAS in the four reactors was 16%, which was slightly greater than the difference reported in CSTR (5–8%) when the biomass concentration was decreased from 20 to 11 g TS/L (Haggensen et al., 2002; Mogensen et al., 2003). The low variation between the degradation rates of LAS among the four reactors in the present study was a result of reduced adsorption of LAS onto the biomass from 33% to 6% and an increase in the LAS content in the effluent from 28% to 47% according to increased bioavailability caused by the reduction in the biomass concentration in the reactor (Table 3). Therefore, the increase

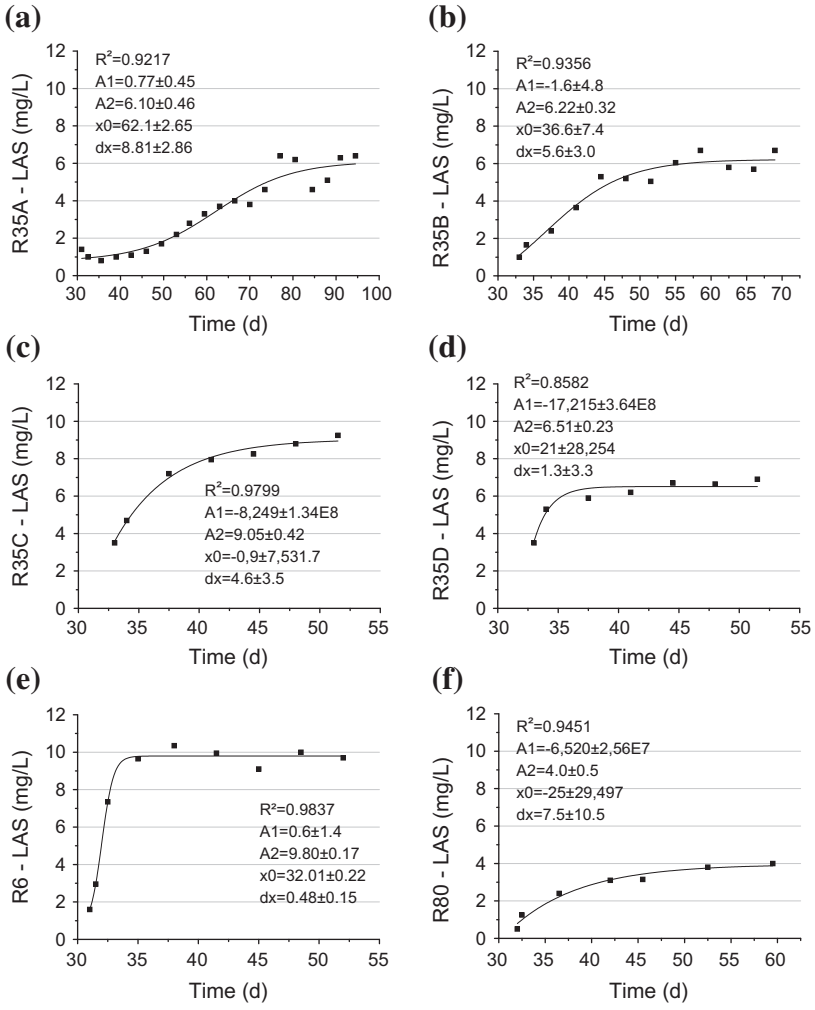


**Fig. 2.** LAS concentrations in effluent (■) fitted to a Boltzmann-type sigmoidal curve for reactors $R_{35A}$ (a), $R_{35B}$ (b), $R_{35C}$ (c), $R_{35D}$ (d), $R_6$ (e) and $R_{80}$ (f).

in the LAS degradation rate corresponding to the bioavailability of LAS was diminished by the contrasting effects of the content of the effluent and of adsorption onto the biomass. The low increase in the degradation rate was demonstrated by a linear regression of the specific LAS loading rate (SLLR), which represents bioavailability, as a function of the specific LAS degradation rate (SLDR), which represents degradation. The coefficient of the slope was 0.487 ± 0.027 (Fig. 3). Linear regression of the SLLR on the SLDR of reactors $R_{35A}$, $R_{35B}$, $R_{35C}$ and $R_{35D}$ resulted in the development of Eq. (1) ($R^2$ = 0.9903)

$$SLDR = 0.487^{*}SLLR - 0.05 \quad (1)$$

The variation in the bioavailability of LAS resulted in a discrete variation in the degradation rate (approximately 16%) due to the contrasting effects of the content of the effluent and the amount of LAS adsorbed onto the biomass. Despite an increased degradation rate of LAS, the retention of biomass was more difficult at low biomass concentrations. Therefore, an optimal biomass concentration is necessary for biomass retention and to increase the degradation rate of LAS sufficiently. Another alternative is the use of arrangements such as immobilized biomass reactors, which can improve biomass retention.

### 3.3. Influence of HRT

The time required to increase the LAS concentration in the effluent was related to the HRT. The LAS effluent concentrations from reactors $R_6$ and $R_{80}$ were fitted to Boltzmann sigmoidal curves as functions of time (Fig. 2e and f, respectively), and the steepness of the curve (measured as *dx*) increased from 0.5 to 7.5 as the HRT was increased from 6 to 80 h. The reduction in the HRT resulted in a more rapid saturation of sorption sites, which occurred because the LAS loading rate was increased from 4 mg/L.d at an HRT of 80 h to 50 mg/L.d at an HRT of 6 h.

The LAS degradation rate increased from 18% to 55% as the HRT increased from 6 to 80 h (Table 3). The low degradation rate of LAS at 18% based on an operating HRT of 6 h indicates that biotransformation of LAS requires a long time. The low LAS concentration in the effluent of reactor $R_{80}$ (2.4 mg/L) demonstrated the transformation of the LAS as a result of a longer HRT of 80 h. However, the degradation rate of LAS in reactor $R_{80}$ was slightly greater (55%) than it was in the reactors operating at an HRT of 35 h; in the other reactors, the degradation rate ranged from 37–53%, except for $R_{LCS}$, which showed a degradation rate of 76% (Table 3). The slight increase in the LAS degradation rate at an HRT of 80 h was a result of the LAS loading rate being inversely proportional to the HRT when the concentration of LAS was kept constant; the LAS loading rate was 9 mg/L.d at an HRT of 35 h, but it was 4 mg/L.d at an HRT of 80 h. Therefore, the amount of LAS added at an HRT of 80 h was approximately half the amount added at an HRT of 35 h, which limited the degradation of LAS in reactor $R_{80}$.

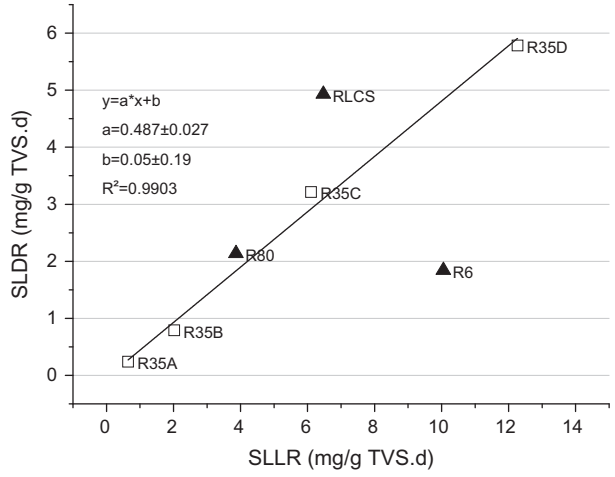


**Fig. 3.** Specific LAS degradation rate (SLDR) according to the specific LAS loading rate (SLLR) in reactors $R_{35A}$, $R_{35B}$, $R_{35C}$, $R_{35D}$ (■), $R_6$, $R_{80}$ and $R_{LCS}$ (▲). Linear regression was conducted on the data obtained from reactors $R_{35A}$, $R_{35B}$, $R_{35C}$ and $R_{35D}$, with an $R^2$ value of 0.9903.

The SLDRs of reactor $R_6$ (operating at an HRT of 6 h) and reactor $R_{80}$ (operating at an HRT of 80 h) were compared to the value from Eq. (1), which was obtained from the reactors operating at an HRT of 35 h. The SLDR of the reactor operating at an HRT of 80 h was slightly greater (17%) than the SLDR that was obtained from Eq. (1), which showed that there was a small increase in the degradation rate of LAS at an HRT that was greater than 35 h (Fig. 3). In contrast, the SLDR of the reactor operating at an HRT of 6 h was remarkably lower (62%) than the SLDR obtained from Eq. (1).

In an expanded granular sludge bed (EGSB) reactor, the removal of LAS based on the effluent LAS concentration relative to the influent LAS concentration was approximately 48% when the HRT was 26 h, while the removal was 64–74% at an HRT of 32 h (Delforno et al., 2012). Moreover, the comparison of results from previous studies to that from Eq. (1) defined for reactors operating at an HRT of 35 h, demonstrates that there was a reduction in degradation rate of LAS at lower HRTs (Table 4). The SLDR of a horizontal anaerobic immobilized biofilm (HAIB) (Duarte et al., 2008) reactor operating at an HRT of 12 h was 26% lower than what was predicted by Eq. (1). The SLDR of an anaerobic stirred sequencing batch reactor (ASSBR) (Duarte et al., 2010) operating at an HRT of 24 h was lower than what was predicted by Eq. (1) by 29–49%, except in stage IV of the reaction process, where the SLDR was 11% greater than what was predicted in Eq. (1). The higher SLDR in stage IV of the ASSBR was due to the withdrawal of co-substrates that increased the degradation rate of LAS.

The adjustment of the HRT from 6 to 80 h resulted in a remarkable change to the LAS degradation rate. A comparison of the results from studies presented in Table 4 shows that there is a positive relationship between the HRT and the LAS degradation rate, except when the co-substrates are being withdrawn from the system. However, the increment in degradation rate of LAS was limited by the LAS loading rate at operating HRTs that were greater than 35 h. Therefore, the optimal contact time for LAS degradation was set at an HRT of 35 h.

### 3.4. Influence of co-substrates

In reactor $R_{LCS}$, the addition of co-substrates such as methanol, ethanol and yeast extract at low concentrations resulted in a high degradation rate of LAS at 76%, according to the mass balance shown in Table 3. The SLDR in reactor $R_{LCS}$ was compared to the value obtained from Eq. (1), defined for reactors at HRT 35 h (as in reactor $R_{LCS}$). The SLDR in reactor $R_{LCS}$ was remarkably greater than what was predicted by Eq. (1) (Fig. 3), which showed that the high degradation rate of LAS was brought about by a low co-substrate concentration.

An LAS degradation rate of 85% was obtained in a UASB reactor when co-substrates were withdrawn from the system, and the degradation rates ranged from 81–99% in fluidized bed reactors (Oliveira et al., 2010; Sanz et al., 2003). A high recirculation flow rate of 16–30 L/h in the fluidized bed reactor increased mass transfer and dilution of the influent, which resulted in a low concentration of VFA (12–16 mg HAc/L) and a greater degradation rate of LAS in the reactor (Oliveira et al., 2010). It was apparent that the low concentration of VFA in the fluidized bed reactor was a result of a low co-substrate concentration in the reactor. It has also

**Table 4**
Comparison of specific LAS degradation rates (SLDR) from the literature with results obtained with Eq. (1).

| Reactor | HRT (h) | LAS (mg/L) | SLLR (mg/g TVS.d) | SLDR reported (mg/g TVS.d) | SLDR obtained with Eq. (1) (mg/g TVS.d) | Error (%) | Reference |
|---|---|---|---|---|---|---|---|
| HAIB | 12 | 14 | 2.80 | 0.97 | 1.31 | −26 | Duarte et al. (2008) |
| ASSBR | | | | | | | Duarte et al. (2010) |
| Stage II | 24 | 22 | 9.80 | 3.33 | 4.72 | −29 | |
| Stage III | 24 | 22 | 6.80 | 1.67 | 3.26 | −49 | |
| Stage IV | 24 | 22 | 8.33 | 4.44 | 4.01 | +11 | |

been reported that there was a greater removal of LAS at VFA concentrations of less than 50 mg HAc/L (Lobner et al., 2005). Therefore, the high degradation rate of the LAS was related to a decrease in co-substrate concentration.

Table 2 shows that the TSS in the effluent of reactor $R_{LCS}$ was the lowest among all reactors, which indicated that there was little loss in biomass. The solids concentration in reactor $R_{LCS}$ was 1.6 g TS/L, which contributed to the low loss of biomass. The COD removal in reactor $R_{LCS}$ was also less at 63% because of the low co-substrate COD concentration of 57 mg/L fed into the reactor.

Despite the requirements of co-substrates for microbial community adaptation and maintenance, a high LAS degradation rate of 76% was related to a low concentration of co-substrates at an SOLR of 0.03 g COD/g TVS.d. The mean TSS concentration in the effluent was 7 mg/L, which indicated a low loss of biomass, favoring the retention of solids in the reactor at a concentration of 1.6 g TS/L. Despite the possibility of a greater loss of biomass when there is a high concentration of solids, the remarkable increase in the degradation rate causes the supply of co-substrates at a low concentration to be more attractive. Moreover, the loss of biomass can be mitigated by system modifications such as use of support materials that promote biomass retention.

### 3.5. Relationships between LAS bioavailability, HRT and co-substrate concentrations

According to the correlation matrix, the SOLR exhibited more influence than other variables on the degradation rate of LAS (Table 5). The correlation coefficient relating LAS degradation rate to SOLR was −0.835 (significance – $p$-value was less than 0.05), indicating that degradation rate was inversely correlated to the SOLR. At the lowest SOLR of 0.03 g COD/g TVS.d used in reactor $R_{LCS}$, the degradation rate of LAS was 76%, while the degradation rates ranged from 18–55% in other reactors at SOLRs ranging from 0.05–0.18 g COD/g TVS.d.

The adjustment of the HRT exhibited a positive correlation with the degradation rate of LAS (coefficient 0.534; $p = 0.22$), while a changing biomass (TS) concentration had a negative correlation with degradation rates (−0.492; $p = 0.26$). Another correlation matrix was determined without the use of any data obtained from reactor $R_{LCS}$ (operating at the lowest SOLR) to determine if HRT or biomass concentration exhibited more influence on the LAS degradation. In this correlation matrix, the influence of HRT on the degradation of LAS was found to be more significant than the biomass (TS) concentration, with a correlation coefficient of 0.819 at $p < 0.05$. The adjustment of the HRT between 6 and 80 h increased the degradation rate from 18% at an HRT of 6 h to a degradation rate of 55% at 80 h. This difference of 37% was greater than the 16% change in the LAS degradation rate observed when the biomass concentration was varied.

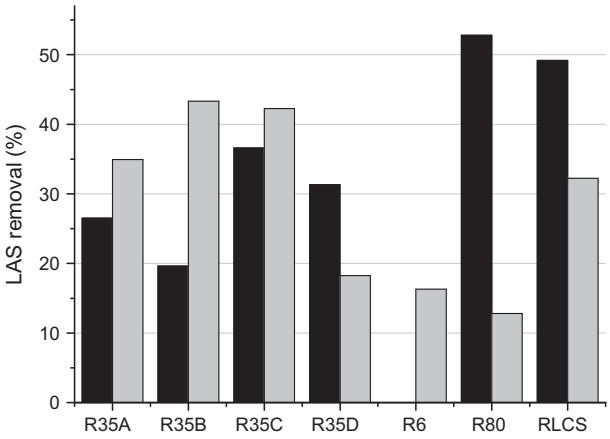


**Fig. 4.** LAS removal according to spatial variation in the phase separator (between the sample point P3 and the effluent ■) and the sludge blanket (between the influent and sample point P3 □). There was no detected removal of LAS in the phase separator from the effluent of reactor $R_6$.

### 3.6. Spatial variation of LAS and VFA in the UASB reactors

The phase separator placed between sample point P3 and the effluent was observed to remove LAS at a rate of 20–53% in all reactors, except in reactor $R_6$ where no removal was observed in the phase separator (Fig. 4). The removal of LAS in the sludge blanket between the influent and sample point P3 ranged between 13 and 43% in all reactors. The removal of LAS in the phase separator could be linearly correlated to the LAS degradation rate with an $R^2$ value of 0.795 (Fig. 5a).

The resazurin added to the reactor showed that the sludge blanket was operating under strictly anaerobic conditions, while

**Table 5**
Correlation matrix relating biomass concentration (total solids-TS), HRT, specific organic loading rate (SOLR) and LAS degradation rate in reactors to determine the influence of these parameters (TS, HRT, SOLR) on LAS degradation.

| | | TS | HRT | SOLR | Degradation rate of LAS |
|---|---|---|---|---|---|
| TS | Pearson Correlation | 1 | | | |
| | Significance (2-tailed) | – | | | |
| HRT | Pearson Correlation | −0.263 | 1 | | |
| | Significance (2-tailed) | 0.569 | – | | |
| SOLR | Pearson correlation | 0.686 | −0.609 | 1 | |
| | Significance (2-tailed) | 0.089 | 0.147 | – | |
| Degradation rate of LAS | Pearson Correlation | −0.492 | 0.534 | −0.835 | 1 |
| | Significance (2-tailed) | 0.262 | 0.217 | 0.019 | – |

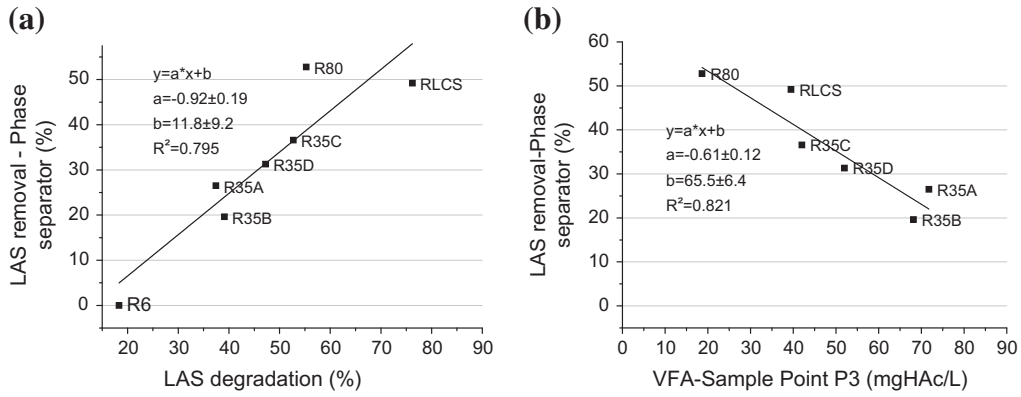


**Fig. 5.** LAS removal in the phase separator (between sample point P3 and the effluent) according to the degradation rate of LAS (a) and according to the concentration of VFA at the sample point P3 (b).

the phase separator was operating under facultatively anaerobic conditions because of the poor oxygen diffusion. Larson et al. (1993) reported the need for LAS to be initially exposed to aerobic conditions for it to be degraded under strictly anaerobic conditions, because the first step of LAS degradation requires the ω-oxidation of the alkyl chain. Additionally, the low concentration of VFA at sample point P3 prior to the phase separator (ranging from 20–70 mg HAc/L, Fig. 5b) stemmed from the

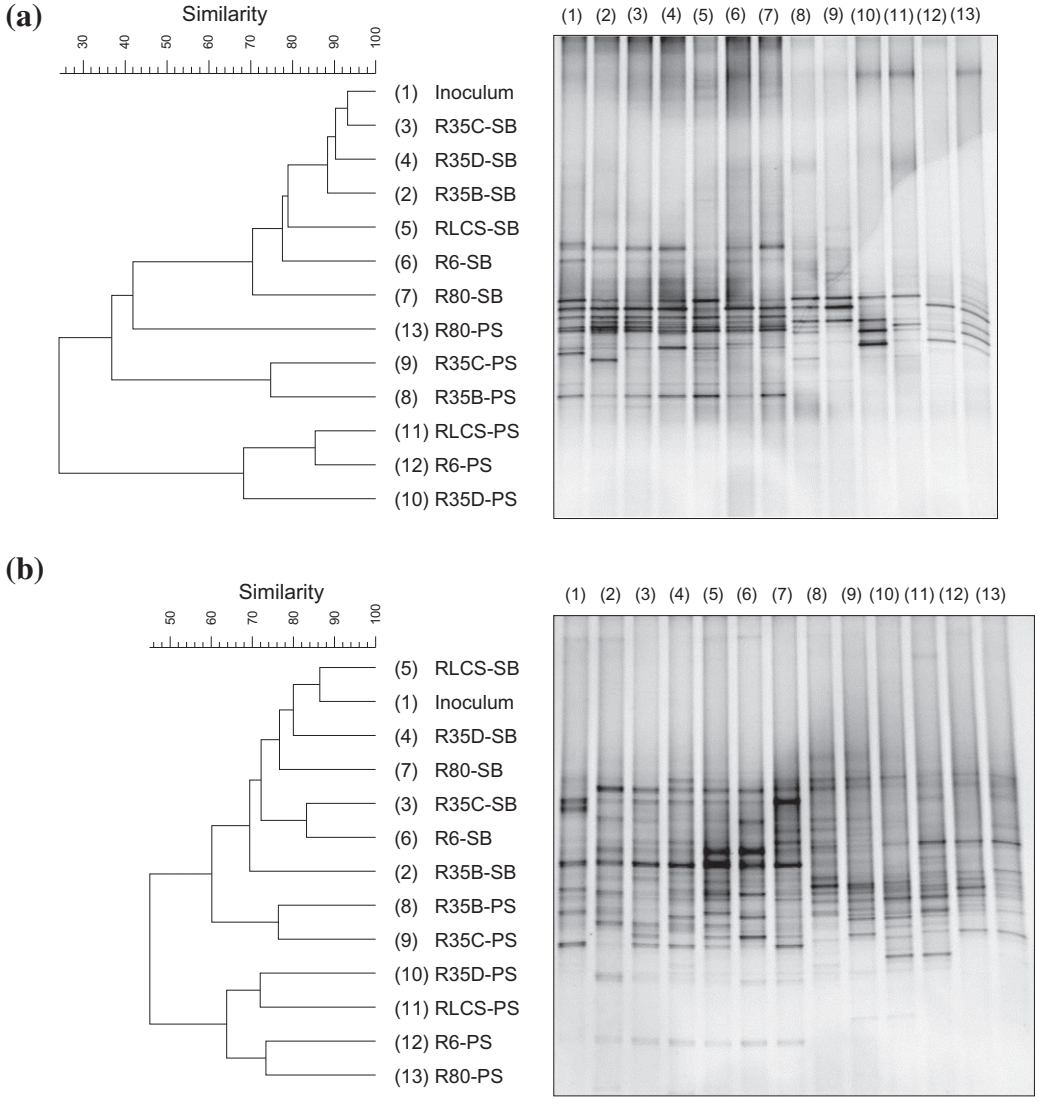


**Fig. 6.** Cluster analysis based on the DGGE profiles for the *Archaea* (a) and *Bacteria* (b) domains in the inoculum, the sludge blanket (SB) and the phase separator (PS).

provision of co-substrates at low concentrations such as what was fed into reactor $R_{LCS}$, which exhibited the highest degradation rate of LAS (76%) among all reactors tested. In fact, there was a linear relationship between the VFA concentrations at sample point P3 and the removal of LAS in the phase separator with an $R^2$ value of 0.821, though this correlation was conducted with the exclusion of data obtained from reactor $R_6$ because the phase separator in this reactor did not exhibit any removal of LAS. The absence of any LAS removal in the phase separator of reactor $R_6$ was related to the short HRT of 6 h at which the reactor was operating. Therefore, the facultative anaerobic conditions and the low concentrations of VFA in the reactors were related to a high removal of LAS in the different phase separators (ranging from 20–53%, Fig. 4).

### 3.7. PCR-DGGE analysis

According to the DGGE dendrogram profile, there were fewer changes in the *archaeal* and *bacterial* communities within the sludge blanket in comparison with the samples obtained from the biomass in the phase separator (Fig. 6). The similarity coefficients for the samples obtained from the sludge blanket were 70–90% for the *Archaea* domain and 69–83% for the *Bacteria* domain. These coefficients were greater than those for the samples obtained from the biomass in the phase separator of 29–86% for the *Archaea* domain and 52–76% for the *Bacteria* domain. Furthermore, the similarity ranges of the biomass samples obtained from the phase separator (57% for the *Archaea* domain and 24% for the *Bacteria* domain) were greater than those of the samples obtained from the sludge blanket (23% for the *Archaea* domain and 18% for the *Bacteria* domain).

A reduced variation in the similarity coefficients indicates that the granule structure in the sludge blanket protects the microbial community from changing significantly according to the adjustment of operating parameters such as the HRT, the SOLR and the biomass concentration. In fact, there was little change reported in the microbial community composition of a sludge blanket used in a UASB reactor to remove LAS, according to a FISH analysis of the *Archaea* and the *Bacteria* domains (Okada et al., 2010). The inoculum exhibited greater similarities with the samples obtained from the sludge blanket than those obtained from the biomass in the phase separator (Fig. 6). The similarity coefficients between the inoculum and the sludge blanket were 70–93% for the *Archaea* domain and 69–87% for the *Bacteria* domain. Furthermore, in the *archaeal* community found in samples obtained from the sludge blanket (Fig. 6a), the adjustment of the HRT and the SOLR resulted in similarity coefficients that were slightly less (70–79% for reactors $R_6$, $R_{80}$ and $R_{LCS}$) than those obtained by changing the biomass concentration (89–90% for reactors $R_{35B}$, $R_{35C}$, $R_{35D}$).

In contrast, the biomass from the phase separator proliferated during the assay and had greater exposure to a wider variety of operating conditions, which resulted in a great range of similarity coefficients. Despite the great range in the similarity coefficients, the samples obtained from the biomass in the phase separator from reactors $R_{35D}$, $R_6$ and $R_{LCS}$ exhibited greater similarity coefficients for the *Archaea* domain (69–86%) than the samples obtained from the biomass in the phase separator from the other reactors (37–75%). The greater similarity coefficients in these samples was attributed to the acetic acid supplied to the biomass in the phase separator, which was 9–11 mg/L at sample point P3, while there was no detectable acetic acid in the other reactors. The presence of acetic acid at sample point P3 indicated the presence of acetoclastic methanogens in the biomass obtained from the phase separators of reactors $R_{35D}$, $R_6$ and $R_{LCS}$.

## 4. Conclusions

The lowest specific organic load rate (SOLR) supplied to the reactor resulted in the highest degradation rate of linear alkylbenzene sulfonate (LAS) (76%), while the LAS degradation rate ranged between 18–55% when adjusting the HRT and between 37–53% according to the bioavailability of LAS. A remarkable removal of LAS (20–53%) was observed in the phase separators, which was related to the facultatively anaerobic conditions and the low local VFA concentrations in that region. According to the DGGE profiles, the microbial communities found in the biomass samples obtained from the phase separators exhibited less similarity than the communities found in the biomass samples obtained from the sludge blanket.

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