Anaerobic degradation of anionic surfactants by indigenous microorganisms from sediments of a tropical polluted river in Brazil

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Received 25-II-2014. Corrected 21-VII-2014. Accepted 21-VIII-2014.

Abstract: Linear alkylbenzene sulfonate (LAS) is widely used in the formulation of domestic and industrial cleaning products, the most synthetic surfactants used worldwide. These products can reach water bodies through the discharge of untreated sewage or non-effective treatments. This study evaluates the ability of the microorganisms found in the Tietê river sediment to degrade this synthetic surfactant. The experiment was conducted in a bioreactor, operated in batch sequences under denitrifying conditions, with cycles of 24 hours and stirring at 150rpm, using 430mL of sediments and 1 070mL of a synthetic substrate consisting of yeast extract, soluble starch, sodium bicarbonate and sucrose. LAS was added at different concentrations of 15mg/L and 30mg/L. The reactor operation was divided into the biomass adaptation to the synthetic substrate without LAS and three experimental conditions: a) addition of 15mg/L of LAS; b) 50% reduction the co-substrate concentration and 15mg/L of LAS, and c) addition of 30mg/L of LAS and 100% co-substrate concentration. The results showed that the degradation efficiency of LAS was directly related to the addition of co-substrates and the population of denitrifying bacteria. The removal of LAS and nitrate can be achieved simultaneously in wastewater with low organic loads. The reduction in the co-substrates concentration was directly influenced by the number of denitrifying bacteria ($2.2x10^{13}$ to $1.0x10^{8}$MPN/gTVS), and consequently, LAS degradation (60.1 to 55.4%). The sediment microorganisms in the Tietê river can be used as an alternative inoculum in the treatment of wastewater with nitrate and LAS contamination. Rev. Biol. Trop. 63 (1): 295-302. Epub 2015 March 01.

Key words: anaerobic, surfactant, degradation, bacteria, nitrate.

Linear alkylbenzene sulfonate (LAS) is the most important anionic surfactant used as an active ingredient in household and industrial cleaning agents. In 2008 the global production of surfactants was 13 million tonnes and approximately 65% of the total production corresponds to anionic surfactants (Olkowska, Ruman, Polkowska, 2014). LAS is a mixture of isomers containing an aromatic ring that is sulfonated at the para position. The LAS homologues contain a linear alkyl chain attached at any position except the terminal carbon (Garcia, Campos, Sanchez-Leal, & Ribosa, 2006). Statistics from the Council of European Surfactant Producers (CESIO) indicate a consumption of two million tons of surfactants in Europe for the year 1999. Within this total, linear alkylbenzene sulfonates (LAS), alkyl ethoxy sulfates (AES), alcohol ethoxylates (AEO) and alkyl sulfates (AS), account for 310 000, 237 000, 220 000 and 102 000tons, respectively. LAS is typically discharged into the environment from sewage treatment stations or directly (Garcia et al., 2006). Currently, LAS

concentration has been reported to vary from 1 to 18mg/L in wastewater treatment plants (WWTP) (APHA, 2005), and up to 10mg/L in coastal waters close to untreated discharges (Leon et al., 2002); nevertheless, LAS can also be found in river sediments (Berna et al., 2007) at concentrations between 0.4 to 4.7mg/Kg (Cavalli et al., 2000). In a review, Olkowska et al. (2014) showed average values of anionic surfactants in sediment samples from rivers, lakes and seas, at a concentration of 0.0002-3.4mg/Kg. LAS concentrations are higher in sediments than in water (Olayemi, Eniola, Awe, & Kayoe-Isola, 2003), thus the bacteria found in sediments are exposed to detergents.

In Brazil over 90% of domestic effluents is not discharged to a sewage treatment system. This untreated sewage is discharged into rivers, as seen by the abundance of xenobiotic surfactants in sanitary sewage (Eichhorn, Rodríguez, Baumann, & Knepper, 2002). The Tietê River, which runs through the city of São Paulo (Brazil), is considered to be one of the most polluted rivers in the world, due to inefficient treatment and launching of clandestine industrial effluents. Mortatti, Moraes and Kiang (2012) analyzed various metals (copper, cobalt, chromium, zinc, nickel and lead) and different depths (0 to 30cm) of the Tietê river sediment. It is observed that the higher metal concentrations were copper (26.6 to 248.9µg/g), chromium (85.8 to 147.8µg/g) and zinc (253.4 to 780.0µg/g).

Nowadays, despite these discharges little is known about the potential of water and metabolism of sediment microorganisms in those rivers (Rocha et al., 2009). As for now, the linear alkylbenzene sulfonate (LAS) concentrations of 1.6mg/L have been reported in the town of Pirapora do Bom Jesus along the Tietê river (Hatamura, Eysink, Bevilacqua, & Moraes, 1993); also the Tamanduateí river in the city of São Paulo, which showed 2.3mg/L of LAS (CESTEB, 1992), Macacu River (state of Rio de Janeiro - Brazil) showed lower concentrations of LAS (14-155µg/L) (Eichhorn et al., 2002). Nevertheless, there is little additional information available about LAS concentration in other Brazilian rivers.

Microbial degradation of organic compounds occurs in anoxic sediments and soils, where the microorganisms utilize different electron acceptors (nitrate, sulfate and carbon dioxide and iron). However, xenobiotics in anoxic environments can be persistent due to the following factors: low solubility, toxicity and low biomass concentration (Elsgaard, 2010). The present study used microorganisms from Tietê river sediments contaminated with detergents, to promote LAS degradation, in a sequence batch reactor in denitrifying conditions.

MATERIALS AND METHODS

Inoculum: Sediment of Tietê River was used as inoculum for the degradation of the anionic surfactant in a bioreactor. An amount of 1.22kg of sediment was collected in the city of Salto-SP (Brazil) (23º 00’ 44.8” S - 47º 00’17.2” W) in July (dry season) using a Van Veen dredge. The location was chosen due to the documented pollution exposure and intense foaming on the surface of the river at this specific point. This sediment was stored in plastic bags and kept refrigerated (6ºC) until use, including characterization of the physicochemical and microbiological properties (Table 1).

Anionic surfactant: The linear alkylbenzene sulfonate used in the present study was a commercial mixture of C10-C13 homologues provided by Aldrich (CAS no. 25155-30-0, technical grade).

Bioreactor: The schema of this bioreactor is shown in figure 1. The reactor was made of borosilicate glass, with a total volume of 1 500mL. Stirring was done with an impeller-type turbine, with three 14cm blades and agitation at 150rpm.

The substrate on the feed line was kept under refrigeration (4°C) for conservation and was heated in a water bath (30°C) before being discharged into the reactor. Peristaltic pumps were used to feed and discharge the effluent.

TABLE 1
Analyzed parameters in the water and sediment of the Tietê River, in Salto-SP, Brazil

Parameters	Results	References
Temperature (ºC)	20.10	
pH	7.08	
Conductivity (mS/cm)	0.373	
Dissolved Oxygen (mg/L)	6.20	
TS (g/L)	159.70	APHA (2005)
TVS (g/L)	31.60	APHA (2005)
LAS dissolved (mg/L)	0.60	Duarte et al. (2006)
LAS adsorbed (mg/g)	<0.30	Duarte et al. (2008)
Nitrate (mg/L)	2.66	APHA (2005)
Nitrite (µg/L)	0.82	APHA (2005)
Denitrifying bacteria(MPNg/TVS)	$7.6x10^{12}$	Tiedje (1982)

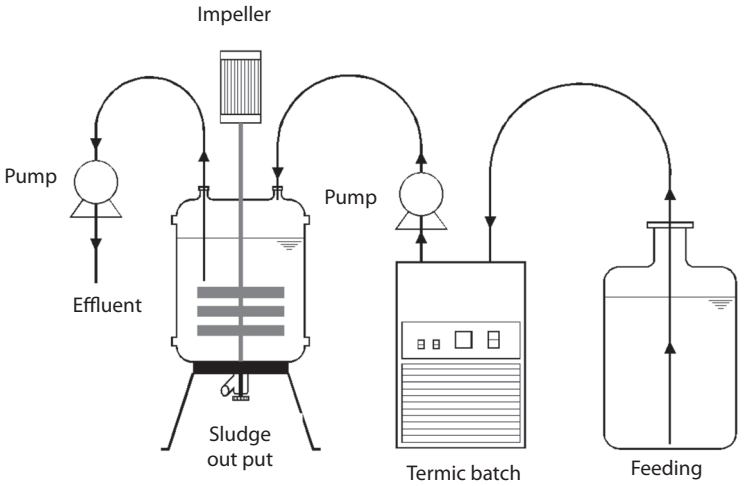

Fig. 1. Scheme of the mechanically stirred anaerobic sequencing batch.

Thus, we used 430mL of Tietê river sediment (inoculum) and 1 070mL synthetic substrate.

The bioreactor was monitored for 144 days with defined 24-hour cycles. Each cycle was divided into four phases: 1) Feed without stirring (15min), 2) Reaction with stirring (23h), 3) Sedimentation without stirring (30min) and 4) Output of treated effluent (drain of reactor) without stirring (15min).

The synthetic substrate in the feed line consisted of yeast extract, sucrose, sodium bicarbonate ($NaHCO_3$), starch, potassium nitrate (KNO_3) and liquid household detergent (quantified as LAS concentration) (Table 2). During the 144 cycles, the bioreactor showed different stages: biomass adaptation to synthetic substrates - 39 days; (A) LAS addition (15mg/L) – 31 days; (B) decrease in co-substrates (50%) – 61 days; (C) increase in LAS concentration (30mg/L) – 43 days. Chemical oxygen demand (COD) (raw and filtered), nitrate and solids were determined according to APHA (2005). The pH of the suspension was determined with a pH meter.

The LAS concentration was periodically measured in the liquid phase (influent and effluent) using high-performance liquid chromatography (HPLC) (Duarte, Oliveira, Buzzini,

TABLE 2
Synthetic substrate composition during stages in sequence batch reactor

Stage	Time (days)	Yeast extract (mg/L)	Sucrose (mg/L)	$NaHCO_3$ (mg/L)	Starch (mg/L)	KNO_3 (mg/L)	LAS (mg/L)
adaptation	39	500	80	400	230	200	–
A	31	500	80	400	230	200	15
B	61	250	40	400	115	250	15
C	43	500	80	400	230	250	30

Adorno, & Varesche, 2006). The adsorbed LAS was extracted with methanol in an ultrasound bath for 30 minutes and analyzed by HLPC HPLC in triplicate (Duarte, Oliveira, Saavedra, Fantinatti-Garboggini, Oliveira, & Varesche, 2008). This extraction protocol had an efficiency of 85% (Duarte et al., 2008). The mass balance for LAS considered the surfactant in the feed (influent), in the effluent and adsorbed on the biomass in the reactor.

The most probable number (MPN) technique was used to estimate the denitrifying bacteria (Tiedje, 1982) in the biomass reactor. The biomass reactor samples were homogenized and diluted in flasks with the feeding solution used in each operational stage. The detection of bacteria was performed after 30 days of incubation at 30ºC. The results were interpreted as detailed by APHA (2005).

RESULTS

The sediment used as inoculum for surfactant degradation showed high density of denitrifying bacteria – $7.6x10^{12}$ MNP/gTVS (Table 1).

In the adaptation stage, the bioreactor showed stability with influent pH of 6.3 and effluent of 7.3. At this stage, the COD removal was 69% for COD influent and effluent of 382mg/L and 129mg/L, respectively. However, the nitrate removal achieved was 98% with an estimation of denitrifying bacteria of $7.6x10^{10}$MNP/gTVS. Solids loss was observed when compared to the initial bioreactor operation conditions (13.6 to 6.9gTVS).

With the addition of 15mg/L of LAS (stage A), the nitrate, COD removal and pH were not affected (Table 3). In this stage, 675mg of LAS were added in the reactor. After this stage, mass balance indicated that the addition of 10% LAS was adsorbed on the biomass and LAS degradation was 60% (Table 4). Denitrifying bacteria population was improved by adding LAS and the population was estimated at $2.2x10^{13}$MPNg/TVS, and the amount of solids in the reactor also increased from 6.9 to 7.3gTVS. Stage B lasted 61 days and was characterized by a 50% decrease of organic sources (co-substrates) and 1 440mg addition of LAS. The effluent pH remained stable; the nitrate removal was reduced to 84% of efficiency; also there was a decrease of COD removal efficiency (37%), LAS degradation (55.4%), estimation of denitrifying bacteria ($1.0x10^{8}$MNP/gTVS) and solids (4.9gTVS) (Table 3).

Due to the decrease in the population of denitrifying bacteria, and in the efficiency of LAS degradation, concentrations of co-substrates were re-established, and the concentration of LAS increased to 30mg/L. Stage C had the highest mass of LAS applied (1 944mg) and showed the highest specific LAS-load rate (9.7mgLAS/gTVS/d). The effluent pH and the most probable number of denitrifying bacteria remained similar to the previous stage, the COD removal efficiency increased to 57%. Even resuming to the previous nutritional conditions, the total volatile solids (3.2g) and nitrate removal (Table 3) decreased, and the degradation efficiency of LAS was the lowest observed during the experiment, reaching 47% (Table 4). It is probable that the addition of 30mg/L LAS and removal of co-substrates were negative concerning LAS removal in the system (Fig. 2).

TABLE 3
Mean values of the monitoring parameters

Parameter	Stages			
	adaptation	A	B	C
COD				
Influent (mg/L)	382±208	350±150	154±61	324±71
Effluent (mg/L)	129±115	130+88	96±43	139±32
Nitrate				
Influent (mg/L)	180±2	200±23	226±23	255±29
Effluent (mg/L)	3±2	15±24	35±12	55±13
LAS				
Influent (mg/L)		15.0±1.0	15.0±1.0	30.0±3.0
Effluent (mg/L)		4.8±1.6	6.1±2.0	14.6±3.4
Specific LAS load rate (mgLAS gTVS/d)		2.06	3.09	9.70
pH				
Influent	6.5±0.7	6.9±0.5	7.7±0.2	7.7±0.3
Effluent	7.3±0.2	7.4±0.3	7.5±0.1	7.7±0.1
Solid Total (g/L)	26.7±1.5	23.6±1.5	14.3±2.8	7.2±8.2
Total volatile (g/L)	6.9±1.0	7.3±1.0	4.9±1.0	3.2±1.0
Denitrified bacteria (MPN/gTVS)	$7.6x10^{10}$	$2.2x10^{13}$	$1.0x10^{8}$	$2.4x10^{8}$

TABLE 4
Mass balance of LAS in sequence batch reactor

Mass balance	Stages		
	A	B	C
Added - influent (mg)	675	1 440	1 944
Effluent(mg)	202	566	953
Adsorbed (mg)	67	77	77
Degraded (mg)	406	798	914
Degradation efficiency (%)	60.1	55.4	47.0
Removal (%)	68.2	59.3	51.2
Time (d)	30	60	42

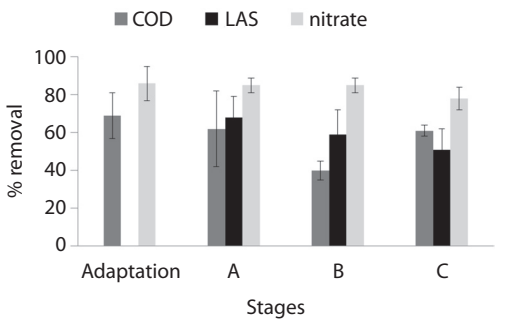

Fig. 2. Percentages of COD, nitrate and LAS removal in different stages.

DISCUSSION

Due to their high consumption and applications, significant amounts of surfactants are released into the environment, and this release causes serious problems in rivers and oceans. High concentrations of surfactants can be found in river sediments receiving untreated effluents due to inefficient degradation of LAS. Eniola and Olayemi (2008) found surfactant concentrations ranging from 45 to 132mg/g in sediments from the Asa River in Nigeria and heterotrophic bacteria $2.9x10^{5}$ and $1.2x10^{7}$CFU/g.

The sediment analyzed in this study showed concentrations of LAS lower than 0.30mg/g of sediment, however, the population of denitrifying bacteria was $7.6x10^{12}$MPN/gTVS, showing that denitrifying bacteria are responsible for nitrate reduction processes in river sediments (Berna et al., 2007).

Also, biological systems using pure cultures or microbial consortia in different fermentation conditions have been used to promote the

degradation of surfactants (Cserháti, Forgács, Oros, 2002). This bioavailability increase was remarkable at the beginning of LAS addition, because of the organic compounds previously adsorbed on the biomass. According to Elsgaard (2010), the presence of LAS (105mg/L) in wastewater does not inhibit nitrate removal, but inhibits iron sulfate removal.

In our study, it is possible that the nutritional conditions enriched some bacteria from the inoculum, increasing microorganisms which use LAS as carbon source. Decreased co-substrates affected the degradation efficiency of LAS (55.4%) possibly due to a lower denitrifying bacteria population estimated ($1.0x10^8$MPNg/TVS). Furthermore, biomass concentration decreased from 7.3gTVS/L (stage A) to 4.9gTVS/L (stage B). Also, mean nitrate removal decreased from 87% (stage A) to 78% (stage B).

LAS degradation under denitrifying conditions observed in this study was higher than any other reported at the same LAS concentration. An anaerobic sequence batch reactor achieved LAS degradation efficiency of 37-53%, related to a LAS concentration in the influent of 22mg/L and specific-LAS load rate ranging from 6.8 to 9.8mgLAS g/TVS, the highest LAS degradation was 53% in the stage without co-substrates (Duarte, Oliveira, Mayor, Okada, & Varesche, 2010). The LAS presence did not inhibit bacteria in an acidogenic Upflow Anaerobic Sludge Blanket (UASB) reactor. After 250 days, LAS degradation was 41%. At hydraulic retention time of 6h, this reactor used lactose (1g/L) as co-substrate. Potassium nitrate was the electron acceptor at 1:1 ratio (LAS: NO3-) and this acceptor was completely consumed (Almendariz, Meráz, Soberón, & Monroy, 2001).

A UASB reactor fed with isotonic solution and LAS (5mg/L) degraded 85% of the surfactant. In this study, another UASB reactor fed with co-substrates showed LAS degradation of 64% (Sanz, Culubret, de Ferrer, Moreno, & Berna, 2003). Moreover, UASB reactors under mesophilic and thermophilic conditions obtained removal rates ranging from 40 to 80% (Lobner, Torang, Batstone, Schmidt, & Angelidaki, 2005). The test batches lasting 165 days, which used marine sediments as inoculum for LAS degradation (concentration ranging from 10 to 50mg/L) resulted in LAS degradation of 79% and identified high phylogenetic variety in the process. Clone library showed the classes Alphaproteobacteria, Gammaproteobacteria (genus *Pseudomonas*), and Sedimentibacter (family Clostridiales) (Lara-Martin, Gomez-Parra, Kochling, Sanz, Amils, & Gonzalez-Mazo, 2007).

Tietê river sediments could be used as inoculum in reactors for the treatment of anionic detergents in denitrifying (anaerobic redox potential) conditions. Denitrifying bacteria are potential candidates for effective anaerobic degradation of LAS. This bacteria group was able to degrade LAS molecule independently of additional carbon sources, while removing chemical oxygen demand and nitrate in anaerobic wastewater treatment plants.

ACKNOWLEDGMENTS

We gratefully acknowledge the Conselho Nacional de Pesquisa (CNPq) for funding this study, (Process 479900/2008-6).

RESUMEN

Degradación anaeróbica de tensioactivos aniónicos en microorganismos autóctonos de los sedimentos de un río tropical contaminado en Brasil. El alquilbenceno sulfonato lineal (LAS) es el tensoactivo sintético más usado en todo el mundo en los produtos de limpeza domestica e industrial y puede llegar a las masas de agua a través de la descarga de aguas residuales sin tratamiento o con un tratamiento ineficaz. El objetivo del estudio consistió en evaluar la capacidad de la microbiota presente en el sedimento del río Tietê en la degradación del tensoactivo anionico – LAS. El experimento se llevó a cabo en un bioreactor de lotes secuenciales en condiciones de desnitrificación con ciclos de 24 horas, agitación de 150rpm, usando 430mL de sedimento y 1 070mL de sustrato sintético constituido por extracto de levadura, almidón soluble, bicarbonato de sodio y sacarosa. El LAS fue añadido a diferentes concentraciones de 15mg/L y 30mg/L. El funcionamiento del bioreactor se dividió en la adaptación de la biomasa con sustrato sintético sin LAS y tres condiciones experimentales: A) adición de 15mg/L

de LAS; B) 15mg/L de LAS y reducción del 50% de la concentración del co-sustrato y C) 30mg/L de LAS y la concentración de 100% de co-substrato. Los resultados obtenidos muestran que la eficiencia en la degradación del LAS está directamente relacionada con la población de bacterias desnitrificadoras y que el sedimento del río Tietê se puede utilizar como inóculo en el tratamiento de LAS en condiciones desnitrificadoras. La población de bacterias fue capaz de degradar el LAS independiente de la fuente de carbón adicionada. La remoción de LAS y de nitrato se puede lograr simultáneamente en aguas residuales con una baja carga orgánica. La reducción de la concentración del co-sustrato fue influenciado directamente por la población de bacterias desnitrificantes ($2.2x10^{13}$ a $1.0x10^{8}$MNP/gTVS) y por lo tanto la degradación de LAS (60.1-55.4%). Los microorganismos en el sedimento del río Tietê se pueden usar como inóculo alternativo para el tratamiento de efluentes contaminados con nitrato y LAS.

Palabras clave: LAS, anaeróbico, tensioactivo, degradación, bacterias, nitrato.

REFERENCES

Almendariz, F. J., Meráz, M., Soberón, G., & Monroy, O. (2001). Degradation of linear alkylbenzene sulphonate (LAS) in an acidogenic reactor bioaugment UIT a *Pseudomonas aeroginousa* (M113) strain. *Water Science Technology, 44*, 183-188.

American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). (2005). *Standard Methods for the Examination of water and wastewater.* Washington DC: American Public Health Association.

Berna, J. L., Cassini, G., Hager, C. D., Rehman, N., López, I., Schowanek, K. D., Sterber, J., Taeger, K., & Wind, T. (2007). Anaerobic biodegradation of surfactants-Scientific Review. *Tenside Surfactants Detergents*, *44*, 312-346.

Cavalli, L., Cassani, G., Vigaró, L., Pravettoni, S., Nucci, G., Lazzarin, M., & Zatta, A. (2000). Surfactants in sediments. *Tenside Surfactactants Detergents, 37*, 282-288.

CETESB - Companhia Ambiental do Estado de São Paulo. (1992). *Água do mar – Teste de toxicidade crônica de curta duração com Lytechinus variegatus, Lamarck, 1816.* Norma Técnica L5.250. São Paulo: CETESB.

Cserháti, T., Forgács, E., & Oros, G. (2002). Biological activity and environmental impact of anionic surfactants. *Environmental International, 28*, 337-348.

Duarte, I. C. S., Oliveira, L. L., Buzzini, A. P., Adorno, M. A. T., & Varesche, M. B. A. (2006). Development of a method by HPLC to determine LAS and its application in anaerobic reactors. *Journal of the Brazilian Chemical Society, 17*, 1360-1367.

Duarte, I. C. S., Oliveira, L. L., Saavedra, N. K. D., Fantinatti-Garboggini, F., Oliveira, V. M., & Varesche , M. B. A. (2008). Evaluation of the microbial diversity in a horizontal-flow anaerobic immobilized biomass reactor treating linear alkylbenzene sulfonate. *Biodegradation, 19*, 375-385.

Duarte, I. C. S., Oliveira, L. L., Mayor, M. S., Okada, D. Y., & Varesche, M. B. A. (2010). Degradation of detergent (linear alkylbenzene sulfonate) in an anaerobic stirred sequencing-batch reactor containing granular biomass. *International Biodeterioration and Biodegradation*, *64*, 129-134.

Elsgaard, L. (2010). Toxicity of xenobiotics during sulfate, iron, and nitrate reduction in primary sewage sludge suspension. *Chemosphere, 79*, 1003-1009.

Eichhorn, P., Rodríguez, S. V., Baumann, W., & Knepper, T. P. (2002). Incomplete degradation of linear alkylbenzene sulfonate surfactants in Brazilian surface waters and pursuit of their metabolites in drinking waters. *Science of Total Environmental*, *284*, 123-134.

Eniola, K. I. T., & Olayemi, A. B. (2008). Linear alkylbenzene sulfonate tolerance in bacteria isolated from sedimento f tropical water bodies polluted with detergents. *Revista Biologia Tropical*, 56, 1595-1601.

Garcia, M. T., Campos, E., Sanchez-Leal, J., & Ribosa, I. (2006). Effect of linear alkylbenzene sulphonate (LAS) on the anaerobic digestion of sewage sludge. *Water Research, 40*, 2958-2964.

Hatamura, E., Eysink, G. J., Bevilacqua, J. E., & Moraes, R. P. (1993). *Enriquecimento das espumas por substâncias químicas como agente de exportação de poluentes no Rio Tietê*. (Relatório Técnico). CETESB.

Lara-Martin, P. A., Gomez-Parra, A., Kochling, T., Sanz, J. L., Amils, R., & Gonzalez-Mazo, E. (2007). Presence, biotransformation and effects of sulfophenylcarboxylic acids in the benthic fish *Solea senegalensis*. *Environment International*, *33*, 565-570.

Lobner, T., Torang, L., Batstone, D. J., Schmidt, J. E., & Angelidaki, I. (2005). Effects of process stability on anaerobic biodegradation of LAS in UASB reactors. *Biotechnology Bioengineering*, *89,* 759-765.

Mortatti, J., Moraes, G. M., & Kiang, C. H. (2012). Distribuição e possível origem de metais pesados nos sedimentos de fundo ao longo da Bacia do alto Tietê: aplicação da normalização geoquímica sucessiva. *Geociências, 31,* 175-184.

Olayemi, A. B, Eniola, K. I. T., Awe, S., & Kayoe-Isola, M. T. (2003). Distribution of Bacteria in three detergent effluent-polluted water bodies in Ilorin, Nigeria. *Nigeria Society for Experimental Journal, 3*, 79-86.

Olkowska, E., Ruman, M., & Polkowska, Z. (2014). Occurrence of surface active agents in the environment. *Journal of Analytical Methods in Chemistry, 2014*, 1-15.

Rocha, P. S., Luvizotto, G. L., Kosmehl, T., Bottcher, M., Storch, V., Braunbeck, T., & Hollert, H. (2009). Sediment genotoxicity in the Tietê River (São Paulo, Brazil): in vitro comet assay versus in situ micronucleus assay studies. *Ecotoxicolology and Environmental Safety, 72,* 1842-1848.

Sanz, J. L., Culubret, E., De Ferrer, J., Moreno, A., & Berna, J. L. (2003). Anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in up-flow anaerobic sludge blanket (UASB) reactors. *Biodegradation*, *14,* 57-64.

Tiedje, J. M. (1982). Denitrification. In A. Page, R. H. Miller, & D. R. Keeney (Eds.), *Methods of soil analysis* (pp. 1011-1026). Winsconsin: American Society of Agronomy.