





## Environmental impact of wastewater discharges containing surfactants and their effect on biomass in anaerobic reactors during treatment

### Impacto ambiental por descargas de aguas residuales con tensoactivos y su efecto en la biomasa de reactores anaerobios durante su tratamiento

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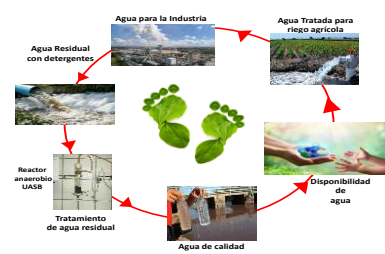
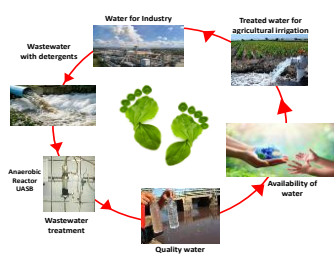


#### Abstract

The widespread use of surfactants in domestic, industrial, and agricultural products has sparked growing concern due to their environmental persistence and potential for bioaccumulation in living organisms. The results showed that the biomass from the acidogenic reactor was able to eliminate this pollutant in terms of its COD (mg/L) by 42.24% and surfactant biodegradation at a concentration of 200 mg/L by 86.65% (experiment V), with a simultaneous desorption and biodegradation dynamic of 39.38 mg/day. At 300 mg/L (experiment VI), these values decreased to 36.6% and 55%, respectively. The methanogenic reactor fed with the effluent from this reactor showed a COD removal and LAS biodegradation efficiency of 83.17% and 13.85% with a bioaccumulation rate of 30.18 mg/day (experiment III). While at a LAS concentration of  $300 \pm 0.05$  mg/L (experiment VI), the LAS accumulation trend in the biomass of both reactors was 29.41 mg/d and 21.07 mg/d.

#### Resumen

El uso masivo de surfactantes en productos domésticos, industriales y agrícolas ha provocado una creciente preocupación debido a su persistencia ambiental y su capacidad de bioacumulación en organismos vivos. Los resultados mostraron que la biomasa del reactor acidogénico logró eliminar este contaminante en términos de su DQO (mg/L) en un 42,24 % y de biodegradación del tensoactivo a una concentración de 200mg/L en un 86,65 % (experimento V) y una dinámica de desorción y biodegradación simultánea de 39,38 mg/día. A 300 mg/L (experimento VI), estos valores se reducen al 36,6 % y 55 %, respectivamente. El reactor metanogénico alimentado con el efluente de este reactor, mostró una eficiencia de eliminación de DQO y de biodegradación de LAS del 83,17 % y del 13,85 % con una tasa de bioacumulación de 30,18 mg/día (experimento III). Mientras que a una concentración de LAS de  $300 \pm 0,05$  mg/L (experimento VI), la tendencia de acumulación de LAS en la biomasa de ambos reactores, fue de 29,41 mg/d y 21,07 mg/d.



**Environmental impact, bioaccumulation, ecosystems, biodiversity, anaerobic reactor, surfactants**

**Impacto ambiental, bioacumulación, ecosistemas, biodiversidad, reactor anaerobio, tensoactivos**

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## Introduction

Surfactants are adjuvant chemical compounds, many of which are used to enhance the effectiveness of biologically active substances such as herbicides or pesticides. Most act at fluid interfaces and have a bipolar structure with hydrophilic and hydrophobic portions that allow them to interact with cell surfaces and biological membranes in general, affecting surface tension and the mobilization of molecules between extracellular and intracellular media. This can cause direct injury to the epithelial membranes of vital organs of aquatic animals, such as the gills; when released into the environment through domestic and industrial wastewater, runoff and leaching from agricultural fields, and by wind action in aerial spraying of agrochemicals (Vázquez, 2024).

According to their structure, they are called surfactants; they have foaming, emulsification, detergency, and particle suspension properties. These types of substances are called emerging contaminants (Gomes *et al.*, 2018). Worldwide, it is estimated that surfactant production increased from 9.25 million tons in 1995 to 11 million tons in 2000, with an average annual growth of 3.5%. Asia is the largest producer of these products, accounting for 45% of the total, followed, in order, by Europe with 28% and the Americas with 22%. It is estimated that national production of surfactants for the year 2002 was approximately 24,053 tons, of which 2,747 were exported, with a total import of 8,126 tons (CENIPALMA, 2005).

Two decades ago, over 4.2 million tonnes (MT) of detergents and 1.2 MT of fabric softeners were used annually in Western Europe (Ivanković & Hrenović, 2010). Global annual surfactant production stood at 7 MT in 2000 (Pettersson *et al.*, 2000), which increased significantly to 12.5 MT in 2006 and 14.1 MT in 2017, with an estimated growth of 18% by 2022 (Johnson *et al.*, 2021). Global demand for surfactants has increased by 300%, surpassing the current global production of 3 million tons per year and consumption of 18 million tons. In Mexico, Procter & Gamble has a production capacity of 180,000 tons per year of powder and liquid detergents; exporting 45% of what is produced in the country (Procter & Gamble Mexico, 2025).

Linear alkylbenzene sulfonate (LAS) [ $C_{12}H_{25}C_6H_4SO_3^-Na^+$ ] are the most commonly used in the production of personal detergents (laundry and cleaning products), and frequently use sodium salts as the sole surfactant in a formulation or together with other anionic, non-ionic or cationic surfactants.

This compound is characterized by an alkyl chain of 11 carbon atoms (C<sub>11</sub>-LAS) to which a sulfonated aromatic ring is attached in the "para" position. Linear alkylbenzenes are obtained from n-paraffins (C<sub>10</sub> to C<sub>14</sub>) either by partial dehydrogenation to obtain olefins, and the subsequent addition of benzene (C<sub>6</sub>H<sub>6</sub>) using hydrogen fluoride (HF) as a catalyst; or by chlorination of the paraffins followed by the Friedel-Crafts reaction, using benzene and an aluminum chloride catalyst (Maloney, 2008).

Within the production of detergents, LAS are the most important in the world and are one of the most widely used sulfonated hydrocarbons with an annual production of approximately 1X10<sup>6</sup> ton/year in the United States out of a worldwide detergent production of 15X10<sup>6</sup> ton/year (Mogensen *et al.*, 2003). Linear alkylbenzene (LAS) as an anionic surfactant, presented a consumption of 270,000 tonnes/year in Europe in 2000 (European Eco-Label, 2002).

Currently, linear alkylbenzene sulfonate (LAS) is one of the most widely used sulfonated hydrocarbons with an annual production of approximately 1X10<sup>6</sup> ton/year in the United States out of a worldwide detergent production of 15X10<sup>6</sup> ton/year (Mogensen *et al.*, 2003).

With an estimated market size of linear alkylbenzene at USD 28.49 Billion in 2023. And the linear alkylbenzene market industry is expected to grow from USD 29.14 Billion in 2024 to USD 35.0 Billion by 2032. With a market growth rate of linear alkylbenzene in the order of 2.31% during the period from 2025 to 2032 (Research Report, 2024) and an increasing demand in Europe, United States, Japan, and developing countries, to reach a global consumption volume in the order of 18 million tons by 2050 (Zhu *et al.*, 2018).

In recent years, an increase in the consumption of anionic surfactants of the sodium alkylbenzene sulfonate (LAS) type has been observed, since they are the most widely used in the synthesis of anionic surfactants, which are used as active agents in various cleaners such as detergents, shampoos, toothpastes, etc., with a per capita consumption in Eastern Europe, USA and Japan, in the order of 3 to 5 g of LAS per inhabitant per day, and consumption in Southern Africa and East Asia, at 1 g per inhabitant per day (Berna *et al.*, 1991).

In Mexico, a production of 528,481 tons/year is reported, internationally, it occupies the 5th place among detergent producers (4.4%), United States (16.5%), Brazil (6.4%), China (5.0%) and Germany (4.7%). This means that per capita consumption in Mexico is high, at 10 kilos per year, a figure higher than that of other countries given the population growth rate, according to INEGI, (National Institute of Statistics, Geography and Informatics, 2013).

In addition to surfactants, detergents contain phosphates (such as sodium tripolyphosphate), optical brighteners, fragrances, and preservatives. The bioaccumulation of surfactants alters the synthesis and stability of photosynthetic pigments, affecting photosynthesis in exposed plants. They can damage cellular proteins or alter their production. Genisel & Eren (2020) found that sodium dodecyl sulfate (SDS) not only reduced pigments but also decreased soluble protein content in barley leaves.

The presence of sulfate, another component of SDS, at high concentrations in cells disrupts the intracellular colloidal structure. In such a case, excess sulfate in plant tissues retards plant growth by disrupting synthesis reactions and cell division. In this study, protein content results have already shown that SDS significantly affects biosynthesis reactions. Irrigation with gray laundry water used in irrigation has negative impacts on soil properties, for example; reduces bean growth and alters the chlorophyll and crude protein content of plants, detergents tend to degrade or alter essential proteins, affecting metabolic processes linked to protein synthesis (Abu-Zreig *et al.*, 2003) mainly associated with the dispersion of aggregates due to the accumulation of sodium (Misra & Sivongxay, 2009).

And the modification of hydrodynamic properties of soils caused by the accumulation of surfactants (Lado & Ben-Hur, 2009). Exposure to high concentrations of detergents in irrigation water triggers oxidative stress responses in corn seedlings, in addition to a decrease in the activities of antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase in SDS-treated barley, accompanied by a strong increase in free radicals and lipid peroxidation (Genisel & Eren, 2020).

Their bioaccumulation can cause serious alterations in plant metabolism: a) Reduction in plant growth; i.e., stem shortening, decreased leaf mass, and b) Interference with nutrient uptake: Due to competitive blockage or root damage. c) Oxidative stress: Production of reactive oxygen species (ROS) that damage lipids and proteins. "The presence of pollutants generates oxidative stress, damaging cell membranes and reducing photosynthetic efficiency" d) Photosynthetic alterations: Loss of chlorophyll, inefficiency in the electron transport chain (Hernández-Baranda, 2023).

Exposure of coastal vegetation to surfactants results in an indirect absorption of sodium chloride and a reduction in water surface tension, caused by the erosion of the epicuticular wax of plants. In hyacinth, it affects the inherent salinity tolerance of water, which is reduced by exposure to the surfactant. In soil, LAS impedes microbial processes, such as bacterial iron reduction. Its continuous application increases the concentration of acid and alkaline phosphatase, also causing a decrease in the activity of arylsulfatase and dehydrogenase. High concentrations of LAS exert selective pressure on the diversity of heterotrophic bacteria; while even at low concentrations, surfactants significantly affect soil physics, chemistry, and biology (Badot *et al.*, 1993).

LAS toxicity data (lethal concentrations, EC<sub>50</sub>) for aquatic organisms range from 1 to 10 mg per liter in short-term tests. LAS is approximately equally toxic to fish and invertebrates, while its toxicity to algae varies widely (Hashim & Kulandia, 1992). At doses of 30 ppm, it induces inhibition of coastal organisms (Bressan *et al.*, 1991).

Exposure of coastal vegetation to surfactants results in an indirect absorption of sodium chloride and a reduction in water surface tension caused by erosion of the epicuticular wax of plants. In hyacinth, it affects the inherent tolerance to water salinity, which is reduced by exposure to the surfactant. Exposed vegetation may receive up to 0.1 mg of anionic detergents, 1.5 mg of petroleum products, and 20 mg of NaCl per square meter of leaf area daily (Badot *et al.*, 1993). Therefore, detergent concentrations in water of 2.5 mg/L affect plant growth and concentrations of 5 to 6 mg/L are toxic to algae and fish in general (Marin, 1995).

This causes water to tend to leave the plant cells, affecting turgor; 1) biochemical alterations, 2) decreased photosynthesis. Damage to chloroplasts reduces the synthesis of chlorophyll a and b, which lowers the efficiency of light capture, that is: Less chlorophyll → less sugar production → less energy for growth (Hernández-Baranda *et al.*, 2023). Damage to membranes and organelles increases the production of free radicals such as  $O_2^{\bullet-}$  and  $H_2O_2$ . These radicals oxidize lipids, proteins and DNA, accelerating cell aging and causing leaf necrosis (Sharma *et al.*, 2012). Anaerobic microorganisms appear to be the most affected by the presence of surfactant (Van Hamme *et al.*, 2006).

The toxicity of surfactants in the environment is affected by various physical, chemical and biological factors that interact with each other, such as temperature and pH. Buhl & Hamilton (2000) suggest that the toxicity of the anionic surfactant sodium dodecyl sulfate (SDS) increases with increasing temperature.

In domestic wastewater average concentrations of LAS have been found from 1 to 20 mg/L in surface waters at 0.5 mg/L and in wastewater from detergent producing industries, average concentrations of 300 mg/L (Fox *et al.*, 2000). Its gross discharge into rivers increases the level of surfactants and other contaminants representing a threat to the resident macro and micro populations (Cirelli & Ojeda, 2008). The toxic effects of surfactants in aquatic organisms are mainly due to their ability to adsorb and penetrate the cell membrane of aquatic organisms (Rebello *et al.*, 2013).

They generate deleterious effects in aquatic organisms by binding to epithelial membranes of their respiratory structures, such as the external gills of amphibians, specialized cell areas of invertebrates, and teleost gills. At the gill level, they can cause histological and ultrastructural lesions, including detachment, necrosis, hyperplasia, hypertrophy, and rupture of the gill epithelium.

These lesions are found in fish exposed to media contaminated with other toxins such as heavy metals, pesticides, organotins, organic solvents, and organic xenobiotics. Depending on their concentration, they are toxic to aquatic organisms because they hinder the transfer of oxygen in the water. High concentrations of surfactants cause death of fish due to; decreased surface tension, tissue destruction and alteration of biomacromolecules (Rosety-Rodríguez *et al.*, 2002). Aquatic plant species are also affected by the toxic effects of surfactants. These break the chlorophyll-protein complex, damaging the cell membrane, which delays metabolism and growth rate (Jardak *et al.*, 2016). In *Azolla pinnata* and *Hydrilla verticillata*, exposed to different concentrations for four weeks, a significant reduction in chlorophyll was observed in the leaves of *A. pinnata* at concentrations above 2 ppm of sodium lauryl sulfate (SLS). Meanwhile, *H. verticillata* turned completely brown at 10, 15, and 20 ppm of SLS. When exposed to sodium dodecylbenzenesulfonate (SDBS), chlorophyll content in both plants decreased at all concentrations (Mousavi & Khodadoost, 2019).

In *macrophytes* such as *Elodea canadensis* and *Myriophyllum spicatum*, photosynthesis was reported to be reduced by 50% after exposure to 1.0 mg/L of LAS (Lewis, 1990). They can also generate adverse effects on terrestrial plants, due to their ability to alter their membranes (Cuevas *et al.*, 2023). Several studies have shown phytotoxic effects derived from exposure to detergents. Hernández-Baranda (2023) reports that, under controlled conditions, increased detergent concentration significantly decreases germination and seedling development, evidenced by shorter stem and root lengths. These effects are visible symptoms such as chlorosis, wilting, necrosis, and reduction in leaf size.

The magnitude of this damage depends on factors such as its concentration, type of detergent, exposure time, and the plant species involved. (Vázquez, 2024). The accumulation of surfactants leads to the production of ammonia, which can affect the synthesis of substances essential for the metabolism of fish, crustaceans, and bacteria, in addition to affecting the growth of phytoplankton (Cardenas & Sanchez, 2013). Their bioaccumulation in aquatic organisms occurs through different mechanisms, largely due to their amphipathic structure, which allows them to interact with cell membranes and fatty tissues.

Therefore, the presence of surfactants in aquatic ecosystems not only represents a threat to biodiversity, but also constitutes a food safety and public health issue that demands urgent monitoring, control, treatment, and mitigation strategies. Over the past 50 years, biodiversity loss has been accelerated by external factors, including global warming, climate change due to anthropogenic activities, and industrial wastewater discharges containing high concentrations of highly toxic pollutants, such as surfactants made from linear alkylbenzene sulfonate (LAS) (Figure 1). Furthermore, the presence of volatile, emerging, and persistent organic compounds represents a new global challenge to water quality in developed and developing countries, posing potentially serious threats to human health, the environment, and ecosystems (WHO/UNICEF, 2015).

Their impacts on aquatic and terrestrial ecosystems can be summarized in three broad categories: a) Eutrophication and loss of water quality (Pérez-García, 2023), b) Alteration of the soil microbiota and c) Phytotoxicity in plants. Plants exposed to detergents show symptoms of chemical stress, such as chlorosis, necrosis, delayed germination, and reduced growth (Cuevas *et al.*, 2023). Hernández-Baranda (2023) documented that exposure of seedlings to household detergents caused a significant decrease in root and stem length, accompanied by visible changes in leaf color. The impact of surfactants on aquatic and terrestrial life depends on their chemical nature (Jardak *et al.*, 2016). In soil, they can modify soil pH, electrical conductivity, and nutrient availability: salinity and pH increase in soils treated with detergents, affecting nitrogen and potassium availability (Sharma *et al.*, 2012).

The phosphates present in some detergents contribute to eutrophication, affecting aquatic plants and surrounding areas. They reduce the surface tension at the air-water interface, which in turn causes a proportional decrease in capillary pressure. In recent years, the problem of contamination of water bodies has been a cause of great concern, due to the toxicity caused by xenobiotic compounds, such as detergents (Mousavi & Khodadoost, 2019). Worldwide, 80% of domestic wastewater that is discharged does not receive prior treatment before being discharged into a natural body of water World Bank Group (2020). Causing an imbalance in ecosystems, modifying the physicochemical parameters of the water resource and the natural characteristics of the fish fauna that receive these contributions. In a monitoring of 7 wastewater treatment plants, concentrations of AS (C<sub>12</sub>-C<sub>15</sub>) were found in their effluents between 0.0012 and 0.012 mg/l, with an average value of 0.0057 mg/L, in the water quality assessment of the Asa River in Ilorin, Kwara State, Nigeria, contamination with industrial detergents was reported (Adekola & Eletta, 2007).

And the waters of the Caspian Sea and the Volga, Terek and Sulak rivers presented a high level of contamination with high concentrations of detergents (Korshenko & Gul, 2005). As well as the coastal areas of the Sea of Okhotsk and the Avacha Bay, on the northwest coast of Russia (Zhuravel *et al.*, 2004). Meanwhile, findings in the waters of the South China Sea showed a concentration of anionic surfactants of 57% in the surface microlayer, and 43% in groundwater (Uning *et al.*, 2022).

Various technologies have been employed for the treatment of wastewater containing high concentrations of anionic, cationic, non-ionic and ampholytic/zwitter ionic surfactants, including; adsorption processes, chemical oxidation, ozonation, hydrogen peroxide, ultraviolet light irradiation, iron salts (Ikehata and El-Din, 2004), advanced oxidation processes, photocatalytic degradation (Bandala *et al.*, 2008), sonochemical processes (Dehghani *et al.*, 2010), ultrasound (Naldoni *et al.*, 2011) electrocoagulation, nanofiltration, aerobic or anaerobic biological processes and electrochemical processes (Korzenowski *et al.*, 2012).

Based on previous studies, the objective of this research was to evaluate the toxic effect and accumulation rate of the surfactant "LAS" in the biomass of UASB-type anaerobic reactors during the treatment of wastewater with high concentrations of detergents.

## 2. Materials and methods

### 2.1. Start-up and operation of the reactors

During the start-up of the anaerobic reactors, the first reactor was fed with lactose as the sole carbon source at a concentration of 1 g/L to promote acidogenic conditions and RAAM mineral medium (Shelton & Tiedje, 1984) (Experiment I) and the second reactor was fed with the effluent from this reactor. The synthetic wastewater was prepared with 1 g/L of lactose and surfactant "LAS" at a concentration of 200 mg/L, increasing to 300 mg/L during experiment VI.

### 2.2 Sample characterization

For the characterization of the synthetic water and process control, the main parameters were evaluated: Chemical oxygen demand (COD) and linear alkylbenzene sulfonate (LAS), according to standard methods (APHA, 2012) and the pH using a potentiometer (Corning pH/ion Analyzer 455).

### 2.3. Inoculum

The biomass used as inoculum for the UASB acidogenic and methanogenic reactor was collected from a UASB reactor that treats wastewater at the Universidad Autónoma Metropolitana Iztapalapa Unit, Mexico City. It presented a concentration of 63.9 g/L TSS, 37.5 g/L VSS, and a specific methanogenic activity (SMA) of 0.15 L CH<sub>4</sub>/g VSS d.

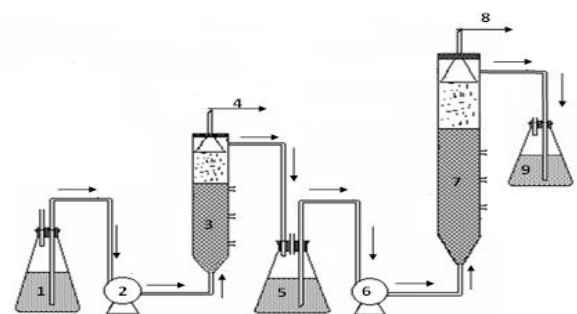
### 2.4. Operating conditions of UASB type acidogenic-methanogenic reactors

The design volume of the first reactor was 0.5 L, with a useful volume of 0.360 L, operated at a hydraulic retention time (HRT) of 0.25 days, fed with RAMM mineral medium (Shelton & Tiedje, 1984)

And lactose as the sole carbon source to promote the development of acidogenesis conditions and the conversion of volatile fatty acids (VFA) during acetogenesis, forming acetate, hydrogen and carbon dioxide and with the acetate produced, promote methanogenic conditions in the second reactor.

The design volume of the second reactor was 1.5 L, useful volume 1.44 L operated at a HRT of 1 day, operating both reactors at an average temperature of the order of 30±0.5°C.

## Box 1



**Figure 1**

Process description: 1) Surfactant wastewater, 2) Peristaltic pump, 3) UASB acidogenic reactor, 4) Biogás pipeline, 5) Treated wastewater by acidogenic reactor, 6) Peristaltic pump, 7) UASB methanogenic reactor, 8) Biogás pipeline, 9) Treated wastewater by methanogenic reactor.

### 2.5 Reactor monitoring

To evaluate the efficiency and performance of the studied system for the simultaneous biodegradation of LAS, 100 mL spot samples of the effluent from each reactor were taken every two days, and the main parameters were analyzed: COD, LAS, pH, and CH<sub>4</sub> produced in the methanogenic reactor. Based on the parameter differences between the influent and effluent, the COD removal rate, LAS biodegradation, and biogas production were estimated.

## 3. Analysis and discussion of results

### 3.1 Characterization of synthetic wastewater

Before addressing the analysis of the results, Table 1 presents the averages of the main parameters evaluated in the synthetic water with surfactant (LAS).

**Box 2****Table 1**

General characteristics of the synthetic wastewater fed to the studied system

| Parameter                | Experiment  |           |            |           |            |           |           |
|--------------------------|-------------|-----------|------------|-----------|------------|-----------|-----------|
|                          | I           | II        | III        | IV        | V          | VI        | VII       |
| Time of operation (days) | 0-62        | 63-120    | 121-174    | 175-244   | 245-290    | 291-343   | 344-374   |
| pH                       | 7.05±0.1    | 7.1±0.11  | 6.94±0.2   | 7.02±0.09 | 7.08±0.12  | 6.94±0.13 | 7.08±0.1  |
| COD (mg/L)               | 1118.6±14.1 | 1499±18.9 | 1479±20.4  | 1123±15.1 | 1503±22.7  | 1709±24.5 | 1074±13.9 |
| BOD <sub>5</sub> (mg/L)  | 655.6±8.1   | 878±10.4  | 866.8±10.1 | 658.2±8.8 | 880.9±12.1 | 1001±14.4 | 629.4±8.1 |
| LAS (mg/L)               | 0           | 200±0.08  | 200±1.3    | 0         | 200±0.09   | 300±0.05  | 0         |

**3.2 Reactor start-up and operation**

Once the system of anaerobic reactors operated in series, the object of this study was launched, the acidogenic reactor was initially fed with lactose (Experiment 1), to achieve the conditions of acidogenesis in the acidogenic reactor and with the conversion of acids volatile fatty acids (VFA) in acetate as the main substrate of the biomass of the methanogenic reactor and start from experiment II, with the feeding of the surfactant and monitor the performance of both reactors on their COD removal capacity and biodegradation of the LAS.

**3.3 Characterization of the water treated**

Tables 2 and 3 show the physicochemical characteristics of the water treated by both reactors.

**Box 3****Table 2**

Characterization of the water treated by the acidogenic reactor

| Parameter               | Experiment |           |           |           |           |           |           |
|-------------------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                         | I          | II        | III       | IV        | V         | VI        | VII       |
| pH                      | 4.4±0.11   | 4.8±0.08  | 4.4±0.11  | 4.5±0.12  | 4.6±0.13  | 4.4±0.1   | 4.4±0.12  |
| COD (mg/L)              | 789±10.8   | 726.8±9.7 | 1035±14.2 | 739±10.4  | 868±11.3  | 1083±14.9 | 717.4±8.9 |
| BOD <sub>5</sub> (mg/L) | 462.4±6.2  | 425±5.4   | 606.6±7.3 | 433.1±5.8 | 508.7±6.4 | 634.7±7.9 | 420.4±5.2 |
| LAS (mg/L)              | N.A        | 38.8±0.7  | 88±0.8    | 12.48±0.9 | 26.7±1.1  | 135±0.9   | 28.05±1.1 |

**Box 4****Table 3**

General characteristics of treated water and methane produced by the methanogenic reactor

| Parameter           | Experiment |           |           |           |           |           |           |
|---------------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                     | I          | II        | III       | IV        | V         | VI        | VII       |
| pH                  | 5.6±0.23   | 6.98±0.12 | 7.25±0.08 | 6.8±0.13  | 7.35±0.1  | 7.1±0.11  | 6.6±0.18  |
| COD (mg/L)          | 372±4.9    | 171±2.54  | 372±5.3   | 130±1.9   | 146±1.84  | 590±6.7   | 449±5.73  |
| LAS (mg/L)          | N.A        | N.A       | 59±0.8    | 4.1±0.5   | 23±0.7    | 115±1.1   | 23±0.9    |
| LCH <sub>4</sub> /d | 0.012±0.03 | 0.26±0.01 | 0.19±0.01 | 0.16±0.01 | 0.23±0.01 | 0.10±0.02 | 0.07±0.03 |

**3.4 COD removal efficiency and LAS biodegradation in the acidogenic reactor**

The presence of toxic compounds also influences the reactor's performance on its biodegradation by inhibiting the activity of the bacterial consortium, if an appropriate biomass acclimatization strategy is not used, in which the use of co-substrates is often not necessary (Terreros *et al.*, 2022).

In a study carried out to degrade three anionic surfactants: linear alkylbenzenesulfonate, sodium dodecylsulfonate and sodium dodecyl sulfate by anaerobic digestion, the inhibitory effect of the surfactant on the methanogenesis process was reported at concentrations higher than 100 mg/L in the sludge of an anoxic reactor, and inhibition at concentrations higher than 50 mg/L in the sludge of an anoxic lagoon.

Other studies have reported that during the anaerobic biodegradation of surfactants made with LAS, the most affected bacteria are the acetoclastic methanogenic bacteria during methane production, associated with the high accumulation of acetate, given the inhibitory effect of LAS (Wagener & Schink, 1988). Therefore, the use of bacterial consortia improves the biodegradation of this type of compounds than a single strain, since they are not capable of mineralizing it.

It is evidenced that the key step to carry it out is in the breaking of the benzene ring, and that its complete mineralization improves when coexisting syntrophy, for example: that which occurs between coastal bacteria and strict aerobic bacteria (Sigouillot & Nguyen, 1992).

The results of COD removal and LAS degradation in the reactor system used in the present investigation are shown below. Figure 4 shows the behavior of the acidogenic reactor (experiments II-VII) on the biodegradation of the surfactant and COD removal at different LAS concentration rates and organic loads.

During the first 60 days of operation (experiment I), both reactors were operated in series, using as a feeding medium a solution made with lactose as a co-substrate at a concentration of  $1000 \pm 1.1$  mg/L and organic load rate of  $4.48 \pm 0.11$  kgCOD/m<sup>3</sup>·d, to achieve acidogenesis conditions in the first reactor from the hydrolysis and fermentation of lactose into simple organic compounds; as short-chain volatile fatty acids (VFA) and intermediate compounds, which are converted into acetate, H<sub>2</sub> and CO<sub>2</sub> by the acetogenic bacteria, acetic acid predominating in relation to the other acids produced (propionic acid and butyric acid), acetate being the main substrate of methanogenic bacteria and at the same time, contribute to the biodegradation of the surfactant in subsequent stages of the experiment.

The literature mentions the use of co-substrates, enriched carbon sources, sodium acetate, yeast extract, the addition of MgSO<sub>4</sub>, CaCl<sub>2</sub>, sources of phosphorus, and/or the addition of micronutrients among others, to acclimatize the biomass of anaerobic reactors in the biodegradation of recalcitrant compounds present in Industrial wastewater (Wosman *et al.*, 2016). From day 64 to day 174 (experiments II-III), the LAS surfactant is again included at an average concentration of  $200 \pm 0.69$  mg/L which represented an average value of the chemical oxygen demand (COD), biological oxygen demand (BOD<sub>5</sub>) and organic load of  $1489 \pm 19.65$  mg/L,  $872.4 \pm 10.25$  mg/L and  $5.92 \pm 0.7$  kgDQO/m<sup>3</sup>·d respectively.

Observing a gradual loss in COD removal efficiency going from 51.51% (experiment II) to 30% during the next 45 days after experiment III began until it became null on day 174 of operation, in addition, a decrease in the biodegradation efficiency of the surfactant of an 80.6% (experiment II) in a 56% (experiment III).

Based on this behavior and to avoid the inhibition of the biomass of the reactor, from day 174 until day 244 (experiment IV) it was decided to suspend the LAS from the feeding medium, which allowed it to improve its performance, achieving COD removal efficiency in a 34.19% and of a 60% of biodegradation of the surfactant present in the biomass of the reactor, appreciating an average concentration of  $12.48 \pm 0.9$  mg/L in the reactor effluent.

This led to the assumption that the surfactant was probably absorbed into the biomass of the reactor. In the literature, it is mentioned that given its chemical nature it can be highly absorbed by the granules and sometimes can represent up to 60% of the total amount (González-Mazo *et al.*, 1998). On the other hand, the primary biodegradation of the different LAS homologs (normally between C<sub>10</sub> and C<sub>13</sub>, but even between C<sub>6</sub> a C<sub>16</sub>), increases as the length of the alkyl chain increases (Swisher, 1981).

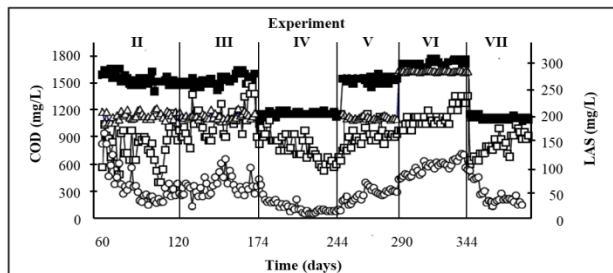
From day 244 to day 290 of operation (experiment V), Again, the surfactant is included in the feed medium at the same surfactant concentration, COD, BOD<sub>5</sub>, and organic loading rate similar to the experiments II-III, and a slight improvement is observed in the degradation of COD in a 42.24% and an 86.65% of LAS respectively. This suggested that, once the biomass was acclimated to the presence of LAS in the presence of lactose as a co-substrate, the performance of the reactor improved. Based on these results, it is decided to increase at will, the concentration of LAS to a concentration of  $300 \pm 0.05$  mg/L from day 291 to day 343 of operation (experiment VI), which represents a concentration of  $1709 \pm 24.5$  mg/L of COD,  $1001 \pm 14.35$  mg/L of BOD<sub>5</sub> and organic load rate of  $6.8 \pm 0.11$  kgCOD/m<sup>3</sup>·d.

With the change in feeding conditions, a slight reduction in the COD removal efficiency and LAS biodegradation was observed in a 36.6% and of a 55% respectively, as well as a concentration of LAS in the effluent of the reactor of  $67.42 \pm 0.9$  mg/L. In the literature, it is mentioned that by increasing the concentration of surfactant, a significant increase in the adaptation period and a decrease in the degradation rate is observed. This fact can be attributed to a bacteria-surfactant interaction effect or a decrease in the solubility of dissolved oxygen in the medium.

There are many examples in the literature in which various compounds are shown in initial concentrations between 20-100 mg/L present inhibitory effects on their degradation process, degrading at lower concentrations (Swisher, 1986).

Therefore, it was decided to definitively suspend the LAS of the reactor feeding medium from day 344 of operation (experiment VII), and as time passed, the presence of LAS was observed in an average of  $28.05 \pm 1.1$  mg/L in the reactor effluent, with a COD removal in a 33.2%, which confirmed, the accumulation of LAS in the biomass of the reactor.

### Box 5



**Figure 2**

Efficiency of COD removal and surfactant biodegradation in the acidogenic reactor (■ COD and △ LAS in influent, □ COD and ○ LAS in effluent)

### 3.5 COD degradation and LAS removal efficiency in the methanogenic reactor

Figure 3 shows the behavior of the methanogenic reactor on the efficiency of COD removal and LAS biodegradation once it is coupled to the effluent of the acidogenic reactor. Taking as a basis the basic principle of anaerobic degradation, which consists of the biochemical transformation of organic matter through different groups of microorganisms, whose efficiency depends directly on the substrate and the possible contaminating agents present in wastewater (Lorenzo Acosta, 2005), as well as the interactions between bacterial consortia and consequently, that the activity of methanogenic microorganisms can be affected by the products generated during fermentation, and even the hydrolysis of organic matter, when using lactose as a co-substrate in the acidogenic reactor. Therefore, the fermentation products are converted into acetate, hydrogen and carbon dioxide (Costello 1991) by obligate hydrogen-producing acetogenic bacteria (OHPA for its acronym in English) (Thiele *et al.*, 1988); the acetate produced contributes significantly to the development of the methanogenic conditions of the second reactor to keep the bacterial consortium active during the biodegradation of the surfactant remaining in the effluent of the acidogenic reactor for its mineralization into methane (McCarty, 1964).

Under this context, due to the acidification of the first reactor (experiment I) due to lactose being an easily hydrolyzable substrate, the effluent of the acidogenic reactor, which in turn was the feeding medium of the methanogenic reactor, had a pH of the order of  $4.4 \pm 0.11$  and due to the overload of volatile fatty acids produced, due to the high concentration of VFA and bicarbonate in the medium, the development of the methanogenic pathway could have been affected, which is why it was decided to separate the methanogenic reactor from the acidogenic reactor from the day 63 and until the day 120 of operation (experiment II), feeding it only with acetate as the only carbon source at a concentration of  $1000 \pm 0.12$  mg/L.

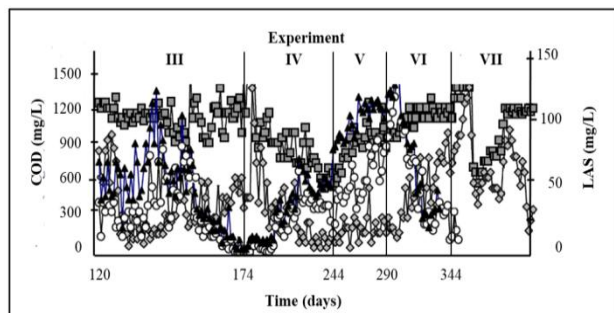
Upon observing an improvement in COD removal by 83.95% during this experimental period, it was decided to couple the methanogenic reactor to the effluent from the acidogenic reactor collected in a container where the pH was adjusted to  $7.01 \pm 0.09$  with  $\text{NaHCO}_3$  to avoid any disturbance in the performance of the methanogenic reactor during experimental development, in the removal of COD and biodegradation of the surfactant due to the acidic pH of the effluent of the acidogenic reactor.

Under this context, from day 121 to day 174 of operation (experiment III), the feeding medium presented a COD concentration of  $1035 \pm 14.2$  mg/L,  $\text{BOD}_5$  of  $606.6 \pm 7.3$  mg/L and organic load of  $4.36 \pm 0.12$  KgCOD/m<sup>3</sup>.d, that due to the presence of the remaining LAS in the effluent of the acidogenic reactor on average  $88 \pm 0.8$  mg/L, the COD removal efficiency decreased significantly in a 64.05% and of a 32.95% of LAS biodegradation.

However, by suspending the LAS from the acidogenic reactor feed medium from day 175 to day 244 of operation (experiment IV), given the average concentration of surfactant present in the effluent of the first reactor  $12.48 \pm 0.9$  mg/L, an improvement is seen in the efficiency of COD removal and LAS biodegradation in an 82.4% and of a 67.14% respectively. By including the LAS again at a concentration of  $200 \pm 0.09$  mg/L and  $1000 \pm 0.6$  mg/L of lactose in the feeding medium of the acidogenic reactor, from day 245 to day 290 of operation (experiment V); The feeding medium of the methanogenic reactor has the following characteristics:

COD of  $868 \pm 11.3$  mg/L and  $BOD_5$  on the order of  $508.7 \pm 6.4$  mg/L, a LAS concentration of the order of  $26.7 \pm 1.1$  mg/L and organic load rate of  $3.56 \pm 0.14$  kgCOD/m<sup>3</sup>·d. During this experimental period, a COD removal efficiency similar to the previous stage was observed, and 13.85% LAS biodegradation. This behavior suggested that the biomass of the methanogenic reactor was more sensitive to LAS than the biomass of the acidogenic reactor, which caused it to not biodegrade and, consequently, to bioaccumulate. Once the concentration of LAS in the feed of the acidogenic reactor was increased to 300 mg/L from day 291 to 343 of operation (experiment VI), The COD present in the effluent of the methanogenic reactor, presented a COD value of  $1083 \pm 14.9$  mg/L,  $BOD_5$  of  $634.7 \pm 7.9$  mg/L, an average LAS concentration of  $135 \pm 0.9$  mg/L and organic load rate of  $4.48 \pm 0.11$  kgCOD/m<sup>3</sup>·d. Appreciating a gradual decrease in both the COD removal efficiency of a 45.52% to 0%, as in the biodegradation of LAS from a 14.81% to 0%.

## Box 6



**Figure 3**

Efficiency of COD removal and surfactant biodegradation in the methanogenic reactor (■ COD and ▲ LAS in influent, ◆ COD and ○ LAS in effluent)

### 3.6 Bioaccumulation of the surfactant "LAS" in the biomass of the acidogenic reactor and the methanogenic reactor

Figures 4 and 5 show the dynamics of the accumulation of the surfactant "LAS" in the biomass of both reactors. In the literature, it is reported that LAS persist in anaerobic environments such as aquatic sediments and digester sludge, in addition, they do not degrade under anaerobic conditions (Federle & Schwab, 1992). Under anaerobic conditions (Maurer, 1965) showed that there is no degradation of LAS, but there is an inhibition of methanogenesis from a concentration of 214 mg/L.

In another study (Federle & Schwab (1992) confirmed these results by comparing the mineralization of radiolabeled LAS in anaerobic sediments from a lagoon receiving wastewater from laundries and sediments from uncontaminated lagoons as a control. LAS did not mineralize in the anaerobic sediments even though the microorganisms had been exposed to the surfactant for more than 25 years.

Based on the results obtained in this study and the studies cited above, it is evident that the system studied under the operating conditions tested in each of the reactors operated in series, confirms the accumulation of the surfactant in the biomass (Wolf & Feijtel, 1998).

These results confirm two relevant things; 1) the sensitivity of methanogenic bacteria to this type of compound and 2) due to the absorption of LAS in both the biomass of the acidogenic and methanogenic reactors, a bioaccumulation of LAS occurred in both bacterial consortia, as reported by the literature, which can be caused by both biotic and abiotic factors.

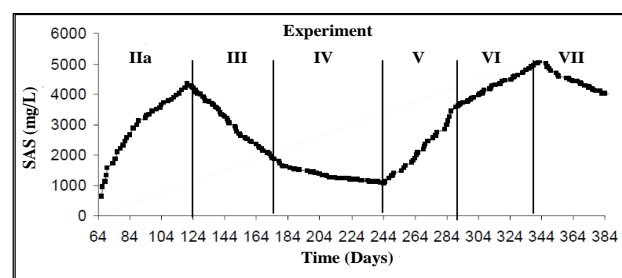
Under this context, it is suggested to acclimatize the biomass of the anaerobic reactors to the presence of this type of compound, at low concentrations of surfactant and gradually increase it until the anaerobic biomass of the biological reactors is acclimated or, failing that, to use hydraulic retention times (HRT) higher than that tested in this study. And it can be seen that the LAS accumulated in the biomass of the acidogenic reactor is desorbed and biodegraded at the same time with an average rate of 39.38 mg/day.

In the methanogenic reactor, LAS tends to bioaccumulate in its biomass at an average rate of 30.18 mg/day (experiment III). While, when it was suspended during experiment IV, both reactors presented a similar desorption-biodegradation behavior in the order of 14.28 mg/d. A greater accumulation trend is observed in the acidogenic reactor at 55.97 mg/d (experiment V), compared to the methanogenic reactor, which was 3.44 mg/d, which is probably explained by the fact that although the biomass of the methanogenic reactor is more sensitive to the presence of LAS, the acetate produced during acetogenesis contributed to the methanogenic reactor in the biodegradation of LAS.

On the other hand, by increasing the concentration of LAS to  $300 \pm 0.05$  mg/L during experiment VI, a similar tendency of LAS accumulation is seen in the biomass of both reactors, this being 29.41 mg/d in the acidogenic reactor and 21.07 mg/d in the biomass of the methanogenic reactor. When the LAS was definitively suspended from the acidogenic reactor feed, a significant difference was observed in the desorption-biodegradation rates of the LAS in both reactors, being of the order of 28.05 mg/d in the acidogenic reactor and 44.35 mg/d in the methanogenic reactor.

This suggested that once the biomass of the methanogenic reactor was acclimated to the presence of the surfactant, it was able to biodegrade the accumulated LAS faster than the biomass of the acidogenic reactor. The acclimatization strategy used is a key factor in achieving the biodegradation of recalcitrant and difficult-to-biodegrade compounds.

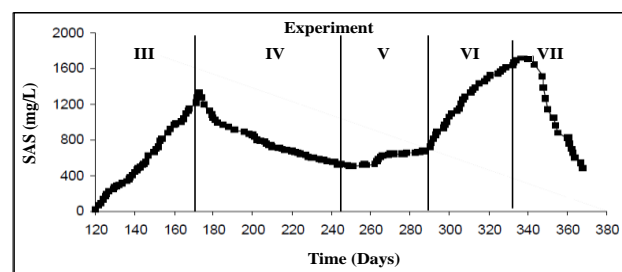
### Box 7



**Figure 4**

Trend in the accumulation of the anionic surfactant sodium alkylbenzene sulfonate (LAS) in acidogenic reactor sludge

### Box 8



**Figure 5**

Trend in the accumulation of the anionic surfactant sodium alkylbenzene sulfonate (LAS) in methanogenic reactor sludge

The bioavailability of LAS, and that of other non-ionic surfactants, has been reported to be low as a result of adsorption to solids and dissolved organic matter (Buhl & Hamilton, 2000). LAS persists in anaerobic environments such as aquatic sediments and digester sludge, and is not degraded under anaerobic conditions (Federle & Schwab, 1992). Under anaerobic conditions (Maurer, 1965), it was shown that there is no LAS degradation, but an inhibition of methanogenesis does occur at a concentration of 214 mg/L.

The LAS was not degraded by this route, leading to its accumulation, which probably decreased biomass production and consequently caused a loss of COD removal efficiency. A greater accumulation of LAS was observed in the acidogenic reactor in relation to the methanogenic reactor, despite this, the acidogenic bacteria did not decrease their metabolic activity as much as the methanogenic bacteria.

It is worth mentioning that during the stages in which the LAS was suspended from the acidogenic reactor feed, a remnant was observed in the effluent of both the acidogenic reactor and the methanogenic reactor, taking an average of 16 days for the activity of the acidogenic bacteria to recover and 36 days for the methanogenic bacteria.

Tables 7 and 8 show the LAS balance in the acidogenic and methanogenic reactors, respectively.

### Box 9

**Table 4**

Balance del surfactante en el reactor acidogénico

| mg/L                        | I       | IIa     | IIb     | III     | IV      | V       | VI      | VII |
|-----------------------------|---------|---------|---------|---------|---------|---------|---------|-----|
| LAS <sub>INFLUENTE</sub>    | 11400   | 10000   |         | 9000    | 15600   |         |         |     |
| LAS <sub>EFLUENTE</sub>     | 2208.25 | 2567.05 | *861.31 | 1201.10 | 3506.31 | *841.45 | *284.05 |     |
| LAS <sub>ACUMULADO</sub>    | 4355.38 | 1912.4  | 1051    | 3569.57 | 5099.01 | 4257.56 | 3973.51 |     |
| LAS <sub>BIODEGRADADO</sub> | 4836.37 | 6120.55 |         | 4229.33 | 6994.68 |         |         |     |

\*Se contempló dentro del LAS acumulado para hacer el balance.

☒ No hubo presencia de LAS

**Box 10****Table 5**

Balance del surfactante en el reactor metanogénico

| mg/L                        | I | IIa | IIb     | III     | IV      | V       | VI      | VII |
|-----------------------------|---|-----|---------|---------|---------|---------|---------|-----|
| LAS <sub>INFLUENTE</sub>    |   |     | 5796.31 | 2266.80 | 2936.95 | 7813.43 | 2065.60 |     |
| LAS <sub>EFLUENTE</sub>     |   |     | 1574.48 | 560.08  | 857.49  | 2238.86 | 712.41  |     |
| LAS <sub>ACUMULADO</sub>    |   |     | 1341.72 | 524.71  | 679.84  | 1808.65 | 478.14  |     |
| LAS <sub>BIODEGRADADO</sub> |   |     | 2880.11 | 1182.01 | 1399.62 | 3765.92 | 875.05  |     |

No hubo presencia de LAS

Suspende el LAS del influente del primer reactor del sistema estudiado

Therefore, it is necessary to consider sustainable and affordable sanitation designs such as the proposal for communities in the San Cristóbal parish, Paute Canton, Azuay Province (Sananay *et al.*, 2025).

**Conclusions**

The implications are equally relevant at the ecological and agricultural levels. The persistence of detergents in arable soils reduces fertility, alters the soil microbiota, and increases salinity, which can decrease agricultural productivity. In natural ecosystems, the phytotoxicity of detergents causes changes in plant composition, affecting sensitive species and disrupting food chains. There is also a risk of biomagnification, as contaminants accumulated in plant tissues can be transferred to herbivores and, ultimately, to humans if the crops are edible. Furthermore, there is the risk of bioaccumulation in the biomass of UASB-type biological reactors during the treatment of wastewater with high concentrations of surfactants.

**Declaration of competing interest**

The authors declare that they have no financial conflicts of interest, nor conflicts in personal relationships that could influence the publication of the work presented in this article.

**Authors' contributions**

All the authors contributed with their experience in the review, analysis and discussion of the results obtained in the study to prepared the manuscript.

**Availability of data and materials**

I put at your disposal the data obtained in this investigation.

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## Differences

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