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**REDOX ENVIRONMENT MODULATION VIA MICROAERATION FOR
INTENSIFIED LAS REMOVAL IN UASB REACTORS: FROM CO-
METABOLISM TO ALTERNATIVE ELECTRON ACCEPTORS**

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Thesis presented to the Graduate Program
in Civil Engineering at the Federal
University of Ceará, as a partial
requirement for obtaining the Doctor
degree in Civil Engineering. Area of
concentration: Environmental Sanitation.

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To my wife, my parents, my brother and
my friends, who gave me all the support
and made me believe I could do it. This
work is ours.

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“Ecology without class struggle is
gardening.”

Chico Mendes

RESUMO

A biodegradação do sulfonato de alquilbenzeno linear (LAS) em sistemas de tratamento de efluentes representa um desafio técnico significativo, especialmente em ambientes anaeróbios, onde a complexidade estrutural do anel benzênico e a toxicidade do surfactante limitam a eficiência biológica. Embora os processos aeróbios sejam consolidados pela alta eficiência na remoção de surfactantes, a rota anaeróbia apresenta maior viabilidade econômica devido ao baixo consumo energético, menor produção de lodo e potencial de recuperação energética através do metano. No entanto, o caráter persistente do LAS em sistemas estritamente anaeróbios, limita sua aplicação prática. Nesse cenário, a microaeração controlada e o uso de mediadores redox emergem como estratégias de intensificação metabólica que ativam rotas oxidativas de quebra do anel aromático e aceleram a transferência de elétrons interespecies, superando as barreiras termodinâmicas da degradação sem comprometer a economia de energia e a robustez do sistema anaeróbio. Esta pesquisa investigou, de forma integrada, o efeito da microaeração associada à seleção de co-substratos (Etapa I), à suplementação do mediador redox antraquinona-2-sulfonato (AQS) (Etapa II) e à otimização do tempo de detenção hidráulica (Etapa III) na remoção de LAS, no desempenho de reatores anaeróbios de manta de lodo (UASB), seleção de consórcios microbianos especializados na desestabilização do anel aromático. Na primeira etapa, os resultados indicaram que a combinação de microaeração com sacarose foi determinante para a otimização do processo, elevando a eficiência de remoção de LAS em 34,3% em comparação aos sistemas estritamente anaeróbios. A análise microbiológica revelou que essa configuração selecionou um consórcio especializado, destacando-se os gêneros *Pleomorphomonas* e *Desulfovibrio*, que atuaram sinergicamente na oxidação parcial do anel benzênico e na dessulfonação do surfactante, sem comprometer a produção de biogás. A produção de biogás aumentou com a microaeração sob alimentação com etanol (2,7 vs. 1,8 L·dia⁻¹), enquanto ambos os reatores apresentaram desempenho semelhante sob alimentação com sacarose (~2,7 L·dia⁻¹). Na segunda etapa, a adição de 50 µM de AQS isoladamente foi capaz de estabilizar a remoção de LAS em 65%, enquanto o controle anaeróbio apresentou performance oscilante em torno de 32%. A sinergia máxima foi alcançada com a coexistência de AQS e microaeração, elevando a remoção para 77% e promovendo o enriquecimento de arqueias metanogênicas hidrogenotróficas (*Methanobacterium*), o que sugere que o AQS atua como uma ponte de elétrons que facilita a transferência

interespecies, mitigando os efeitos inibitórios do LAS. A terceira etapa demonstrou que o aumento do TDH para 16h sob microaeração criou uma "janela cinética" essencial para a atividade de enzimas oxigenases, permitindo que processos de ω -oxidação ocorressem de forma mais completa. Esta configuração não apenas maximizou a biotransformação do LAS (64%), mas também dobrou a eficiência de recuperação energética, atingindo um rendimento específico de metano de 0,55 L_{CH₄}.g_{DQO_{apl}}⁻¹, com a coexistência estável de bactérias oxidativas (*Smithella*, *Thauera*) e metanógenos acetoclásticos. De maneira integrada, os resultados demonstram que o gerenciamento do ambiente redox, através da combinação estratégica de microaeração e mediadores (AQS) constitui uma ferramenta robusta para transformar reatores UASB em sistemas de alta performance para o tratamento de efluentes contendo surfactantes, garantindo estabilidade funcional e sustentabilidade energética.

Palavras-chave: Surfactantes Aniônicos, Transferência Extracelular de Elétrons, Valorização do Biogás, Ecologia Microbiana, Intensificação de Processos, Rotas de Biotransformação

.

ABSTRACT

The biodegradation of linear alkylbenzene sulfonate (LAS) in wastewater treatment systems poses a significant technical challenge, especially in anaerobic environments, where the structural complexity of the benzene ring and the surfactant's toxicity limit biological efficiency. Although aerobic processes are well-established for their high efficiency in surfactant removal, the anaerobic route offers greater economic viability through lower energy consumption, reduced sludge production, and potential for energy recovery through methane. However, the persistent nature of LAS in strictly anaerobic systems limits its practical application. In this scenario, controlled microaeration and the use of redox mediators emerge as metabolic intensification strategies that activate oxidative pathways for aromatic ring cleavage and accelerate interspecies electron transfer, overcoming the thermodynamic barriers of degradation without compromising energy efficiency and the robustness of the anaerobic system. This research investigated, in an integrated manner, the effect of microaeration associated with the selection of co-substrates (Step I), the supplementation of the redox mediator anthraquinone-2-sulfonate (AQS) (Step II), and the optimization of hydraulic retention time (Step III) on LAS removal, in the performance of upflow anaerobic sludge blanket (UASB) reactors, and the selection of microbial consortia specialized in the destabilization of the aromatic ring. In the first step, the results indicated that the combination of microaeration with sucrose was crucial for process optimization, increasing LAS removal efficiency by 34.3% compared to strictly anaerobic systems. Microbiological analysis revealed that this configuration selected a specialized consortium, highlighting the genera *Pleomorphomonas* and *Desulfovibrio*, which acted synergistically in the partial oxidation of the benzene ring and in the desulfonation of the surfactant, without compromising biogas production. Biogas production increased with microaeration under ethanol feeding (2.7 vs. 1.8 L·day⁻¹), while both reactors performed similarly under sucrose feeding (~2.7 L·day⁻¹). In the second stage, the addition of 50 µM AQS alone stabilized LAS removal at 65%, whereas the anaerobic control showed fluctuating performance around 32%. Maximum synergy was achieved with the coexistence of AQS and microaeration, increasing removal to 77% and promoting the enrichment of hydrogenotrophic methanogenic archaea (*Methanobacterium*), suggesting that AQS acts as an electron bridge to facilitate interspecies transfer and mitigate the inhibitory effects of LAS. The third stage demonstrated that increasing the HRT to 16h under microaeration created an

essential "kinetic window" for the activity of oxygenase enzymes, enabling ω -oxidation to proceed more completely. This configuration not only maximized LAS biotransformation (64%) but also doubled energy recovery efficiency, achieving a specific methane yield of 0.55 LCH₄.gCOD_{apl}, with the stable coexistence of oxidative bacteria (*Smithella*, *Thauera*) and acetoclastic methanogens. In an integrated manner, the results demonstrate that managing the redox environment through the strategic combination of microaeration and mediators (AQS) constitutes a robust tool for transforming UASB reactors into high-performance systems for treating effluents containing surfactants, ensuring functional stability and energy sustainability.

Keywords: Anionic Surfactants, Extracellular Electron Transfer, Biogas Valorization, Microbial Ecology, Process Intensification, Biotransformation Pathways.

LIST OF ABBREVIATIONS AND ACRONYMS

AFBR	Anaerobic Fluidized Bed Reactor
AQDS	Anthraquinone-2-Disulfonate
AQS	Anthraquinone-2-Sulfonate
COD	Chemical Oxygen Demand
DEHA	Department of Hydraulic and Environmental Engineering
DO	Dissolved Oxygen
EC	Emerging Contaminants
EGSB	Expanded Granular Sludge Bed
EPS	Extracellular Polymeric Substances
HRT	Hydraulic Retention Time
KO	KEGG Orthology
LABOSAN	Environmental Sanitation Laboratory
LAS	Linear Alkylbenzene Sulfonate
MCr	M reductase
OLR	Organic Loading Rate
OS	Operating System
TCA	Tricarboxylic acid
TFS	Total Fixed Solids
TS	Total Solids
TVS	Total Volatile Solids
UASB	Upflow Anaerobic Sludge Blanket
UMSB	Upflow Microaerobic Sludge Blanket
UFC	Federal University of Ceará
VSS	Volatile Suspended Solids
VFA	Volatile Fatty Acid
WWTPs	Wastewater Treatment Plants

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1. INTRODUCTION

The detection of emerging contaminants (ECs) in wastewater treatment plants (WWTPs) has become a growing global concern. Even when present at residual concentrations, ECs exhibit high persistence and significant ecotoxicological potential (Motteran, Varesche, Lara-Martin, 2022; Terreros-Mecalco et al., 2024a). Among these substances, surfactants stand out for their widespread use in domestic and industrial sectors, with Linear Alkylbenzene Sulfonate (LAS) being the predominant anionic surfactant worldwide (Mecalco; Hilario; Faviel, 2024b; Villabona-Ortíz; Tejada-Tovar; De-La-Rosa-Jiménez, 2020; Wang et al., 2024a). As a primary ingredient in laundry detergents, cleaning agents, and personal care products, LAS accounts for nearly 80% of global detergent consumption (Palmer; Hatley, 2018; Ran et al., 2024; Zhu et al., 2020a).

Consequently, LAS is frequently detected in domestic wastewater, with concentrations in WWTP influents typically ranging from 0.8 to 21 mg·L⁻¹ (Costa et al., 2024; Motteran et al., 2018a). Due to its widespread use and incomplete removal by conventional treatment processes, the compound is often found in final effluents and nearby water bodies, with levels in marine sediments reported to reach up to 500 mg·kg⁻¹ (Corada-Fernández et al., 2013; Luo et al., 2023). Its presence in aquatic environments triggers several physical-chemical disruptions, including increased surface tension at the air–water interface, reduced dissolved oxygen, and diminished light penetration due to foam formation (Teran et al., 2022; Villabona-Ortíz; Tejada-Tovar; De-La-Rosa-Jiménez, 2020).

The available metabolic pathways fundamentally dictate the biodegradation efficiency of LAS. In aerobic systems, degradation is rapid and efficient, initiated by oxygenase enzymes that use molecular oxygen to destabilize the alkyl chain and the benzene ring (Lara-Martín et al., 2010a). Conversely, under strictly anaerobic conditions, the consortium must rely on reductive pathways, which are thermodynamically less favorable and significantly slower (Motteran et al., 2018a). This kinetic bottleneck often leads to the accumulation of intermediates and inhibition of methanogenesis, representing the primary challenge for LAS mineralization in conventional UASB reactors (Terreros-Mecalco et al., 2024a).

Furthermore, LAS exhibits environmental persistence and toxicity even at low concentrations, raising concerns about its bioaccumulation potential (Luo et al., 2023). Although biodegradable under specific conditions, its degradation in WWTPs is often incomplete, particularly under anaerobic conditions (Ogawa; Kawase, 2021).

Commercial LAS mixtures are inherently complex, consisting of various homologues with different alkyl chain lengths and positional isomers. This structural complexity contributes to its resistance to biological treatment in the absence of Oxygen (Terreros-Mecalco et al., 2024a). Moreover, at moderate to high concentrations ($2 - 15 \text{ mg}\cdot\text{L}^{-1}$), LAS can act as a potent inhibitor of microbial activity, negatively affecting the overall performance of treatment systems (Wu et al., 2020).

1.1. A Biochemical Pathways and Mechanisms of LAS Degradation

Upflow Anaerobic Sludge Blanket (UASB) reactors are widely established for the treatment of domestic and industrial effluents due to their upward-flow configuration, which promotes the development of a dense, metabolically active, and efficient sludge bed (Collivignarelli et al., 2021; Mariraj Mohan; Swathi, 2022). Beyond conventional applications, these anaerobic systems have demonstrated the capacity to remove recalcitrant xenobiotics, although their performance is typically lower and more variable than that of aerobic processes (Cui et al., 2022; Teran et al., 2022).

In biological treatment, LAS removal can occur through precipitation, adsorption, or biodegradation, with microbial transformation generally being the most significant route. While aerobic degradation is the most efficient and well-documented pathway (Costa et al., 2024), anaerobic environments can sustain LAS biotransformation through direct utilization as a carbon source or via co-metabolic processes involving complex biochemical reactions (Motteran et al., 2018a). The proposed primary anaerobic degradation pathway involves a putative initial addition of fumarate to the alkyl chain (fumarate addition pathway), followed by sequential β -oxidation reactions, aromatic ring cleavage, and eventual desulfonation (Lara-Martín et al., 2010a).

A metabolically complementary consortium sustains these intricate biochemical steps. Members of the *Synergistota* phylum are frequently implicated in the degradation of toxic, structurally complex compounds (Motteran et al., 2018a). Furthermore, *Pseudomonas* species can degrade aromatic intermediates while interacting syntrophically with strictly anaerobic partners to facilitate downstream transformations (Unuofin, Okoh, Nwodo, 2019a). The genus *Desulfovibrio* is also consistently identified as a dominant taxon, playing a crucial role in the transformation of sulfonated intermediates (Braga et al., 2015; Delforno et al., 2019a; Wei et al., 2024a).

However, despite this microbial potential, strictly anaerobic biodegradation often remains below 20%, largely due to limited bioavailability resulting from the formation of

insoluble chemical species and the biochemical complexity of degradation intermediates (Cui et al., 2022; Terreros-Mecalco et al., 2024a). Consequently, LAS removal efficiencies in UASB reactors vary widely, from 30% to 91%, depending, besides its own concentration, on the hydraulic retention time (HRT), electron acceptor availability, and the presence of co-substrates (Costa et al., 2024; Mecalco; Hilario; Faviel, 2024b; Okada et al., 2013b).

Operational studies illustrate this variability: Granatto et al. (2019) achieved 60 ± 30 % removal in an EGSB reactor with a 36-h HRT and a LAS concentration of 6.2 ± 3.2 mg.L⁻¹, while Carosia et al. (2014a) reported 48 ± 10 % removal in a fluidized bed reactor with a 15-h HRT and 14 ± 3 mgLAS.L⁻¹. A critical factor in enhancing these rates is the presence of readily fermentable carbon sources, such as sucrose or ethanol. These co-substrates provide the necessary electrons and metabolic intermediates that sustain syntrophic partnerships, accelerate β -oxidation, and mitigate surfactant-induced metabolic stress (Motteran et al., 2018a; Terreros-Mecalco et al., 2024a). Without such intensification strategies, the recalcitrance of various LAS homologues and microbial inhibition can compromise effluent quality and system stability (Teran et al., 2022; Wu et al., 2020).

1.2. Microaeration as a Catalyst for Enhanced Biodegradation

Traditional aerobic treatment of LAS is often limited by high operational energy costs due to elevated oxygen (O₂) requirements and significant foaming issues caused by direct aeration (Liu et al., 2018; Zhang et al., 2023). In this context, microaeration—the strategic introduction of low, controlled oxygen doses into anaerobic systems—emerges as a superior alternative. By supplying O₂ levels ranging from 0.005 to 5 L_{O₂}.L_{feed}⁻¹.d⁻¹, depending on substrate composition and treatment goals, this strategy creates a metabolic bridge that stimulates facultative and microaerotolerant taxa (Guimarães de Oliveira et al., 2021a; Nguyen; Khanal, 2018). This "oxidative boost" enables partial oxidation of xenobiotics without disrupting the low-redox conditions required for stable methanogenesis and biogas production (Li et al., 2024).

The versatility of microaeration as a process intensifier is evidenced by its success in enhancing the removal of diverse recalcitrant pollutants. Previous studies have demonstrated that airflow rates between 0.5 and 4.0 mL·min⁻¹ can increase the removal of BTEX compounds to above 83% and hormones or pharmaceuticals to 90–94%, significantly outperforming strictly anaerobic reactors (Nascimento et al., 2021; Siqueira

et al., 2018). Furthermore, microaeration has proven effective for antibiotic degradation, achieving up to 91% efficiency for sulfamethoxazole (Buakaew; Ratanatamskul, 2024), and for the complete removal of aromatic amines from textile dyes, resulting in effluents 16 times less toxic than those from anaerobic controls (Carvalho et al., 2020). Beyond xenobiotic removal, microaeration is also widely applied for hydrogen sulfide oxidation in biogas treatment (Khadir et al., 2025).

The fundamental mechanism by which microaeration intensifies LAS removal is through the activation of oxygenase enzymes (mono and dioxygenases). These enzymes are kinetically more efficient than the anaerobic fumarate addition pathway, catalyzing the terminal hydroxylation of the alkyl chain (ω -oxidation) and the subsequent cleavage of the benzene ring (Cui et al., 2022; Liu et al., 2024a). This oxidative priming converts the hydrophobic surfactant into more hydrophilic carboxylated intermediates, effectively reducing the thermodynamic barrier to aromatic degradation and allowing the anaerobic consortium to mineralize via the TCA cycle (tricarboxylic acid cycle) (Lara-Martín et al., 2010a; Xu et al., 2023).

Beyond enzymatic activation, the combination of microaeration with fermentable co-substrates, such as sucrose, promotes a strategic functional selection within the microbial community. This process favors the enrichment of specialized microaerophilic-syntrophic consortia, notably dominated by the phylum *Pseudomonadota* and genera such as *Pleomorphomonas* and *Desulfovibrio* (Braga et al., 2015; Delforno et al., 2019a). This specialized structure facilitates a coupled metabolic sequence: an initial oxidative attack on the LAS molecule, followed by anaerobic desulfonation and methanogenesis, supported by oxygen-tolerant archaea such as *Methanobacterium*. Consequently, microaeration bridges the efficiency of aerobic pathways with the energy recovery potential of anaerobic digestion without compromising overall process stability (Costa et al., 2024).

1.3. Metabolic and Operational Strategies to Enhance LAS Removal in Anaerobic Digestion

The success of LAS removal in anaerobic systems relies on overcoming kinetic barriers that conventional metabolic pathways cannot bypass in isolation. In this context, integrating nutritional, chemical, and operational factors creates a favorable environment for surfactant mineralization without compromising microbial consortium stability or biogas production (Costa et al., 2024; Zhang et al., 2020).

1.3.1. Nutritional and Chemical Intensification: The role of Co-substrates

The availability of readily fermentable carbon sources, such as ethanol and sucrose, is a primary determinant of efficiency in UASB reactors, raising removal rates to 30%-91% (Costa et al., 2024; Mecalco; Hilario; Faviel, 2024b; Okada et al., 2013b). These carbon sources act as essential electron donors that sustain the syntrophic partnerships required for LAS biodegradation. By providing a stable energy flux, co-substrates help mitigate the metabolic stress induced by surfactant exposure on the microbial matrix, ensuring the maintenance of methanogenesis even under inhibitory conditions (Motteran et al., 2018a; Terreros-Mecalco et al., 2024a).

From a mechanistic perspective, the role of co-substrates extends beyond mere energy supply; they influence the thermodynamic feasibility of the initial LAS attack (Motteran et al., 2018a; Okada et al., 2013b). The fermentation of sucrose or ethanol generates a high flux of reducing equivalents, which are essential for the reductive pathways of the benzene ring. Furthermore, these co-substrates promote the secretion of extracellular polymeric substances (EPS), which can modulate the bioavailability of the surfactant. By altering the sludge matrix's hydrophobicity, co-substrates may reduce the direct toxic exposure of methanogenic cell membranes to the LAS alkyl chain, facilitating a more resilient syntrophic degradation environment (Zhou et al., 2018b).

1.3.2. Chemical Intensification: Redox Mediators as Electron Shuttles

Complementary to nutritional support, the use of redox mediators has emerged as a promising strategy to overcome the degradation limits of structurally complex compounds in anaerobic environments (Zhang et al., 2020). Redox mediators are chemical compounds that facilitate extracellular electron transfer between microbial cells and electron acceptors or donors, thereby increasing the biodegradation rates of various pollutants (Do Nascimento et al., 2021b).

Quinone-based mediators, such as anthraquinone-2-sulfonate (AQS) and anthraquinone-2,6-disulfonate (AQDS), have attracted attention for their chemical stability, low toxicity, and reversible redox properties. By functioning as electron shuttles, these compounds accelerate reductive transformation processes for several recalcitrant classes, including dyes, chlorinated organics, and polycyclic aromatic hydrocarbons (He et al., 2017; Zhou et al., 2018a). Practical applications underscore their efficacy: the

addition of AQS has been noted to increase azo dye removal by 15% in up-flow reactors (Lu et al., 2021), while even a low dose of AQDS (10 μ M) enhanced antibiotic removal by approximately 50% in anaerobic batch tests (He et al., 2017).

The influence of redox mediators on LAS biodegradation primarily stems from their reduction of the activation energy required for extracellular electron transfer. Due to its amphiphilic nature, LAS often accumulates at the solid-liquid interface, creating a physical barrier for direct microbial contact. Mediators like AQS circumvent this limitation by acting as mobile electron carriers that can penetrate the biofilm or sludge flocs. By undergoing reversible cycles of reduction and oxidation, these quinones bypass the rate-limiting step of slow biological electron transport, accelerating the destabilization of the aromatic ring, a structure characterized by high resonance energy and significant resistance to anaerobic cleavage.

1.3.3. Operational Control: The Kinetic Window of HRT

Finally, the success of these co-metabolic and mediated processes is governed by the HRT, which defines the kinetic window available for complex biochemical reactions, such as aromatic ring destabilization, to reach completion. The wide discrepancy in removal efficiencies across different reactor configurations evidences the importance of HRT as a master operational parameter. For instance, Granatto et al. (2019) achieved 60 ± 30 % LAS removal in an EGSB reactor with an extended HRT of 36 h, whereas Carosia et al. (2014) reported only 48 ± 10 % in an AFBR at 15 h.

This discrepancy highlights a critical operational trade-off: while shorter HRTs are economically desirable, they often provide insufficient contact time for specialized consortia to stabilize the degradation of aromatic surfactants. In many developing countries across Latin America and Asia, UASB reactors are typically operated at short HRTs of 6–8 h for domestic wastewater treatment due to cost-effectiveness (Chernicharo et al., 2015b). However, the kinetic limitations imposed by these short residence times are significant; Okada et al. (2013c) reported only 18% LAS removal at a 6-h HRT. Similarly, Carneiro et al. (2024a) observed removal efficiencies below 15% for recalcitrant aromatic pharmaceuticals, such as diclofenac and naproxen, under a 6-h HRT, reinforcing the need for adequate hydraulic retention time (HRT) to facilitate ring cleavage. Consequently, optimizing the HRT is essential to enhance LAS biotransformation while maintaining stable biogas production and microbial resilience (Costa et al., 2024; Granatto et al., 2019).

The interaction between HRT and LAS removal is deeply rooted in the surfactant's physical-chemical properties, particularly its high sorption coefficient. A restricted HRT not only limits the time for enzymatic ring cleavage but also leads to rapid saturation of the sludge's adsorption sites, resulting in immediate breakthrough of LAS into the effluent. Mechanistically, an optimized HRT ensures that hydraulic shear stress does not exceed the rate of microbial aggregation, allowing the development of dense granules or flocs capable of retaining the specialized, slow-growing, oxidative and syntrophic bacteria required for complete mineralization. Thus, the HRT serves as a regulator of both the surfactant mass transfer to the biocatalyst and the metabolic synchronization of the anaerobic consortium (Chernicharo et al., 2015).

1.3.4. Towards a Holistic Redox Management: Synergistic Intensification and Research Gaps

While the individual effects of co-substrates, redox mediators, and HRT have been documented in isolated studies, their integrated influence on the anaerobic biotransformation of LAS remains unexplored. A holistic view suggests that these parameters do not act independently; rather, they form a complex biochemical-operational nexus. For instance, the metabolic advantage provided by a specific co-substrate may only be fully realized if the HRT is sufficient to prevent the washout of the specialized syntrophic groups it promotes. Similarly, the efficiency of redox mediators like AQS is intrinsically linked to the system's redox potential, which in turn can be finely tuned through controlled microaeration.

The synergy between these factors creates a "metabolic bridge" that is missing in conventional UASB reactors. Microaeration acts as a catalyst by providing the trace oxygen required for the initial oxygenase-mediated attack on the aromatic ring. In contrast, co-substrates provide the energetic flux for subsequent anaerobic steps, and redox mediators accelerate electron transfer between these stages. Despite the potential of this integrated approach, the literature currently lacks a comprehensive evaluation that combines microaeration, co-substrate selection, redox shuttling, and hydraulic optimization within a single treatment platform. This research addresses this critical gap, moving beyond isolated parameter analysis to establish a multidimensional strategy to intensify LAS degradation, thereby offering a more resilient, high-performance solution for surfactant-rich wastewater treatment.

In this context, the present thesis is structured to investigate the role of

microaeration as a selective and catalytic factor in UASB reactors, considering not only its effects on LAS biodegradation and mineralization, but also its implications for methanogenesis stability and energy recovery. By systematically evaluating different redox intensification strategies (co-substrates, redox mediators, and alternative electron acceptors) in combination with hydraulic operational conditions, this work aims to advance an integrated understanding of anaerobic consortia behavior under surfactant-induced toxicity and to support the development of optimized, resilient treatment strategies for detergent-rich wastewater.

This research is grounded in the hypothesis that the modulation of the redox environment through controlled micro-oxygenation, integrated with diverse operational strategies, acts as a biochemical driver to activate oxygenase-mediated pathways. By overcoming the kinetic bottlenecks of aromatic ring cleavage, this synergistic approach is expected to enhance LAS mineralization while maintaining or improving methane yields and the overall robustness of anaerobic systems treating surfactant-rich effluents.

Research Questions

Based on this general hypothesis, the following research questions are addressed in this study:

1. How does microaeration, applied under different co-metabolic and redox-mediated strategies, act as a selective pressure to influence LAS mineralization, organic matter removal, and the functional structure of anaerobic consortia?
2. How do sucrose and ethanol, as co-substrates of varying complexity, affect the metabolic pathways and the resilience of microaerated systems exposed to high surfactant loads?
3. How does the addition of the redox mediator AQS interact with micro-oxygenation to accelerate extracellular electron transfer and the biotransformation of the LAS molecule?
4. Can the detrimental effects of surfactant toxicity on methanogenesis be mitigated by increasing the HRT to provide a wider kinetic window for aromatic ring stabilization?
5. Does the integration of these multi-redox strategies compromise or enhance the energy balance (methane purity and yield) of UASB reactors compared to strictly anaerobic or fully aerobic systems?

6. Through which specific metabolic pathways does the integration of microaeration and redox mediators bypass the kinetic bottlenecks of LAS mineralization in anaerobic consortia?
7. To what extent does the synergistic application of micro-oxygenation and chemical additives select for specialized functional groups, and how does this ecological restructuring correlate with reactor stability?
8. Can targeted batch assays isolate the specific contributions of abiotic processes, co-substrate-driven fermentation, and redox-shuttling toward the overall biotransformation of LAS, providing a mechanistic baseline for continuous-flow observations?
9. How do the proposed intensification strategies affect the overall system performance, specifically regarding COD removal efficiency, biogas composition, and the specific methane yield, compared to conventional strictly anaerobic UASB reactors?

2. OBJECTIVES

2.1 General objective

The primary objective of this thesis is to evaluate and optimize the performance of UASB reactors for the treatment of LAS-rich wastewater by implementing process intensification strategies, including microaeration, redox mediation, and the variation of HRTs. Moreover, this work aims to establish a comprehensive understanding of how these strategies influence surfactant biodegradation pathways, microbial community structure, and overall system performance, with a specific focus on enhancing biogas quality and methane yield while maintaining anaerobic process stability.

2.2 Specific objectives

To achieve the general objective, the research was divided into the following specific goals, corresponding to the chapters of this thesis:

- To investigate the influence of co-substrate complexity (sucrose vs. ethanol) and microaeration on the stability and LAS removal efficiency in UASB reactors (Chapter 4).
- To assess the synergistic effect of combining microaeration with the redox mediator AQS on the kinetics of organic matter removal and surfactant degradation (Chapter 5).

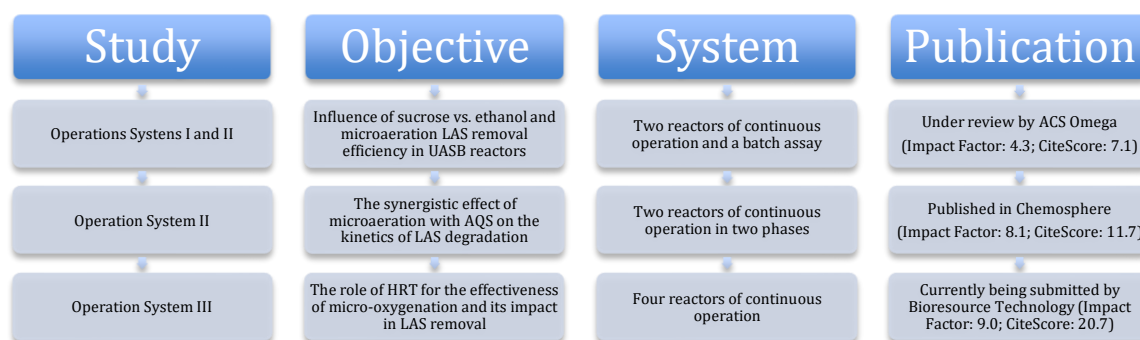
- To determine the role of HRT (8 h vs. 16 h) as a modulating factor for the effectiveness of micro-oxygenation and its impact on specific methane yields (Chapter 6)
- To characterize the shifts in microbial community structure and the selection of specialized functional groups (e.g., syntrophic and oxidative taxa) under different co-metabolic, redox-mediated, and hydraulic conditions (Chapters 4, 5, and 6).

3. MATERIAL AND METHODS

The research was developed at the Environmental Sanitation Laboratory (LABOSAN), affiliated with the Department of Hydraulic and Environmental Engineering (DEHA) at the Federal University of Ceará (UFC), and divided into three main stages, each outlining a subsequent chapter in the thesis structure.

Distinct process intensification strategies and controlled redox conditions were applied to the experimental units to evaluate their effects on anaerobic consortia adaptation, LAS biodegradation kinetics, methanogenic stability, and energy recovery (Figure 1).

Figure 1 – Schematic summary of the experiments.



Source: Prepared by the author.

3.1. Operation System I (OS I)

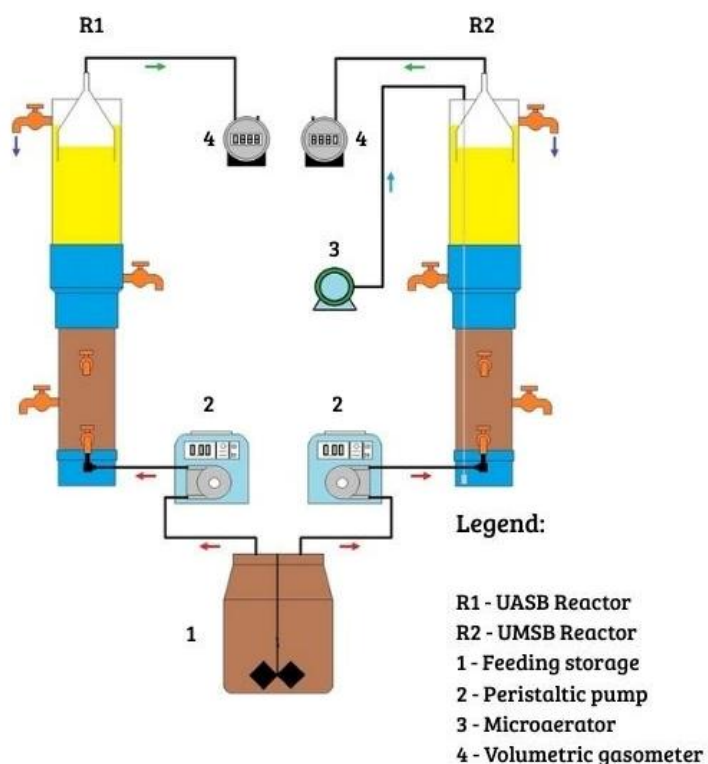
The present system involved the continuous operation of two reactors to evaluate the influence of microaeration and the application of different co-substrates (ethanol and sucrose) on LAS removal. The second experiment consisted of a batch assay designed to preliminarily assess the role of hydrolytic fermentative microorganisms (represented by sucrose), acidogenic fermentative microorganisms (represented by ethanol), and acetoclastic methanogenic archaea (represented by acetate), as well as abiotic and

endogenous processes, in relation to LAS removal efficiencies in continuous-flow experiments under the tested operational conditions.

3.1.1. Experiment set-up

The two cylindrical acrylic reactors UASB (Figure 2) were operated in parallel as a laboratory-scale system with a working volume of 3.25 L. Both reactors were inoculated with 1.6 L of sludge and operated under the same conditions, differing only in the microaeration insert: R1, traditional UASB, R2, microaerated UASB (renamed to UMSB – Upflow Microaerobic Sludge Blanket).

Figure 2 – Schematic of the experimental set-up.



Source: Prepared by the author.

The inoculum was sludge from a UASB reactor treating sewage in Caucaia, Brazil. Total solids (TS), total volatile solids (TVS), and total fixed solids (TFS) in the inoculum sludge were 48 ± 3 , 25 ± 1 , and 23 ± 1 g·L⁻¹, respectively.

Microaeration in R2 was performed with synthetic air (80% N₂:20% O₂, White Martins, Brazil) at its base using a mass flow controller (GFC17, Cole-Parmer, USA). The microaeration air dose used in R2 was approximately 0.7 L_{air}·min⁻¹, corresponding

to $0.1 \text{ L}_{\text{O}_2} \cdot \text{L}_{\text{feed}}^{-1} \cdot \text{d}^{-1}$, which was kept constant during the experimental period. The microaeration dose was chosen based on the best results found by Do Nascimento et al. (2021b), who evaluated the removal of emerging sulfur components. The reactors were fed by two peristaltic pumps (ColeParmer MasterFlex L/S 7522-30, USA) at a flow rate of $6.8 \text{ mL} \cdot \text{min}^{-1}$, resulting in an HRT of 8 hours, a value similar to that used in sewage treatment plants (Chernicharo et al., 2015).

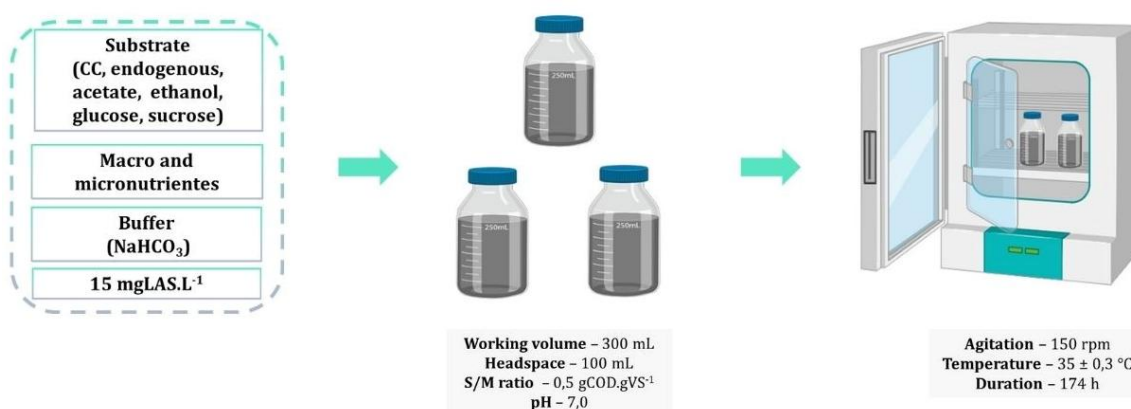
The experiment was conducted in two sequential phases, each lasting 45 days, with a 45-day stabilization interval in between. In the first phase, ethanol was used as the co-substrate, and in the second phase, sucrose was introduced to evaluate its impact on system performance. During the stabilization interval, the reactors were fed with ethanol in the absence of LAS to reset microbial activity and ensure a clear transition between phases. This structured approach allowed for a systematic comparison of the effects of different co-substrates on organic matter degradation and LAS removal under both anaerobic and microaerobic conditions.

3.2. Operation System II (OS II)

At the end of the first operational phase, sludge samples from both reactors were collected, homogenized, and used in the presence of acetate, ethanol, and sucrose to assess LAS degradation and methanogenesis. The experiment was conducted in triplicate, following the methodology described by Angelidaki et al. (2009). Anaerobic reactors consisted of 300 mL borosilicate glass flasks with a working volume of 200 mL and a 100 mL headspace, maintained under continuous agitation at 150 rpm and $35 \pm 1 \text{ }^{\circ}\text{C}$.

A substrate-to-microorganism ratio (S/M) of $0.5 \text{ gCOD} \cdot \text{gVS}^{-1}$ was adopted, based on a final concentration of $1 \text{ gCOD} \cdot \text{L}^{-1}$ for the substrate and $2.0 \text{ gVS} \cdot \text{L}^{-1}$ for the inoculated sludge. The basal medium included LAS ($15 \text{ mg} \cdot \text{L}^{-1}$), macro- and micronutrients, and sodium bicarbonate ($1 \text{ g} \cdot \text{L}^{-1}$) as a buffering agent. Two control groups were established: (i) endogenous control (containing LAS, sludge, and basal medium, but no additional carbon source), and (ii) chemical control (containing only LAS and distilled water), as illustrated in Figure 3.

All reactors were sealed with butyl rubber stoppers and purged with nitrogen (N_2) for approximately one minute to ensure anaerobic conditions. The experiment was conducted over 174 hours, a period determined based on the cessation of CH_4 production, indicating the completion of the anaerobic digestion process in batch mode.

Figure 3 – Schematic of the batch experiments.

Source: Prepared by the author.

3.3. Operation System III (OS III)

3.3.1. Experimental set-up

Two lab-scale UASB reactors (working volume of 3.5 L) (Figure 2), inoculated with anaerobic sludge [Total solids (TS) = $50 \pm 3 \text{ g} \cdot \text{L}^{-1}$, total volatile solids (TVS) = $28 \pm 1 \text{ g} \cdot \text{L}^{-1}$ and total fixed solids (TFS) = $23 \pm 1 \text{ g} \cdot \text{L}^{-1}$], were operated, in parallel, at an HRT of 8 h and room temperature of approximately 28 °C.

Both reactors operated under the same conditions except for microaeration. R1 was a conventional UASB reactor, while R2 included microaeration and was called UMSB (Upflow Microaerobic Sludge Blanket). The experiment had two 45-day periods, differing only in the addition of AQS. In Period I, no AQS was added (R1_{AQS-} and R2_{AQS-}). In Period II, both systems (R1_{AQS+}, R2_{AQS+}) were supplemented with 50 μM AQS (98%, Sigma-118 Aldrich, USA) to evaluate its impact on system performance. This structured approach allowed for a systematic comparison of the effects of AQS in organic matter degradation and LAS removal under both anaerobic and microaerobic conditions.

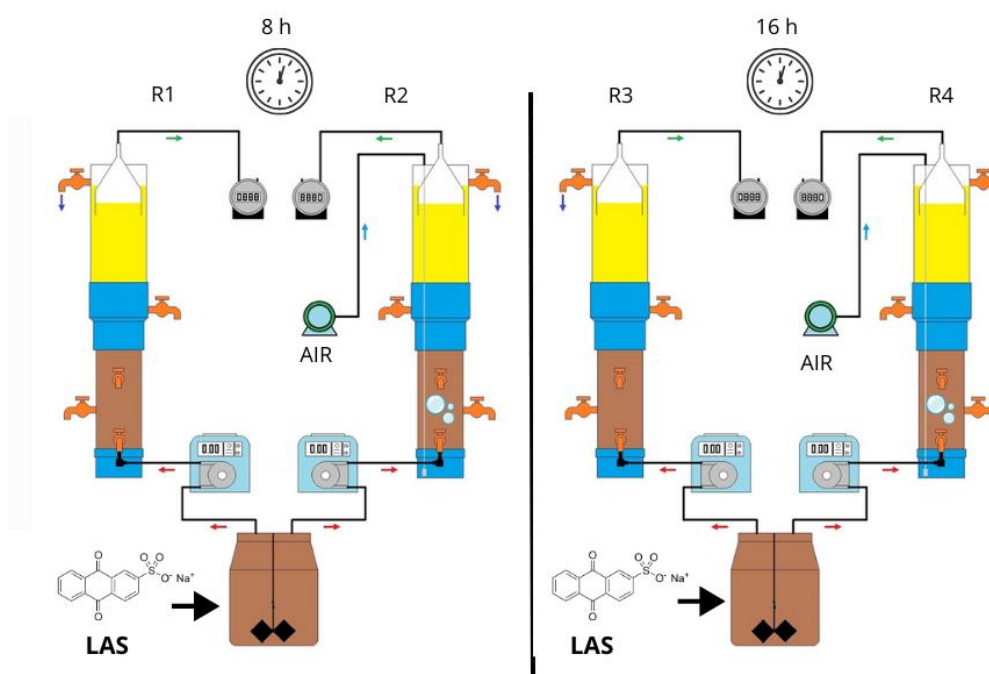
Microaeration in R2 was performed with synthetic air (80% N₂:20% O₂, White Martins, Brazil) at its base by means of a mass flow controller (GFC17, Cole-Parmer, USA). The microaeration air dose used in R2 was approximately $1.5 \text{ L}_{\text{air}} \cdot \text{min}^{-1}$, corresponding to $0.2 \text{ L}_{\text{O}_2} \cdot \text{L}_{\text{feed}}^{-1} \cdot \text{d}^{-1}$, which was kept constant during the experimental period. Based on both the literature (Do Nascimento et al., 2021b) and the limited LAS removal achieved in the previous stage, the microaeration rate was doubled in Chapter V to enhance the system's oxidative capacity and overall performance.

3.4. Operation System IV (OS IV)

3.4.1. Experimental set-up

Four lab-scale UASB reactors (working volume of 3.5 L) (Figure 4), inoculated with anaerobic sludge [Total solids (TS) = $55.1 \pm 3.5 \text{ g} \cdot \text{L}^{-1}$, total volatile solids (TVS) = $30.6 \pm 1.4 \text{ g} \cdot \text{L}^{-1}$ and total fixed solids (TFS) = $24.7 \pm 1.1 \text{ g} \cdot \text{L}^{-1}$], were operated, in parallel, at a room temperature of approximately 28 °C.

Figure 4 – Schematic of the OS IV continuous experimental set-up.



Source: Prepared by the author.

The reactors were operated under the same conditions except for microaeration and HRT. R1 and R3 were conventional UASB reactors, while R2 and R4 included microaeration and were called UMSB (Upflow Microaerobic Sludge Blanket). Regarding hydraulic retention time, R1 and R2 had an HRT of 8 h, while R3 and R4 had an HRT of 16 h. The 16-h HRT was selected as a strategic benchmark to confirm that any performance limitations at 8 h were kinetic rather than due to inoculum or system instability. This approach is based on the group's previous research, which showed that short residence times led to only 34.3% LAS removal in the microaerobic system.

Microaeration was performed with synthetic air (80% N₂:20% O₂, White Martins, Brazil) from the bottom of the reactor, using a mass flow controller (GFC17, Cole-Parmer, USA). The microaeration air dose used in R2 was approximately $1.5 \text{ L}_{\text{air}} \cdot \text{min}^{-1}$,

corresponding to $0.2 \text{ L}_{\text{O}_2} \cdot \text{L}_{\text{feed}}^{-1} \cdot \text{d}^{-1}$, which was kept constant during the experimental period. The microaeration dose was selected based on the best results reported by Do Nascimento et al. (2021a) for the removal of emerging sulfur-containing compounds.

3.5. Synthetic Wastewater

The reactors were fed with a synthetic wastewater formulated to simulate domestic or industrial effluents contaminated with surfactants. The base composition consisted of Linear Alkylbenzene Sulfonate (LAS) as the target pollutant (Sodium dodecylbenzenesulfonate, 100%, Sigma-Aldrich, USA) at a constant concentration of $15 \text{ mg} \cdot \text{L}^{-1}$. This concentration was selected to represent the upper range of LAS typically found in domestic wastewater, which generally varies between 2 and $15 \text{ mg} \cdot \text{L}^{-1}$. By adopting this concentration, the study aims to simulate a high-load scenario, providing a robust assessment of the system's capacity to handle peak surfactant concentrations often encountered in real-scale treatment plants."

To evaluate the influence of co-metabolism (OS I), the carbon source was varied according to the experimental phase: ethanol ($1 \text{ g COD} \cdot \text{L}^{-1}$) was used during Phase I of the initial study, while sucrose ($1 \text{ g COD} \cdot \text{L}^{-1}$) served as the primary co-substrate for all subsequent stages, including the redox mediation (period II of OS III) and hydraulic optimization assays (OS IV). The influent was supplemented with a basal medium containing essential macro and micronutrients, prepared according to Firmino et al. (2010), and buffered with sodium bicarbonate ($1 \text{ g} \cdot \text{L}^{-1}$) to maintain the pH near neutrality (7.0 ± 0.2). All reagents were used as purchased without further purification. To prevent premature biodegradation of the organic matter, the synthetic influent was stored in refrigerated tanks at $4 \text{ }^{\circ}\text{C}$ throughout the experimental periods.

3.6. Analytical methods

Chemical oxygen demand (COD), pH, total suspended solids (TSS), and total solids (TS) content were determined according to APHA (2012). The quantification of CH_4 , CO_2 , H_2 , and H_2S in the biogas was determined by gas chromatography with ionization detection by dielectric barrier discharge (GC BID-2010 Plus, Shimadzu Corporation, Japan), equipped with GS-GASPRO column ($60 \text{ m} \times 0.32 \text{ mm}$) (Agilent Technologies Inc., USA). Helium gas was used as the carrier gas (White Martins LTDA, Brazil) at a flow rate of $2 \text{ mL} \cdot \text{min}^{-1}$, with a run time of 9 min. Oven, injector, and detector temperatures were 50, 100, and $250 \text{ }^{\circ}\text{C}$, respectively. O_2 and N_2 were quantified by gas

chromatography with thermal conductivity detection (GC-TCD) (GC-17A, Shimadzu Corporation, Japan). The biogas sample (1.0 mL) was injected in splitless mode, and chromatographic separation was performed on a Mol Sieve 5A PLOT column (30 m, 0.32 mm ID) (Restek Corporation, USA). Oven, injector, and detector temperatures were 35, 40, and 230 °C, respectively. Helium (White Martins LTDA, Brazil) was used as the carrier gas at a flow rate of 7 mL·min⁻¹, and the run time was 5 min.

LAS concentrations were determined according to the methodology adapted from Silva et al. (2017) by high-performance liquid chromatography (HPLC) using a Shimadzu system (CBM-20A, SPD-20A, CTO-20A, SIL-20A, and LC-20AT) equipped with a reversed-phase C18 column from Shimadzu. The mobile phase was water with 5 mmol·L⁻¹ ammonium acetate (solvent A) and acetonitrile (solvent B); the mobile phase flow rate was 1 mL·min⁻¹, the sample volume was 50 µL, the temperature of the thermostated column compartment was 40 °C, and UV-Vis detection at 230 nm. A gradient was applied to the mobile phase to separate the LAS constituents. It started with 45% solvent B, and its concentration began to rise after 5 minutes, reaching 60% in 20 minutes. The concentration then returned to 45% after 1 minute and remained constant for 6 minutes.

3.7. Determination of Adsorbed LAS in Batch Sludge Samples

The final mass balance of LAS in the system was calculated based in the quantification of adsorbed LAS from the biomass and support media that were removed from the reactor at the end of the experiment to quantify the total solids.

To quantify the fraction of LAS adsorbed onto the anaerobic biomass during the batch assays, a solid-phase extraction protocol was adapted from Duarte et al. (2008). At the end of the experimental period, sludge samples were collected in triplicate and subjected to an extraction process using methanol as the organic solvent. The mixture was placed in an ultrasound bath (5 min, 55 kHz) to promote the desorption of LAS from the microbial extracellular polymeric substances (EPS) and the sludge matrix.

The resulting extracts were centrifuged (10000 rpm, 10 min) and the supernatant was filtered through 0.22 µm membranes and analyzed via HPLC.

3.8. Microbial community analysis

Sludge samples are collected at the beginning and end of operation for all systems to analyze microbial changes. For this purpose, sludge samples were collected at different reactor heights to characterize a composite biomass sample. 0.25 g of fresh sample (total

wet weight) was used to extract DNA from microorganisms using the PowerSoil® DNA Isolation Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Based on the 16S rRNA region V4 gene, the amplicon sequencing was prepared in triplicate according to Illumina protocol (2013), using specific primers (515F/806R) (Caporaso et al., 2012; Parada; Needham; Fuhrman, 2016).

After indexing, the PCR products (amplicons) were purified using Agencourt AMPure XP-PCR purification beads (Beckman Coulter, Fisher Scientific, USA), according to the product manual, and quantified using the dsDNA BR assay kit (Invitrogen, ThermoFisher Scientific, USA) on a Qubit 2.0 fluorimeter (Invitrogen, ThermoFisher Scientific, USA). The amplicons were sequenced using the MiSeq 300-cycle chemistry reagent kit (Illumina, USA) on the MiSeq desktop sequencer (Illumina, USA), located at the Genomics and Bioinformatics Center (CeGenBio) at UFC.

The sequencing data were analyzed using bioinformatics tools. The DADA2 package v1.28.0 (Callahan et al., 2016) was used to filter and assemble reads into amplicon sequence variants (ASVs). The obtained sequences were taxonomically classified using version 5.1 of the MiDAS database, which catalogs microorganisms identified in anaerobic digesters (Dueholm et al., 2023), with the assign Taxonomy method of the DADA2 package. Alpha diversity indices were estimated with the estimate_richness method of the Phyloseq package v1.44.0 (McMurdie & Holmes, 2013). The raw sequence data obtained in this study have been deposited in the National Center for Biotechnology Information (NCBI) BioProject database, ID PRJNA836880.

To infer the metabolic capabilities of the microbial communities present in the reactor's samples, the ASV-based 16S rRNA abundance data were analyzed using PICRUST2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). Functional predictions were generated based on KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways, using EC (Enzyme Commission) numbers and KO (KEGG Orthology) identifiers to assign putative gene functions (Douglas et al., 2020; Kanehisa et al., 2016; Kanehisa and Goto, 2000; Langille et al., 2013).

3.9. Statistical methods and kinetic parameters

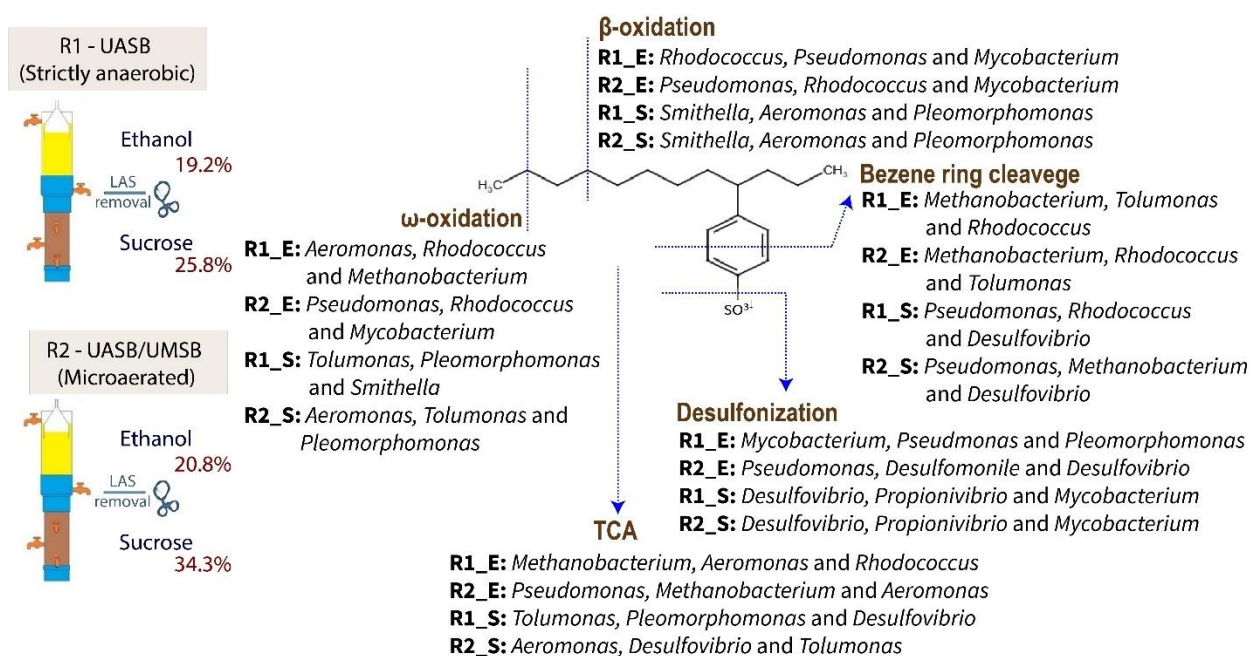
The Mann-Whitney and Kruskal-Wallis nonparametric tests, which do not require specific distributional assumptions, were used to compare reactor performance across the different experimental periods at the 5% significance level.

The full version of this paper is under review in ACS Omega (2026) under the same name as this subsection.

4. MICROBIAL DYNAMICS DURING THE REMOVAL OF LINEAR ALKYL BENZENE SULFONATE (LAS) UNDER ANAEROBIC AND MICROAEROBIC CONDITIONS WITH DIFFERENT CO-SUBSTRATES

This pioneering study evaluates the microbiological pathways and metabolisms of LAS biodegradation in UASB (Upflow Anaerobic Sludge Blanket) reactors operated under microaerated conditions compared to strictly anaerobic systems (Figure 5). Furthermore, the effects of different co-substrates (ethanol and sucrose) on LAS removal efficiency and biogas production were also evaluated. The results indicate that microaeration, combined with sucrose as a co-substrate, significantly improves LAS removal, reaching 19.2% (R1) and 20.8% (R2) with ethanol and with sucrose, efficiencies increased to 25.8% (R1) and 34.3% (R2). The study also revealed that microaeration influenced biogas production, reducing the methane concentration but resulting in a higher overall volumetric production, increasing with microaeration under ethanol feeding (2.7 vs. 1.8 L·day⁻¹), while both reactors performed similarly under sucrose feeding (~2.7 L·day⁻¹). Microaeration significantly influenced the microbial structure and function of the systems, depending on the co-substrate used. In the presence of ethanol, both systems showed a predominance of fermentative and methanogenic communities, with no significant differences in LAS removal. In contrast, with sucrose, microaeration promoted greater removal efficiency, associated with the selection of a specialized consortium composed of *Pleomorphomonas* and *Desulfovibrio*, capable of performing partial oxidation of the benzene ring and desulfonation of the surfactant. This condition activated oxidative and sulfur metabolism pathways, while maintaining functional methanogenesis, resulting in a less diverse but metabolically more targeted and efficient community in LAS degradation. These findings highlight the potential of microaeration and co-substrate selection as strategies to optimize the anaerobic treatment of LAS-containing wastewater while maintaining stable reactor performance.

Figure 5 – Graphical abstract of the OS I.



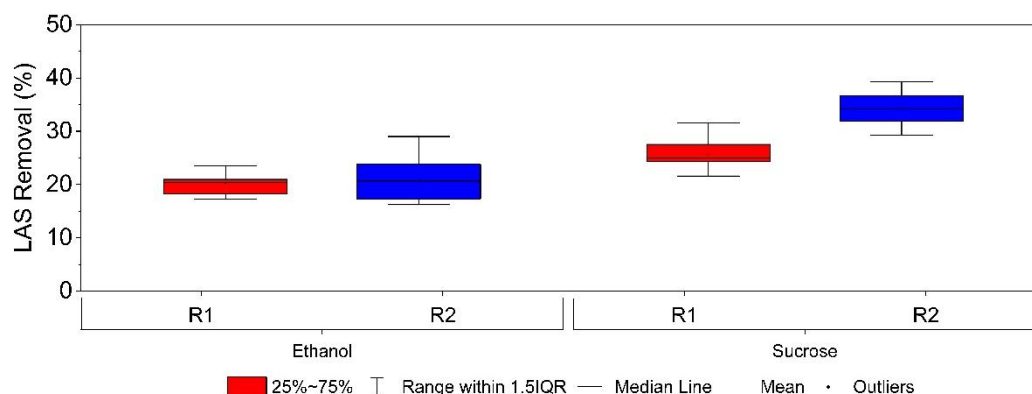
Source: Prepared by the author.

4.1. Linear alkylbenzene sulfonate (LAS) removal

Linear Alkylbenzene Sulfonate (LAS) removal was assessed throughout the operational period (Figure 6), demonstrating distinct efficiencies depending on the co-substrate and aeration conditions. Under ethanol feeding, R1 (conventional) achieved an average LAS removal of $19.2 \pm 3.2\%$, while the microaerated reactor (R2) reached $20.8 \pm 4.1\%$. No statistically significant differences were observed between the reactors during this stage ($p < 0.05$). Nonetheless, these values are higher than those reported by Okada et al. (2013), who observed $9.0 \pm 2.4\%$ LAS removal under conditions of $12 \text{ mg} \cdot \text{L}^{-1}$ LAS, a 6 h HRT, and a co-substrate mixture containing methanol, ethanol, and yeast extract. This allows to infer that the operational parameters used in this study were sufficient to increase LAS removal, obviously without forgetting the role of the inoculum source.

When sucrose was used as a co-substrate, LAS removal efficiency in R1 increased from 19.2% to 25.8%. A more pronounced improvement was observed in R2, where the average removal efficiency during the ethanol phase was 20.8%, but rose to 34.3% with sucrose addition. These results indicate that sucrose enhanced LAS removal, likely by promoting the development of a more diverse and metabolically active microbial community capable of degrading complex organic compounds.

Figure 6 – Removal of LAS in anaerobic (R1) and microaerobic (R2) reactors throughout the experiment.



Source: Prepared by the author.

Similar observations were reported by Okada et al. (2013), who found that LAS removal increased when a more complex organic source was supplied. In their study, a UASB reactor operated at a 24 h HRT and fed with short-chain alcohols (ethanol and methanol) achieved lower removal efficiencies, whereas the addition of yeast extract, a complex co-substrate, raised LAS removal to 50%. When only ethanol and methanol were used, removal remained below 41%, emphasizing the role of co-substrate complexity in enhancing LAS degradation.

Macedo et al. (2015) also evaluated LAS removal in commercial laundry wastewater (18.2 ± 11.5 to 25.4 ± 8.8 mg·L⁻¹) and reported that the type of co-substrate influenced degradation performance. In contrast to the present findings, their study showed better results with ethanol. Using a fluidized bed reactor with effluent recirculation, they observed that LAS removal efficiency increased from $51.7 \pm 23.9\%$ to $72.9 \pm 18.8\%$ when switching from a sucrose–ethanol mixture (50:50) to ethanol alone, which was accompanied by changes in bacterial community composition.

These discrepancies can be largely attributed to differences in reactor configuration and operating conditions. The fluidized bed reactor adopted by (Macedo et al., 2020a) promoted intense mixing and substrate recirculation, which tend to mitigate LAS concentration peaks, reduce local toxicity, and stabilize microbial dynamics, thereby favoring populations adapted to gradual substrate exposure. In contrast, the UASB reactors in the present study operated at a shorter HRT of 8 h, a condition that may have enhanced LAS removal when sucrose was used as co-substrate.

Sucrose, as a readily fermentable carbohydrate, can stimulate a broad range of anaerobic and facultative microorganisms because it is rapidly hydrolyzed into glucose and fructose, which enter central carbon metabolism through glycolysis (Embden–Meyerhof–Parnas pathway), the pentose phosphate pathway, and, in some bacteria such as *Pseudomonas* spp., the Entner–Doudoroff pathway (Nikel et al., 2015). In contrast, ethanol metabolism is more restricted, proceeding mainly through sequential oxidation by alcohol dehydrogenase and aldehyde dehydrogenase to acetyl-CoA (Du; Wang; Wu, 2025). Therefore, sucrose degradation generates a more diverse pool of intracellular intermediates and may provide higher reducing power in the form of NADPH via the oxidative branch of the pentose phosphate pathway, which can support biosynthesis and cellular stress responses.

This metabolic versatility promotes syntrophic interactions and supports the activity of facultative groups recruited by microaeration, which can initiate LAS degradation via oxygenases—an energy-intensive process more effectively supported by sucrose metabolism than by the more specific alcohol dehydrogenase route of ethanol (Abboud et al., 2007; Braga et al., 2015a).

The faster generation of VFAs from sucrose likely supported methanogenic activity and improved LAS biodegradation kinetics, whereas ethanol, metabolized through more specific enzymatic routes involving alcohol dehydrogenases, provides a slower and more selective metabolic response. Therefore, the superior performance obtained with sucrose under shorter HRT conditions reflects the combined effect of substrate fermentability, microbial versatility, and enhanced metabolic coupling between fermentative and methanogenic pathways.

4.2. Volumetric Biogas Production

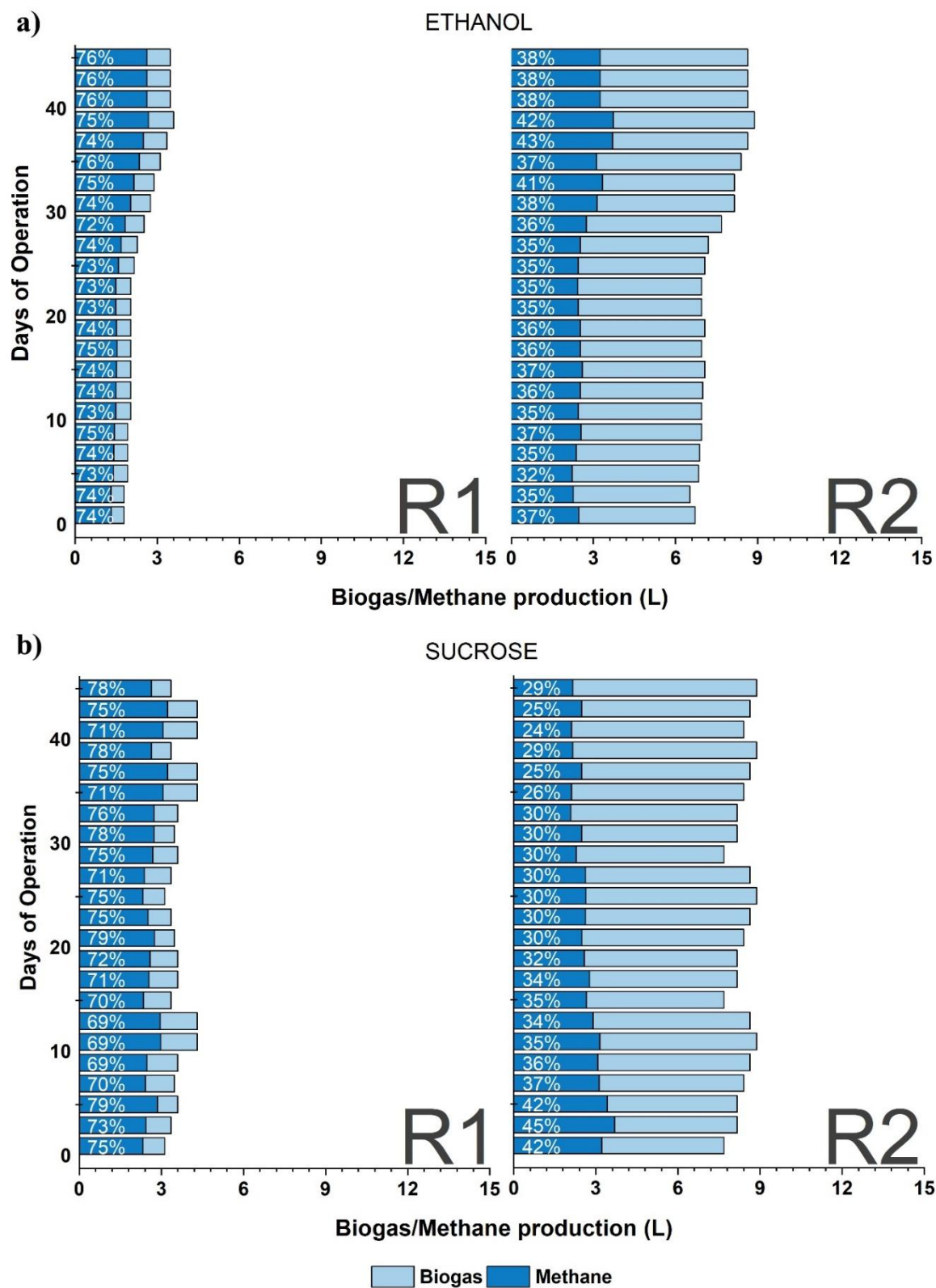
Figure 7 presents volumetric biogas and methane production in reactors operated with sucrose and ethanol as substrates. Monitoring data show that both reactors achieved higher volumetric methane production with sucrose. Under ethanol feeding, R1 (conventional UASB reactor) produced an average of $1.8 \pm 0.4 \text{ L} \cdot \text{day}^{-1}$, whereas the microaerated reactor (R2) reached $2.7 \pm 0.5 \text{ L} \cdot \text{day}^{-1}$. With sucrose, both reactors showed similar performance: R1 yielded $2.7 \pm 0.3 \text{ L} \cdot \text{day}^{-1}$, and R2 achieved $2.7 \pm 0.4 \text{ L} \cdot \text{day}^{-1}$. These findings indicate that sucrose, as a more complex substrate, supported more uniform methane production across configurations.

Sucrose, a disaccharide composed of glucose and fructose, is hydrolyzed by hydrolytic bacteria into readily fermentable monosaccharides. These are processed through glycolysis into organic acids, which are subsequently transformed into acetate and methane by acetoclastic methanogens (Braga et al., 2015). Additionally, monosaccharides are converted into hydrogen (H_2) and carbon dioxide (CO_2), which hydrogenotrophic methanogens further reduce to methane. Ethanol, in contrast, undergoes a narrower metabolic route, being directly oxidized into acetate or $H_2 + CO_2$ by acetogenic bacteria prior to methanogenesis (Zhang et al., 2024). Wang et al. (2020) investigated magnetite-enhanced methanogenesis in UASB reactors and reported higher methane output when sucrose was used as a co-substrate compared to acetate, propionate, or butyrate.

Average methane concentration in biogas produced by R1 reached 73.9% during ethanol and 73.4% during sucrose operation, values exceeding typical ranges for conventional anaerobic digestion (50–70%) (Mecalco; Hilario; Faviel, 2024a), reflecting the absence of oxygen and dominance of methanogenic activity.

In contrast, microaerated systems showed lower methane content, averaging 36.6% (ethanol) and 32.7% (sucrose). For biogas to be considered suitable as an energy source, methane concentration must exceed 45% (Cruz-Salomón et al., 2017). This reduction is primarily due to dilution from air injection, along with possible shifts in microbial community structure resulting from the presence of oxygen. These trends were consistent with findings by Nascimento et al. (2021) and Guimarães et al. (2021b), who reported that microaeration enhances organic matter removal but compromises methane purity. For systems requiring elevated oxygen availability to improve pollutant removal, using pure oxygen in microaeration is a potential strategy to minimize biogas dilution.

Figure 7 – Biogas and methane production from reactors throughout the Ethanol (a) and Sucrose (b) phases.



Source: Prepared by the author.

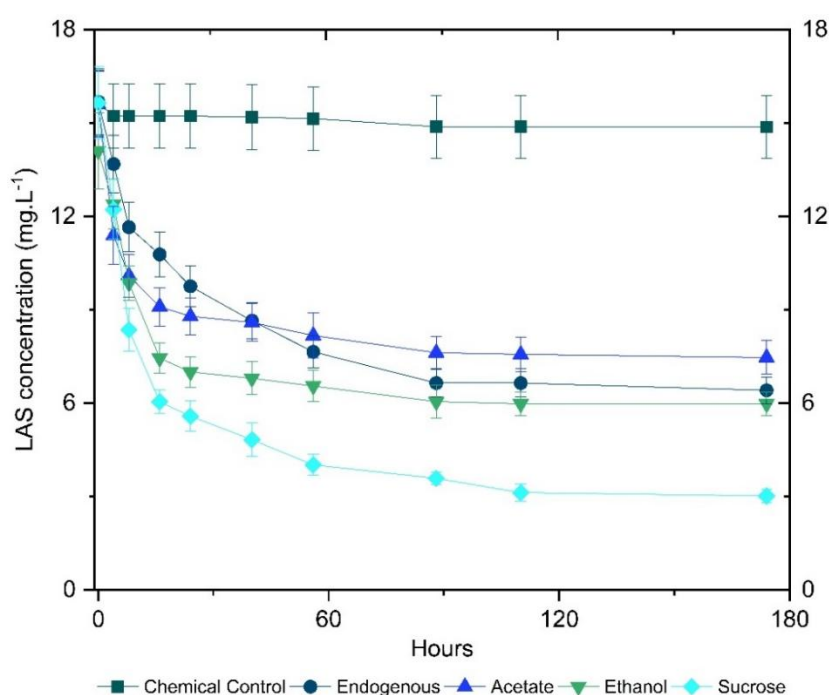
4.3. Batch Experiment

A batch experiment was conducted under anaerobic conditions to preliminarily assess the role of hydrolytic fermentative microorganisms (represented by sucrose), acidogenic fermentative microorganisms (represented by ethanol), and methanogenic pathways, as well as abiotic and endogenous processes, in relation to LAS removal efficiencies in continuous-flow experiments under the tested redox conditions. Acetate was used to represent the acetoclastic route, which is crucial for the final mineralization of organic carbon, while the overall stability and hydrogen consumption were monitored as indicators of the hydrogenotrophic route's performance.

Endogenous controls revealed that 20% of LAS was adsorbed onto the biomass under all tested conditions, whereas the chemical control showed no abiotic LAS removal. Therefore, LAS removal was attributed to biological processes, whose efficiency depended on the type of co-substrate present.

LAS removal efficiencies from the liquid phase after 174 hours of reactor operation were $80.7 \pm 4.2\%$, $57.6 \pm 2.9\%$, $52.2 \pm 2.5\%$, and $59.1 \pm 3.2\%$ for the sucrose, ethanol, acetate, and endogenous series, respectively (Figure 8). In contrast, no LAS removal was observed in the chemical control series, which lacked both co-substrate and biomass, indicating that LAS is not self-biodegradable under the conditions tested.

Figure 8 – LAS removal during the batch experiment.



Source: Prepared by the author.

LAS removal rates were $0.26 \pm 0.4 \text{ mg} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ (sucrose), $0.16 \pm 0.2 \text{ mg} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ (ethanol), $0.18 \pm 0.3 \text{ mg} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ (acetate), and $0.16 \pm 0.2 \text{ mg} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ (endogenous). These results suggest that co-substrates influence the assimilation rate of organic matter, with sucrose supporting higher LAS degradation rates than the other conditions, including the endogenous control. These findings are consistent with those observed in the continuous reactor experiment, where sucrose, being a more complex substrate, offered multiple metabolic pathways for the entire fermentation process and promoted more consistent methane production.

Among the tested co-substrates, the acetate series exhibited the lowest LAS removal efficiency. This may be attributed to the metabolic limitations or sensitivity of microorganisms, particularly acetoclastic methanogens, to the anionic surfactant (Motteran et al., 2018a). Granatto et al. (2021) also noted that acetoclastic methanogens are more susceptible to surfactant toxicity, whereas hydrogenotrophic archaea are generally more resilient.

An important point to note was the high LAS removal observed in the endogenous control, indicating that in the absence of more efficient substrates for co-metabolic processes, such as sucrose or ethanol, LAS may be directly utilized by the microorganisms present or degraded through alternative co-metabolic pathways involving exopolysaccharides (EPS) or substrates derived from endogenous metabolism. Besides, it is important to reinforce that there are two important aspects in the LAS degradation in anaerobic condition: the first refers to the low availability of this compound in the medium due to adsorption in the sludge

4.4. Molecular biology

4.4.1. Microbial community richness and diversity

Metagenomic analysis allowed us to understand the influence of microaeration and co-substrate type on the structure and functionality of the microbial community in the evaluated anaerobic reactors, providing support for interpreting the different performances observed in LAS removal. In general, although richness and diversity indices were higher in the Bacteria domain, they showed distinct behaviors within the Archaea (Figure 9a) and Bacteria (Figure 9b) domains, modulated by the nature of the co-substrate and the presence of residual oxygen introduced in R2.

For the Archaea domain, a consistent pattern was observed, regardless of the co-substrate used. The conventional reactor (R1) showed greater richness and diversity,

suggesting a more complex and balanced methanogenic community under strictly anaerobic conditions. R1 showed higher Chao1 richness and Shannon diversity, suggesting not only a higher number of taxa but also a more even distribution (equitability) of methanogenic populations under strictly anaerobic conditions. The greater diversity of archaea in R1 may be associated with greater functional redundancy and trophic stability typical of consolidated anaerobic systems, but did not necessarily reflect greater efficiency in LAS degradation. This is because methanogenic archaea, although essential in the final stage of anaerobic digestion, act predominantly in the conversion of simple intermediates into methane, not directly participating in the oxidative reactions involved in LAS breakdown (Granatto et al., 2021; Okada et al., 2013a).

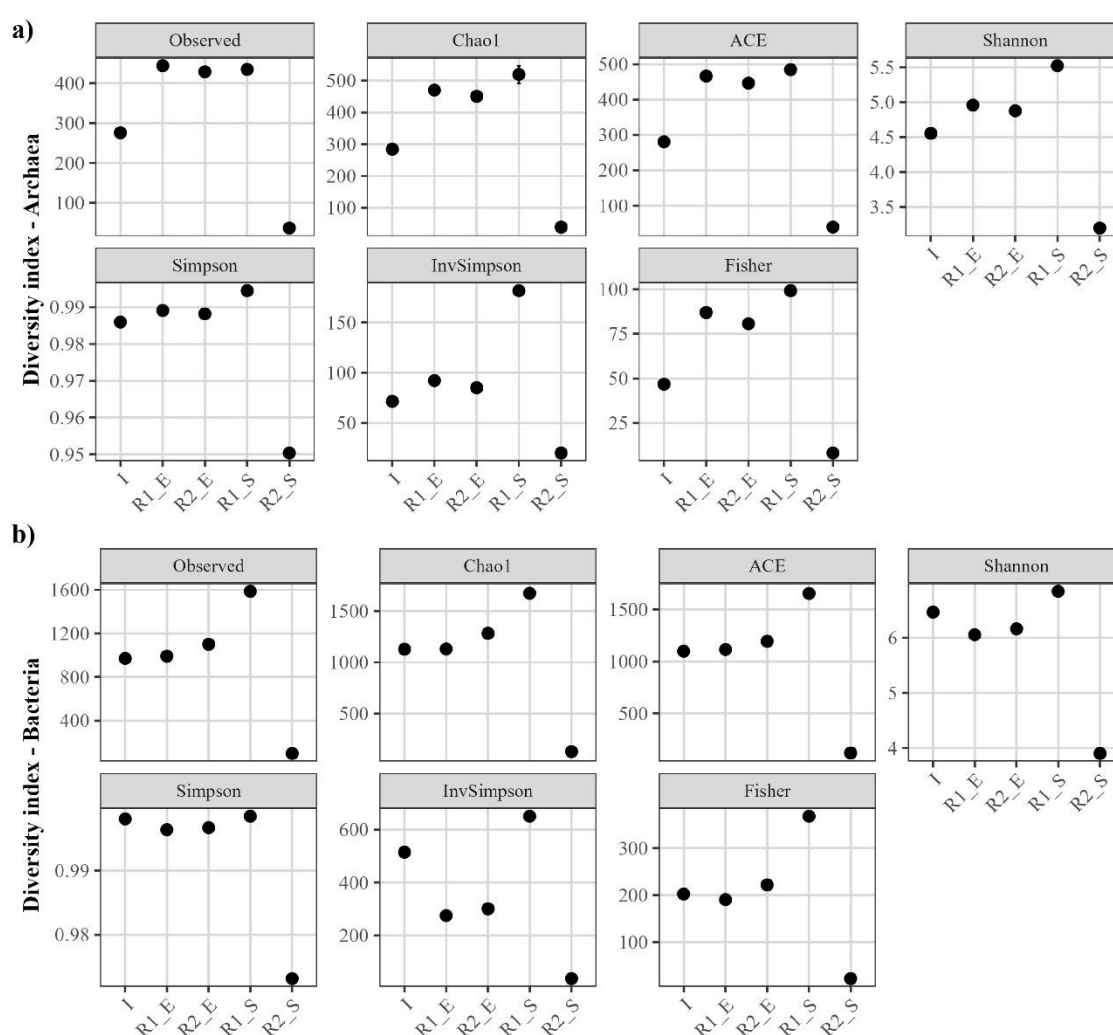
In the bacterial domain, however, the response was more sensitive to the type of co-substrate. When ethanol was used, the microaerated reactor (R2) showed greater bacterial richness and diversity, indicating that the controlled availability of oxygen favored the growth of facultative and microaerophilic groups capable of coexisting with strict anaerobes. This increased diversity may have broadened the metabolic spectrum of the system, although, in this case, LAS removal did not show a statistically significant difference between the reactors. This result suggests that, under ethanol, LAS degradation occurred mainly through anaerobic mechanisms, dependent on specific consortia already well adapted to the surfactant, and that the presence of residual oxygen did not represent a significant metabolic advantage. Furthermore, ethanol, being an easily assimilated substrate, tends to direct metabolism towards rapid fermentative and acetogenic pathways, reducing the residence time of aromatic intermediates derived from LAS and, therefore, limiting the potential benefit of microaeration in the partial oxidation of these compounds (Macedo et al., 2015, 2019).

In the presence of sucrose, a pronounced shift was observed. In the microaerated reactor (R2), both richness and diversity indices decreased markedly. The reduction in the Simpson index, coupled with the decrease in Shannon diversity, indicates a clear process of microbial dominance. This suggests that sucrose and microaeration acted as a selective pressure, favoring the 'bloom' of specialized functional groups (e.g., *Pseudomonas*) that outcompeted rare taxa. While in the ethanol phase, the R2 maintained a more 'generalist' and diverse community (high Shannon/Chao1), with sucrose, R2 transitioned to a specialized consortium.

This pattern is consistent with the fact that sucrose (hydrolyzed into glucose and

fructose) is rapidly assimilated by bacteria equipped with highly efficient metabolic machinery for sugar utilization. As these sugars provide high energetic yield and enable fast ATP and biomass generation, microorganisms with higher growth rates tend to outcompete slower-growing taxa, leading to reductions in diversity, richness, and, in some cases, increased dominance within the community. Importantly, this community streamlining coincided with a substantial increase in LAS removal, particularly in R2, suggesting that the selective advantage conferred by sucrose assimilation favored microbial groups metabolically aligned with the degradation pathways of the surfactant.

Figure 9 – Diversity and richness indices separated by the a) Archaea and b) Bacteria domains.



Source: Prepared by the author.

This inverse relationship between diversity and performance suggests a process

of functional selection, in which microaeration, associated with a more complex substrate such as sucrose, favored the enrichment of populations with a greater capacity to degrade aromatic compounds. Limited oxygenation possibly stimulated the activity of facultative bacteria involved in the initial steps of benzene ring oxidation, promoting the conversion of recalcitrant intermediates into molecules more assimilable to subsequent anaerobes, as already observed by other authors (Atashgahi et al., 2018; Zheng et al., 2021). Thus, the reduction in diversity reflected a more targeted and functionally efficient community, adapted to the degradation of LAS under controlled microaeration conditions.

It should also be considered that sucrose, being a carbohydrate with a more complex structure and less toxic, may have provided greater metabolic stability, allowing the coexistence of fermentative and oxidative populations in a wider redox gradient. (Braga et al., 2015) also observed that sucrose favored a certain degree of stability under strictly anaerobic conditions, allowing the coexistence of different microbial groups involved in the biodegradation process of LAS.

In the microaerated reactor, this gradient possibly intensified, creating complementary niches between microaerobic and anaerobic zones, which favors syntrophic pathways between partial oxidants and residual sulfate or nitrate reducers, as well as oxygen-tolerant methanogens. Thus, the greater removal of LAS observed in R2 with sucrose results not only from the presence of oxygen, but also from the interaction between the type of co-substrate and the consequent restructuring of the microbial community towards a more specialized and synergistic consortium.

The analysis of the Venn diagram reinforces these inferences obtained from the index analyses (Figure 10). The high number of unique ASV in R1 with sucrose (1762) indicates that, under strictly anaerobic conditions and with a more complex substrate, a highly heterogeneous microbial community developed, composed of multiple functional niches. This heterogeneity is consistent with the higher richness and diversity indices previously observed for R1, suggesting that, in the absence of oxygen, the system maintained a wide range of coexisting populations, but possibly with high functional redundancy and diluted metabolic efficiency among different trophic pathways.

Figure 10 – Vennon diagram for each system operated, including the starting inoculum (I – inoculum; R1_E – Ethanol (R1); R2_E – Ethanol (R2); R1_S – Sucrose (R1); R2_S – Sucrose (R2)).

The fact that only 16 ASV were common between the inoculum and the reactors under both conditions reinforces that both the type of co-substrate and microaeration acted as the main selective forces. Thus, while ethanol maintained more similar communities across the systems, sucrose, in the presence of low oxygen levels, induced a strong ecological reorganization, resulting in a less diverse but functionally more efficient community.

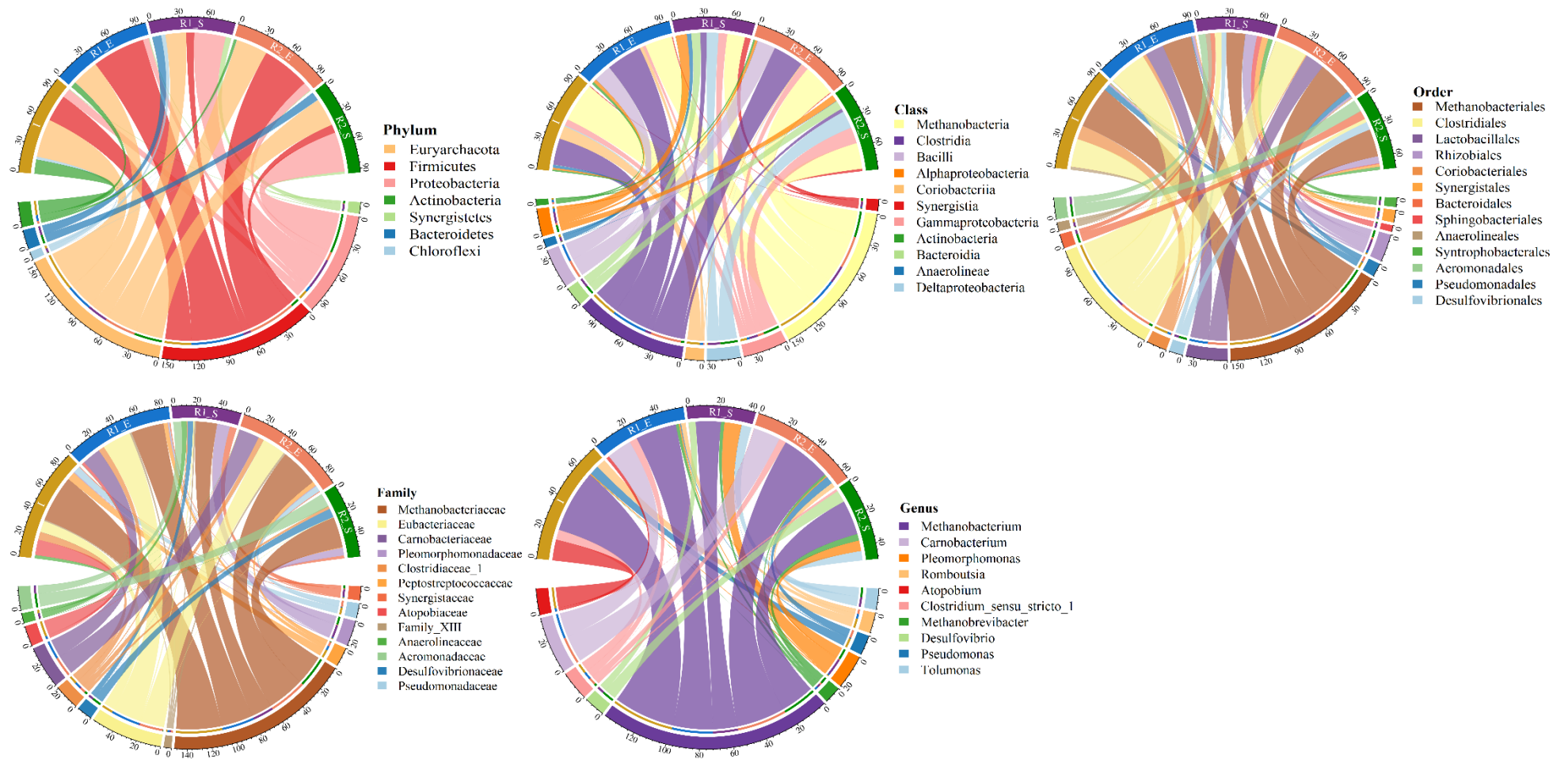
Microbial structure analysis revealed that the type of co-substrate and the presence of microaeration were determinants in the composition and functionality of the communities, directly reflecting the efficiency of LAS removal (Figure 11).

In systems with ethanol, the phylum *Bacillota* was the most dominant, especially

in R1, and was mainly represented by the classes *Clostridia* and *Bacilli* and the families *Eubacteriaceae* and *Carnobacteriaceae*. In the anaerobic LAS removal experiments by (Costa et al., 2024) a predominance of the phylum *Bacillota* was also observed. (Delforno et al., 2020) also found a greater abundance of *Bacillota* in the biomass of the bed of an expanded granular bed sludge reactor treating effluent with $14 \text{ mg} \cdot \text{L}^{-1}$ of LAS, ethanol, and methanol.

The predominance of *Clostridium* and *Carnobacterium* genera in these reactors suggests the potential prevalence of typical fermentative pathways, focused on the conversion of simple substrates into volatile fatty acids, H_2 , and CO_2 . These groups play a relevant role in the acidogenic stage, oxidizing organic acids such as acetate, butyrate, and propionate, but are inefficient in degrading recalcitrant molecules like LAS (Braga et al., 2015; Motteran et al., 2025).

Figure 11 – Structure of the microbial community in terms of phylum, class, order, family, and genus.



Source: Prepared by the author.

The slight reduction of *Clostridium* with microaeration suggests that residual oxygen exerted a selective pressure against strict anaerobes, without, however, introducing groups capable of degrading LAS more effectively. This explains the absence of a statistical difference in LAS removal between R1 and R2 with ethanol. Under these conditions, the metabolic network likely remained centered on redundant fermentative pathways, with a presumably limited contribution from initial oxidative pathways, which were insufficient to overcome the recalcitrance of the surfactant in the ethanol-fed system.

With the replacement of the co-substrate with sucrose, a strong microbiological transition occurred. The phylum *Pseudomonadota* became dominant, mainly in the microaerated R2, accompanied by an increase in the *Alpha*-, *Gamma*-, and *Desulfovibrionia* classes. This change is directly associated with the increase in LAS removal, since these groups harbor genera with a recognized capacity to oxidize aromatic compounds and surfactants. *Pseudomonadota* has been the most abundant phylum commonly found in anaerobic systems treating effluents with surfactants, especially with sucrose as a co-substrate (Braga et al., 2015; Costa et al., 2024; Wei et al., 2024a).

In R2_S, the increase in the genera *Pleomorphomonas* (family *Pleomorphomonadaceae*, *Alphaproteobacteria*) and *Desulfovibrio* (family *Desulfovibrionaceae*, *Desulfovibrionia*) evidences the formation of a specialized microaerophilic-syntrophic consortium. *Pleomorphomonas* includes species capable of metabolizing aromatic compounds under microaerobic conditions, while *Desulfovibrio* is known to act in sulfate reduction processes and degradation of sulfonated intermediates, being considered one of the most abundant genera associated with LAS degradation (Braga et al., 2015; Moura et al., 2019; Wei et al., 2024a). The joint presence of these genera under microaeration suggests a sequential degradation of LAS, with an initial oxidation of the benzene ring mediated by *Pleomorphomonas* followed by the removal of sulfonate groups by *Desulfovibrio*. This synergy explains the superior performance of R2_S in removing LAS, in contrast to R1_S, where the absence of oxygen limited the formation of more biodegradable intermediates.

While traditionally recognized for its nitrogen-fixing capabilities, *Pleomorphomonas* species are versatile chemoorganotrophs capable of utilizing diverse organic compounds (Wang et al., 2024a). In the context of LAS degradation, this genus likely plays a strategic role in the ω -oxidation of the alkyl chain, providing the initial enzymatic attack that converts the terminal methyl group into a carboxyl group. Furthermore, its organotrophic nature supports the metabolism of aromatic intermediates

derived from benzene ring cleavage. The presence of *Pleomorphomonas* suggests a metabolic contribution that goes beyond nitrogen cycling, participating in the complex syntrophic network required for the mineralization of recalcitrant surfactants under low oxygen tension.

Furthermore, the stimulation of *Pseudomonadaceae* and the pattern observed for the genus *Pseudomonas* reinforce the importance of microaeration for the establishment of populations with facultative aerobic metabolism. *Pseudomonas* was detected only in R2 with ethanol, indicating that, despite the potential capacity to degrade LAS, the low complexity of the substrate (ethanol) and the lower availability of aromatic structural carbon did not favor its activity. However, the inhibition of this genus in R2 with sucrose may be associated with competition with other microorganisms adapted to the microaerobic regime and the accumulation of metabolites that inhibit the growth of *Pseudomonas*. According to Unuofin et al. (2019), *Pseudomonas* species are strong degraders of aromatic compounds, but their abundance and activity can be influenced by competition with other representatives of *Pseudomonadota*, especially under microaerobic or partially redox conditions.

The presence of *Synergistaceae* and *Anaerolineaceae*, associated with the phyla *Synergistota* and *Chloroflexi*, respectively, and predominant in R1_S, suggests the involvement of fermentative and syntrophic pathways in intermediate stages of anaerobic degradation. These groups frequently act in the fermentation of amino acids, organic acids, and complex compounds, contributing to the digestion process, but do not directly participate in the initial degradation of LAS. *Synergistota* has been frequently found in anaerobic systems treating effluents with LAS and is reported as an efficient degrader of toxic compounds (Motteran et al., 2018a). However, enzymatic pathways may not be active and prevent the removal of pollutants.

The predominance of these taxa in the reactor without microaeration, combined with the greater overall diversity observed under sucrose, indicates that the complexity of the co-substrate favored a diverse community, but one that is functionally less directed towards surfactant degradation.

The phyla *Bacteroidota* and *Synergistota*, stimulated only with sucrose, reinforce this interpretation. *Bacteroidota*, although active in the degradation of polymers and high molecular weight compounds (Wei et al., 2024b), exhibited similar abundances between R1 and R2, suggesting a more auxiliary role in the conversion of LAS byproducts.

Actinomycetota, on the other hand, was inhibited under both conditions, reflecting

its sensitivity to sulfonated compounds and microaeration. The complete inhibition of *Atopobiaceae* (*Actinomycetota*) and its restriction to the inoculum reinforce this toxic and selective effect of LAS.

The predominance of *Methanobacterium* (family *Methanobacteriaceae*, class *Methanobacteria*, phylum *Methanobacteriota*) in all systems reflects the maintenance of hydrogenotrophic methanogenesis, essential for H₂ consumption and redox balance (Li et al., 2020). However, the abundance of this genus was reduced in relation to the inoculum, especially with sucrose, suggesting that the accumulation of oxygenated or sulfonated intermediates from the partial degradation of LAS exerted competitive inhibition on the archaea.

The persistence of methanogenic activity in the microaerated reactor (R2) can be explained by a combination of ecological and molecular mechanisms. Ecologically, the formation of complex biomass aggregates (granules/biofilms) creates a dissolved oxygen gradient, where facultative bacteria in the outer layers consume the residual O₂, shielding the strictly anaerobic archaea in the inner core. At the molecular level, the identification of genera such as *Methanobacterium* suggests the presence of antioxidant defense systems, including Superoxide Reductase (SOR) and Catalase. These enzymatic pathways allow methanogens to neutralize reactive oxygen species (ROS) and maintain cell viability, facilitating the metabolic coupling between the initial aerobic oxidation of LAS and the final anaerobic mineralization to methane.

Nevertheless, the greater presence of *Methanobacterium* in the microaerated reactors (R2) indicates that microaeration did not compromise methanogenesis, but allowed functional coexistence between methanogenic populations and oxidative bacteria. This interaction is consistent with the greater removal of LAS in R2 with sucrose, where archaea likely acted in the final mineralization of carbon from the initial oxidative degradation of the surfactant.

Nevertheless, the greater presence of *Methanobacterium* in the microaerated reactors (R2) indicates that microaeration did not compromise methanogenesis, but allowed functional coexistence between methanogenic populations and oxidative bacteria. This interaction is consistent with the greater removal of LAS in R2 with sucrose, where archaea likely acted in the final mineralization of carbon from the initial oxidative degradation of the surfactant.

The preservation of methanogenesis in microaerated systems is attributed to the spatial organization of the granular sludge. The layered architecture allows facultative

microorganisms to colonize the outer surface, where they consume residual oxygen and create a protected, strictly anaerobic niche for acetogens and methanogenic archaea within the granule core (Baloch et al., 2008; Picioreanu; Batstone; Van Loosdrecht, [S.d.]

Overall, the observed taxonomic pattern indicates that microaeration and sucrose acted as complementary selective forces, reducing diversity but promoting a functionally specialized community for LAS degradation. While R1 maintained predominantly fermentative populations (*Clostridium*, *Carnobacterium*, *Synergistaceae*, *Anaerolineaceae*), R2 with microaeration and sucrose favored the establishment of oxidative and syntrophic consortia (*Pleomorphomonas*, *Desulfovibrio*, *Methanobacterium*), capable of performing partial degradation and subsequent mineralization of the surfactant. The coexistence of these groups suggests a coupled metabolic sequence, in which the initial oxidation of LAS under microaerobic conditions is followed by the anaerobic removal of intermediates, resulting in the greater efficiency observed in R2_S.

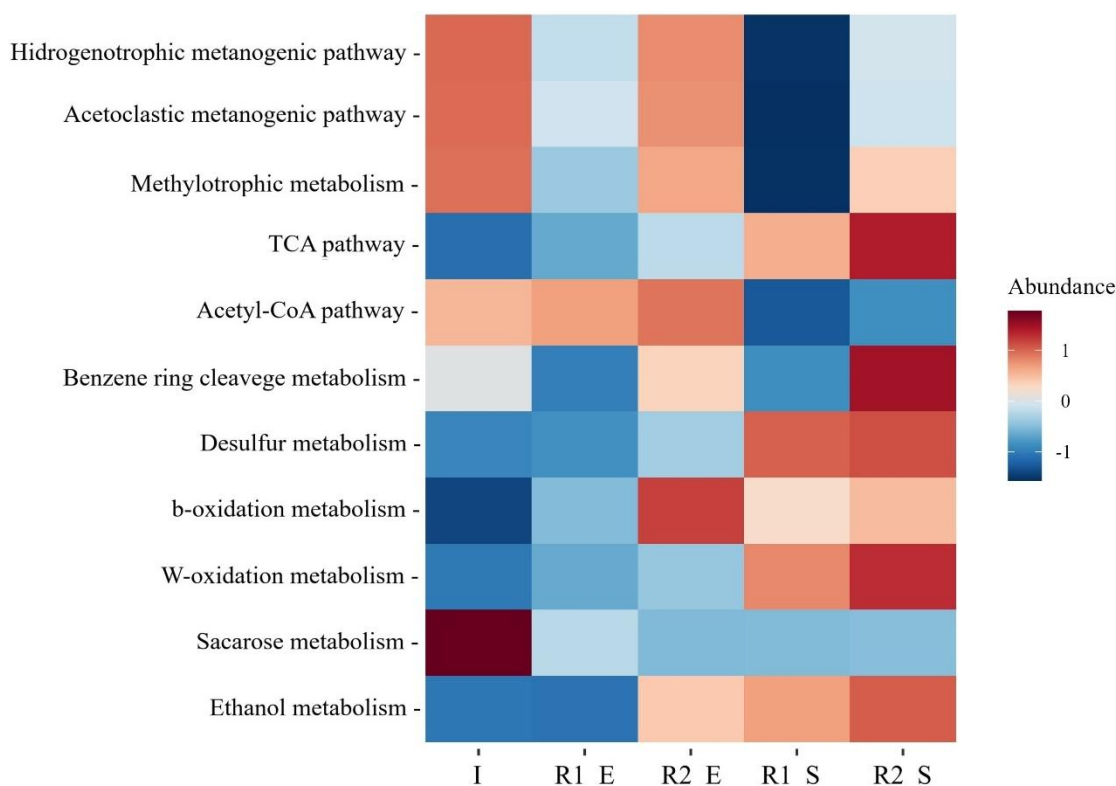
4.4.3. Intracellular metabolism from different substrates used under strictly anaerobic and microaerobic conditions

According to Lara-Martín et al. (2010a), the anaerobic degradation of LAS involves four main pathways: (i) fumarate addition to the alkyl chain, (ii) subsequent β -oxidation reactions, (iii) aromatic ring cleavage, and (iv) desulfonation process. However, other metabolic pathways may be correlated and influence the entire process. Thus, a functional prediction of genes from microbial communities was performed in samples from each system, comparing the relative abundance of KO found in the biomass of each reactor with the KEGG database for different biodegradation pathways.

Initially, from the analysis of functional abundance using the z-score, it was possible to identify the most prominent metabolic pathways in each operated system, including the inoculum (Figure 12). The z-score standardization centers the abundance values within each pathway (mean = 0) and scales them to a unit standard deviation (sd = 1), allowing the heatmap to represent deviations relative to the intrinsic average of each metabolic route. In this framework, negative z-scores indicate samples in which the expression of a given pathway is lower than the pathway's mean expression across all samples, whereas positive z-scores denote above-average expression. This normalization removes differences in absolute magnitude between pathways and highlights relative

shifts in functional potential, enabling a more accurate comparison of metabolic trends driven by the operational conditions.

Figure 12 – Z-score of the relative abundance of metabolic pathways involved in the biological degradation process of LAS.



Source: Prepared by the author.

In the inoculum, the predominance of pathways related to sucrose metabolism, hydrogenotrophic and acetoclastic methanogenic pathways, methylotrophic metabolism, and the Acetyl-CoA pathway indicates a diverse community that is not yet directed by operational selective pressures, but with broad metabolic potential for sugar fermentation and subsequent conversion to methane. The simultaneous presence of methanogenic and sucrose metabolism pathways suggests an active microbiota in the degradation of simple and complex organic compounds, providing a basis for the establishment of populations in the reactors (Braga et al., 2015).

In the systems with ethanol, it was observed that both R1_E and R2_E maintained a predominance of methanogenic pathways (acetoclastic and hydrogenotrophic), but with differences regarding auxiliary pathways. In R1_E, the Acetyl-CoA pathway and sucrose metabolism stood out, even though the co-substrate used was ethanol, suggesting the

occurrence of cross-fermentation and the reuse of metabolic intermediates, probably derived from the partial degradation of LAS or other complex organic compounds. The use of ethanol by certain microbial groups aids in the degradation of LAS through the reduction of the alkyl chain, either by β -oxidation or γ -oxidation, allowing the release of short-chain compounds such as organic acids that are converted to Acetyl-CoA or pyruvate (Motteran et al., 2018a).

In R2_E, the most abundant routes included β -oxidation and hydrogenotrophic methanogenesis, indicating that microaeration may have favored the partial oxidation of organic compounds and surfactants, such as LAS, resulting in the generation of intermediates that are more easily convertible to methane. This condition is consistent with the observation that, although the removal of LAS with ethanol did not show a statistical difference between R1 and R2, the slightly oxidizing microenvironment of the microaerated reactor may have promoted greater stability in the process, allowing the coexistence of anaerobic and microaerophilic microorganisms.

With the replacement of the co-substrate with sucrose, the predominant metabolic pathways were distinct and more related to oxidative degradation and the metabolism of aromatic compounds. In R1_S, the most representative routes were sulfur metabolism, ω -oxidation, ethanol metabolism, and the tricarboxylic acid (TCA) cycle. This configuration indicates a system more directed towards the catabolism of complex and possibly sulfonated compounds, such as LAS, albeit in a predominantly anaerobic manner. The simultaneous presence of desulfurization and ω -oxidation pathways is particularly relevant, as these pathways are involved in the cleavage of sulfonate groups and alkyl chains, fundamental initial steps in the biodegradation of LAS (Atashgahi et al., 2018; Delforno et al., 2020; Wei et al., 2024b).

In the microaerated sucrose reactor (R2_S), the activation of the TCA cycle, benzene ring cleavage, ω -oxidation, desulfurization, and ethanol metabolism pathways indicated a more complete and integrated metabolism, capable of promoting both the opening of the aromatic ring of LAS and the subsequent oxidation of its intermediates. The prominent presence of the benzene ring cleavage pathway only in R2_S reinforces the positive role of microaeration in inducing aerobic or microaerobic degradation routes for aromatic compounds, which are often limiting in strictly anaerobic environments. This condition explains the better performance of the microaerated reactor in removing LAS with sucrose, since the initial oxidation of the aromatic ring, catalyzed by oxygen-dependent mono- and dioxygenase enzymes, is a crucial step for the complete

biodegradation of the surfactant (Liu et al., 2024a).

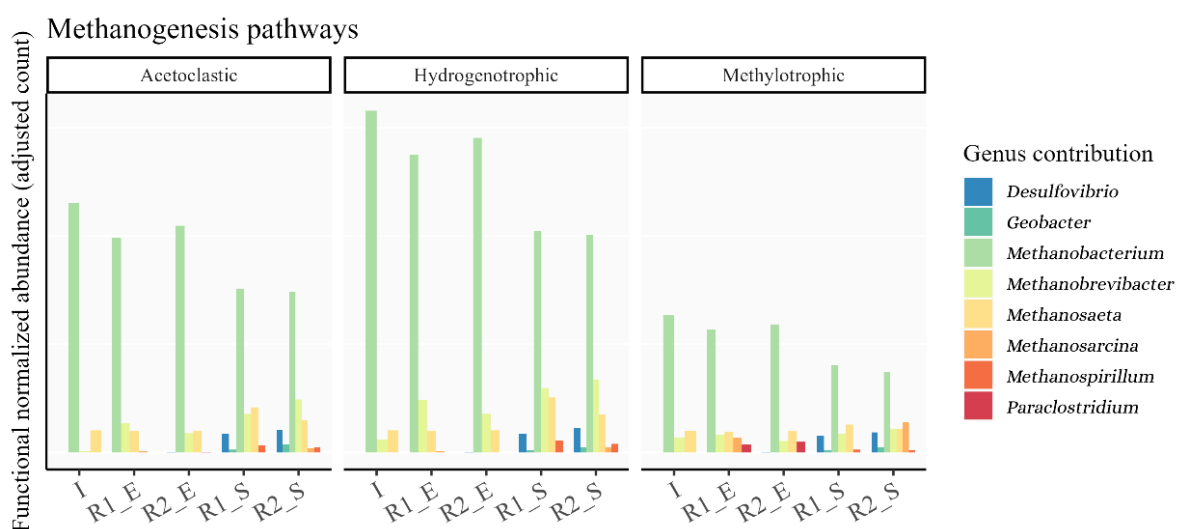
Therefore, while ethanol predominantly maintained fermentative and methanogenic pathways, sucrose stimulated more diverse pathways, associated with the oxidation of aromatic compounds and sulfur metabolism, especially under microaeration. Thus, the superior performance observed with R2_S in LAS removal can be attributed to the synergy between the sucrose co-substrate, which may have favored the expression of desulfurization and oxidation pathways, and the controlled supply of oxygen, which may have allowed the advancement of the initial aerobic steps of LAS degradation before the final conversion of intermediates by methanogenic anaerobic microorganisms.

4.4.4. Gene-enzyme linkage analysis and correlation between microbial community structure and functional genes involved in different metabolisms

4.4.4.1 Methanogenic acetoclastic hydrogenotrophic and methylotrophic pathways

The integrated analysis of the acetoclastic, hydrogenotrophic, and methylotrophic methanogenic pathways revealed a strong influence of the co-substrate type and microaeration on the functional potential (predicted gene abundance) and the overall performance of the systems in LAS removal (Figure 13).

Figure 13 – Contribution of the most significant genus in methanogenic pathways.



Source: Prepared by the author.

In general, the microorganisms carrying the genes for these pathways were predominantly from the genus *Methanobacterium*, typical of the hydrogenotrophic

pathway. These genomic findings, coupled with the stable methane production observed during the operation, suggest that methanogenesis via CO₂ reduction by H₂ potentially represented a key carbon conversion mechanism in the systems evaluated. However, it is important to consider that the presence of these taxa indicates a metabolic potential that likely supported the system's resilience, especially under the selective pressure of LAS and microaeration.

This predominance was particularly evident in the reactors with ethanol, in which the availability of electrons from the partial oxidation of this co-substrate favored the activity of hydrogenotrophic microorganisms. According to Zhang et al. (2024), the partial oxidation of ethanol generates hydrogen, which is then consumed by these methanogens, boosting methane production and favoring the degradation of complex substrates such as LAS.

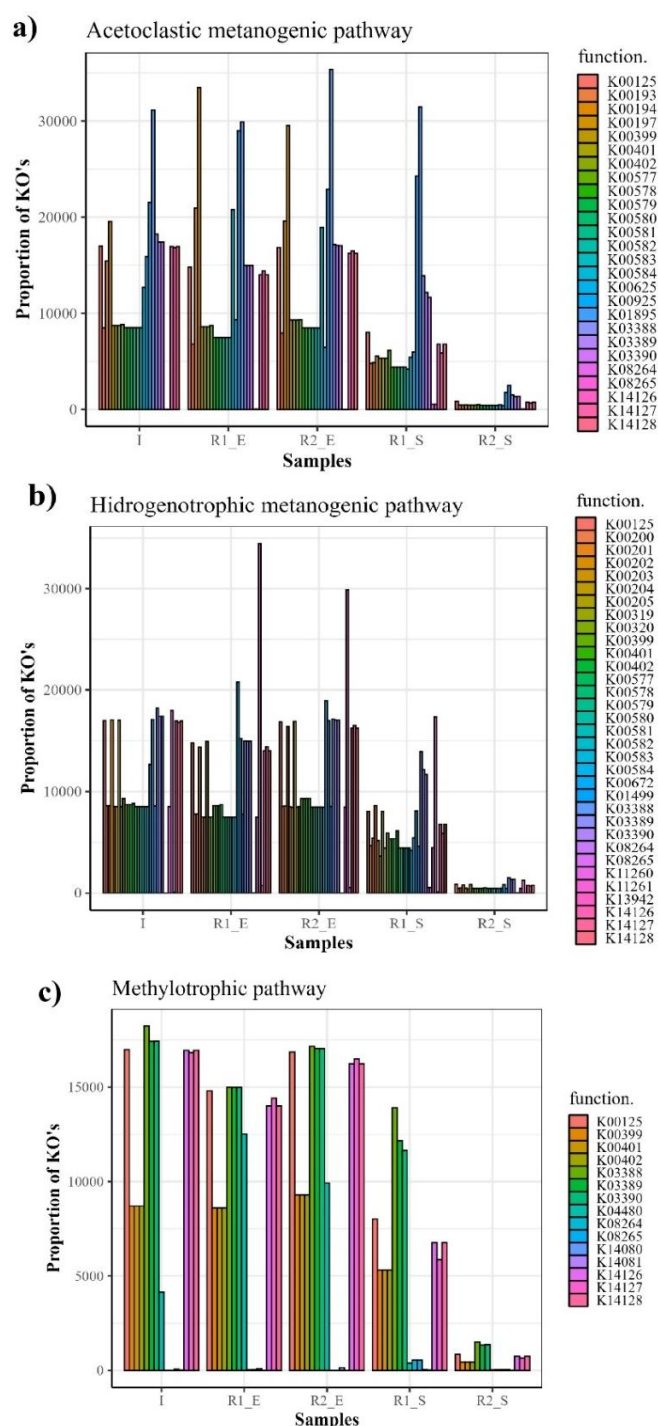
Furthermore, even under microaerated conditions, the R2_E reactor maintained high number of methanogenic genes, indicating that moderate microaeration did not inhibit methanogenesis, but may have promoted a redox environment favorable to the coexistence of oxidative and methanogenic populations, which justifies the stable performance of these systems regarding LAS removal, even without statistical differences between R1_E and R2_E. Do Nascimento et al. (2021a) also found that microaeration did not impair methanogenesis and favored the abundance of different functional species.

In the acetoclastic pathway, the main KO (KEGG Orthology) observed were K00197, K01895, K00925 and K03388 (Figure 14), which encode enzymes associated with the conversion of acetate to methane and CO₂, such as Acetyl-CoA synthetase and Acetyl-CoA decarbonylase/synthase (Mehta et al., 2021; Zhou et al., 2023). Systems with ethanol showed higher overall predicted abundance, especially in R2_E, which exhibited the highest number of KO in this pathway, notably the increase in K01895, which surpassed K00197 in abundance. This predominance indicates that, with ethanol, the microbial community directed its metabolism towards the efficient conversion of acetylated intermediates to methane, reflecting the high fermentative activity typical of this co-substrate. However, the high flux of simple carbon may have limited the use of LAS as a secondary source of carbon and electrons, reducing its effective degradation. According to Delforno et al. (2019a), this enzyme is mainly involved in acetate oxidation via the acetoclastic methanogenesis pathway, although it can act in other metabolic pathways.

In systems with sucrose, the abundance of these KO was lower, mainly in R2_S,

although R1_S had a more significant number of genes or metabolic pathways related to acetoclastic methanogenesis. In this case, K01895 was also the most predominant, followed by K00925, and K03388 emerged as the third most relevant, suggesting the involvement of enzymes associated with activation pathways of more complex compounds.

Figure 14 – Analysis of the main genes in methanogenic pathways: a) Acetoclastic, b) Hydrogenotrophic and c) Methylotrophic.



Source: Prepared by the author.

This configuration suggests that, with sucrose, some of the carbon was redirected to oxidative and fermentative pathways potentially associated with LAS degradation. This shift in the metabolic profile appeared to favor a higher overall efficiency of surfactant removal, especially in the microaerated reactor, even if it implies a different carbon distribution compared to strictly methanogenic pathways

The hydrogenotrophic pathway showed similar behavior, with higher gene predicted abundance in systems with ethanol and a significant reduction in R2_S, suggesting that the presence of sucrose and the introduction of microaeration redirected microbial metabolism towards oxidative and LAS degradation pathways at the expense of classical methanogenesis.

In almost all systems, except the inoculum and R2_S, K11261, related to ferredoxin-dependent hydrogenase (Chen et al., 2023), was the most abundant, followed by K03388, indicating intense conversion of H₂ to methane in strictly anaerobic environments. However, in R2_S, the dominant of KO K03388, K03389, and K03390 suggests the presence of enzymatic complexes involved in both CO₂ reduction and partial oxidation of organic compounds, which is consistent with the more oxidative nature of this system and its greater efficiency in LAS removal.

Finally, the methylotrophic pathway showed the lowest relative presence among the methanogenic pathways, with emphasis on the KO K03388, K03389, and K03390, which encode subunits of methyl-coenzyme M reductase (Mcr), a central enzyme in the final step of methane production (Delforno et al., 2019a; Macedo et al., 2020). These KO were detected in all systems, but with greater abundance under conditions with ethanol, indicating that methylotrophic metabolism was favored by more reduced carbon sources. With sucrose, a slight increase in K03388 was observed compared to the others, suggesting the maintenance of active methylotrophic microorganisms, although with a smaller relative contribution to the total carbon balance.

In addition to *Methanobacterium*, the genera *Methanobrevibacter*, *Methanosaeta*, and *Methanosarcina* were also identified as the main carriers of methanogenic genes. *Methanobrevibacter*, associated with the hydrogenotrophic pathway, reflects the typical activity under conditions of high H₂ availability (Li et al., 2020). *Methanosaeta* and *Methanosarcina*, in turn, are classic representatives of the acetoclastic pathway, with the latter also capable of operating through methylotrophic pathways (Motteran et al., 2025).

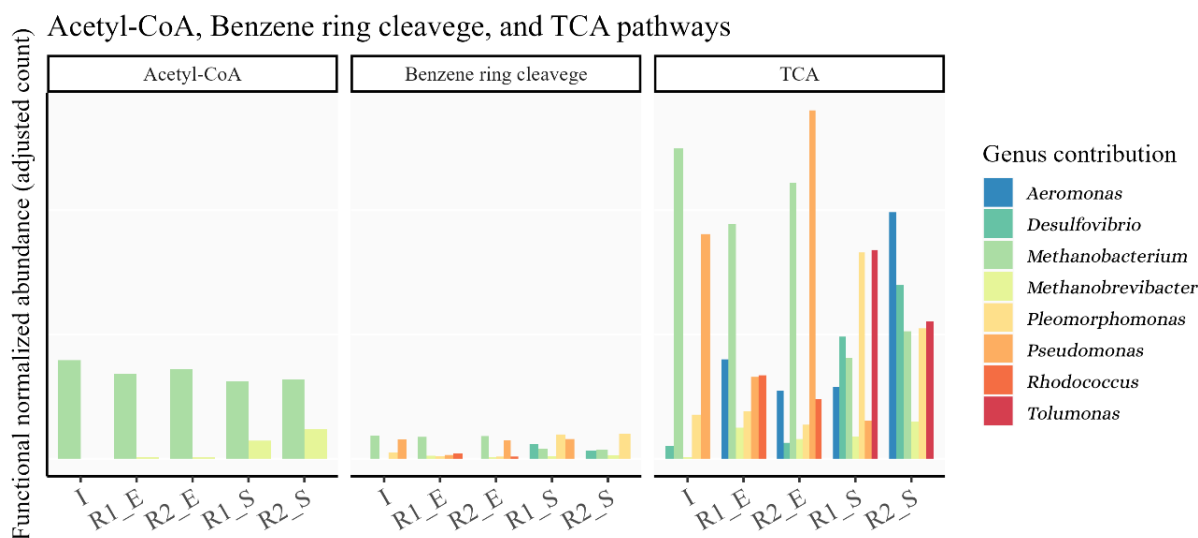
The presence of these archaea indicates a functionally diverse community, capable of converting a variety of organic intermediates into methane.

Taken together, the results demonstrate that methanogenic pathways remained active in all systems, but with intensity modulated by the type of co-substrate and the presence of microaeration. Ethanol promoted greater prevalence of KO linked to methane production, while sucrose and microaeration reduced this expression, favoring the activation of pathways associated with the oxidation and degradation of LAS. Thus, the better performance of R2_S in surfactant removal is related to a metabolic rebalancing, in which methanogenic activity gives way to oxidative and hydrolytic processes more relevant to the breakdown of alkyl chains and the aromatic ring of LAS.

4.4.4.2 TCA and Acetyl-CoA pathways and Benzene ring cleavage metabolism

In the tricarboxylic acid (TCA) cycle pathway, a greater diversity of microbial genera was observed, indicating a broad participation of heterotrophic microorganisms in the central carbon metabolism (Figure 15). The systems fed with ethanol showed a predominance of *Methanobacterium* and *Pseudomonas*, with the former being more abundant in the conventional UASB and the latter in the microaerated system, suggesting that the controlled presence of oxygen favored microorganisms with more oxidative metabolism. With sucrose, the behavior was distinct between the systems. In R1, *Tolumonas* and *Pleomorphomonas* stood out, while in R2 *Aeromonas* and *Desulfovibrio* prevailed, indicating greater variation in microbial structure according to the operating conditions.

Figure 15 – Contribution of the most significant genus to the TCA, Acetyl-CoA, and benzene ring cleavage metabolism pathways.



Source: Prepared by the author.

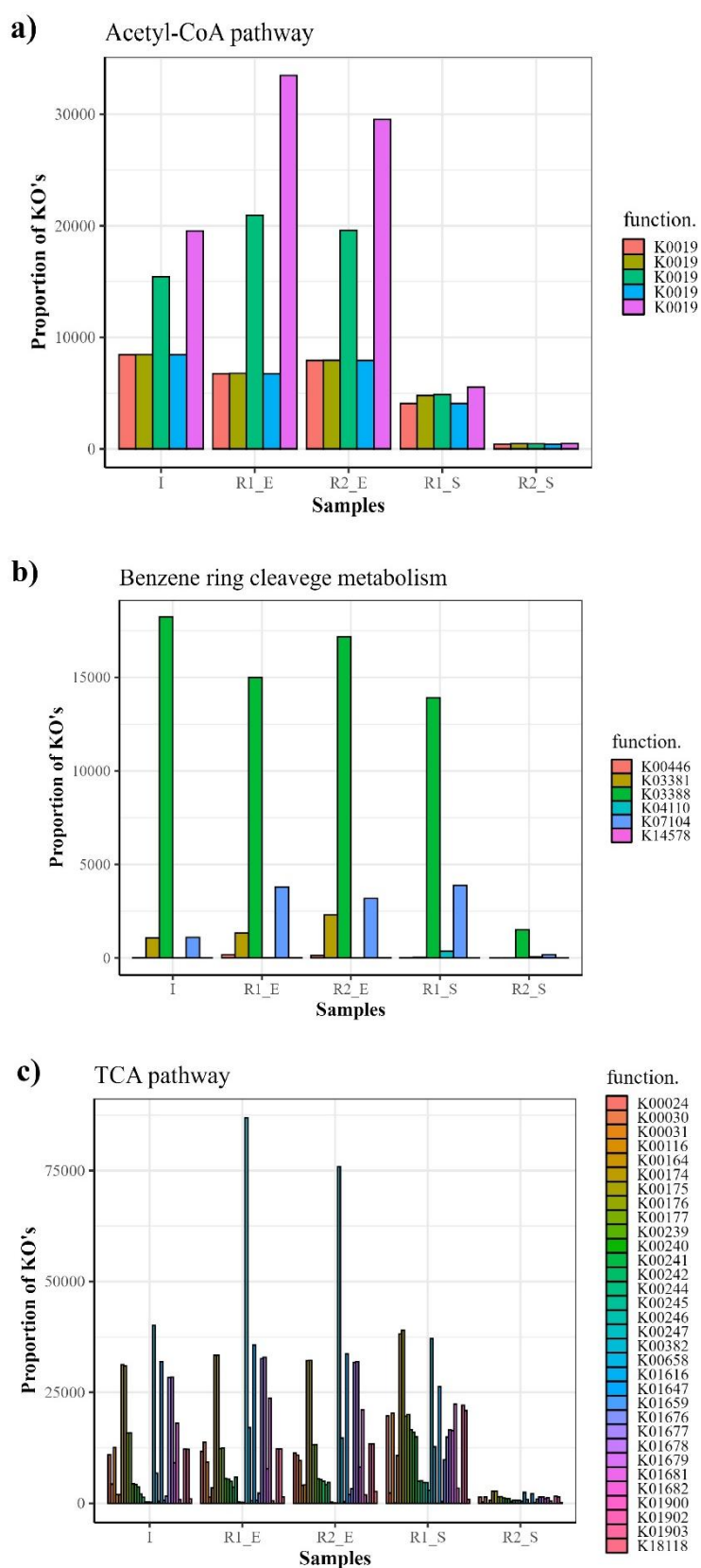
In general, the microaerated systems presented a greater number of KO in this pathway, suggesting greater metabolic activity associated with the intermediate oxidation of organic compounds (Figure 16). In terms of KO abundance, reactors with ethanol showed higher values than those with sucrose, with K00247 being the most representative, followed by K00174 and K00175. In systems with sucrose, the pattern was reversed, with K00175 being the most abundant, followed by K00174 and K00247.

In the benzene ring cleavage pathway, reactors fed with sucrose showed higher KO abundance, indicating greater potential for the degradation of aromatic compounds under this condition. With ethanol, the most associated genus was *Methanobacterium*, while with sucrose *Pleomorphomonas* stood out, both related to processes of breaking aromatic structures and transforming recalcitrant byproducts, possibly derived from LAS (Atashgahi et al., 2018). In all systems, the main KO identified were K03388 and K07104, with the R2_E reactor standing out, showing the great importance for these routes, suggesting a relevant role of microaeration combined with ethanol in the activation of oxidative pathways complementary to surfactant degradation.

In the Acetyl-CoA pathway, similar behavior was observed among the systems, with *Methanobacterium* as the main genus involved, although the reactors with sucrose showed greater total abundance, especially R2_S, which also showed an increase in *Methanobrevibacter*. This predominance reinforces the importance of these archaea in the final conversion of intermediates into methane, especially via the acetoclastic pathway. In all reactors, except for R2_S, K00197 was the most abundant KO, followed by K00194, representing central steps in acetate activation (Delforno et al., 2019a; Liu et

al., 2024a). Among the systems, R1_E showed the highest abundance of KO, while R2_S had the lowest, suggesting that ethanol favored greater metabolic flux via Acetyl-CoA and, consequently, greater energy efficiency and LAS degradation, while sucrose partially limited this route, possibly by promoting the accumulation of fermentative intermediates.

Figure 16 – Analysis of the main genes in the a) Acetyl-CoA, b) benzene ring cleavage metabolism and c) TCA pathways.



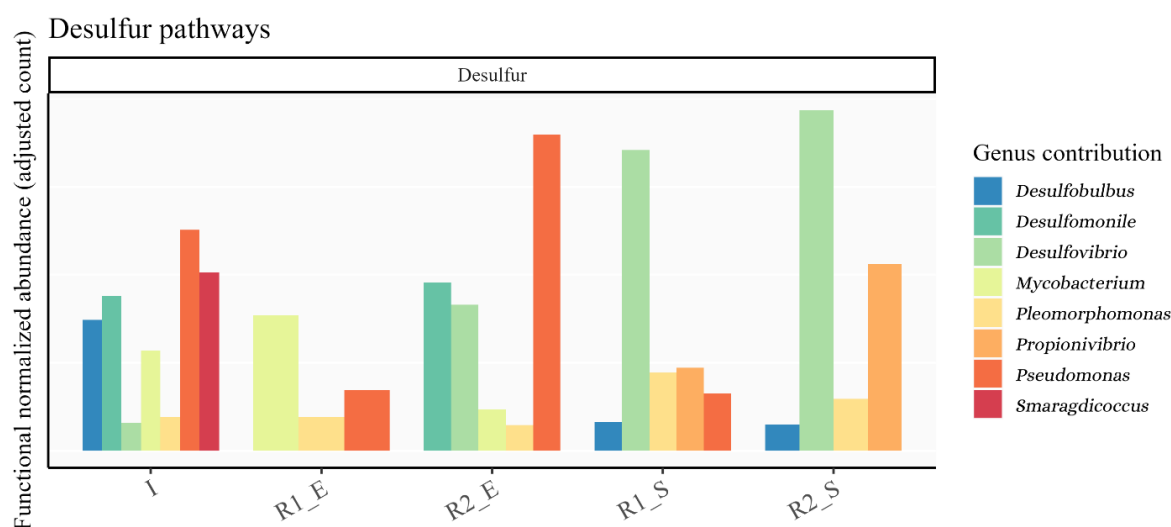
Source: Prepared by the author.

4.4.4.3 Desulfur metabolism

Analysis of sulfur metabolism suggests that desulfonation was a central component in LAS biodegradation and that the way this pathway was operationalized

directly conditioned the reactor performance (Figure 17). In taxonomic and functional terms, the systems with sucrose showed a profile that favors the reduction of the sulfonate group. *Desulfovibrio* and *Propionivibrio* (R1_S and R2_S) were the dominant genera, with R2_S showing the highest overall abundance of microorganisms associated with this route. The high presence of *Desulfovibrio* is mechanistically relevant, as these sulfate-reducing bacteria perform final steps of sulfite/sulfonate reduction to sulfide, releasing the organic skeleton for subsequent anaerobic mineralization steps (Braga et al., 2015; Macedo et al., 2020). This provides a direct explanation for why the sucrose-containing systems, and in particular R2_S, exhibited greater LAS removal. That is, active desulfonation makes LAS intermediates more accessible to subsequent catabolism.

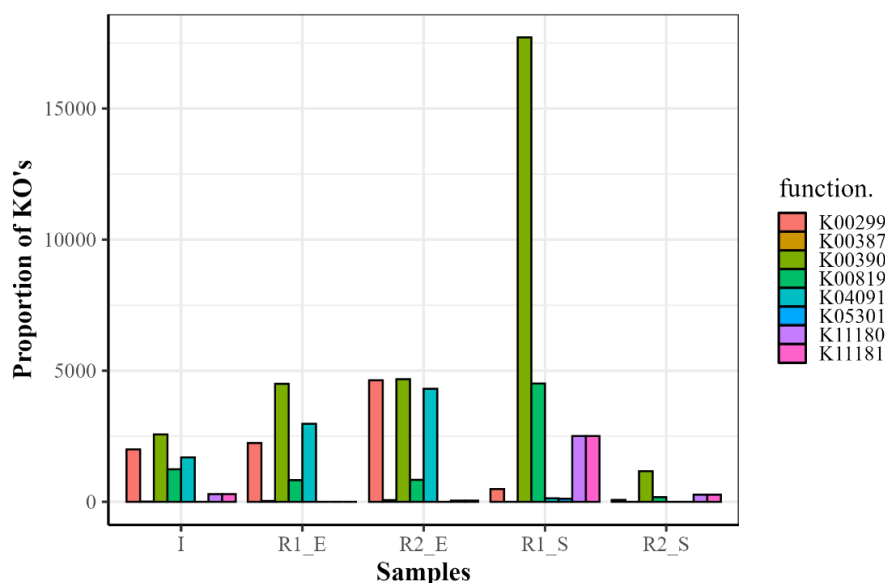
Figure 17 – Contribution of the most significant genus to desulfur metabolism.



Source: Prepared by the author.

As for KO, K00390 (ferredoxin-dependent sulfite reductase) stood out in all systems, especially in the sucrose-containing reactors (Figure 18). The high level of K00390 in R2_S and R1_S indicates a possible enzymatic capacity to convert sulfite into sulfide (a critical step in LAS desulfonation) and corroborates the functional importance of this pathway for surfactant removal (Zheng et al., 2023). Furthermore, R1_S showed the highest total abundance of sulfur metabolism KO, suggesting great overall enzymatic potential for desulfonation even in the absence of microaeration.

Figure 18 – Analysis of the main genes in desulfur metabolism.



Source: Prepared by the author.

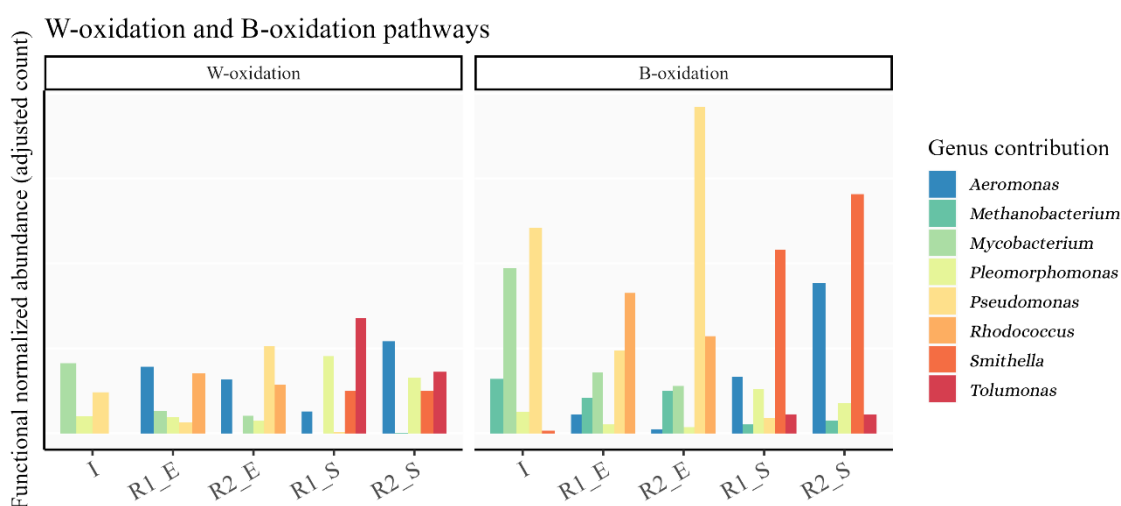
However, the high identification and/or inferences of metabolic functionality through number of KO does not guarantee removal efficiency of LAS. The difference between R1_S and R2_S assumes that desulfonation needs to be coupled with initial oxidative steps for LAS mineralization to be effective. T. Wei et al. (2024) and Yadav et al. (2001) point out that alkyl chain cleavage and aromatic ring opening precede or coincide with desulfonation.

In R1_S, despite many KO inferred and identified (including K00390), the absence of oxidative support limited by strict anaerobiosis restricts the generation of intermediates that sulfate-reducing microorganisms can fully transform, explaining why R1_S had lower removal than R2_S. In contrast, R2_S combines a high presence of sulfur-reducing microorganisms with oxidative microzones induced by microaeration, which promotes both the necessary partial oxidation and subsequent desulfonation, resulting in the highest removal observed. In the systems with ethanol, the profile identified was different. There was a greater balance between K00299 (relating to enzyme APS reductase), K00390 and K04091 (related to enzyme cysteine desulfurase), and the communities included *Pseudomonas*, *Mycobacterium*, and *Desulfomonile* (R2_E). This enzymatic composition indicates that both sulfate activation (K00299) and sulfite reduction (K00390) and sulfur transfer (K04091) were present (Zheng et al., 2023). Even so, LAS removal did not show a statistically significant difference between R1_E and R2_E. This likely occurred due to carbon competition and metabolic prioritization, which limit the impact on LAS removal.

4.4.4.4 LAS degradation by ω - and β -oxidation metabolism

The analysis of inferences for the ω -oxidation and β -oxidation pathways highlights the decisive role of these routes in the degradation of alkyl groups in LAS (Figure 19), complementing the ring cleavage and desulfonation routes discussed previously, where the former is responsible for activation and the latter for sequential degradation (Eichhorn; Knepper, 2002; Lara-Martín et al., 2010a).

Figure 19 – Contribution of the most significant genus to the ω -oxidation and β -oxidation pathways.



Source: Prepared by the author.

According to the results of gene prediction, β -oxidation proved to be more expressive than ω -oxidation in all systems, reflecting the possible predominance of progressive side-chain degradation mechanisms, which is expected, since LAS has long linear alkylbenzene chains that can be sequentially shortened by the removal of two-carbon units (Delforno et al., 2019a; Wang et al., 2024b). The greatest global abundance of these pathways was observed in R2_S, the same system that showed the greatest LAS removal, reinforcing the direct correlation between the activation of oxidative pathways (even in a microaerobic environment) and the efficiency of the process.

In ω -oxidation, the differences between cosubstrates and reactors were relevant regarding to metabolic inference analyses. In systems with ethanol, the activity of this pathway was mainly sustained by *Aeromonas* and *Rhodococcus* in R1_E, while in R2_E the presence of *Pseudomonas* promoted a significant increase in the predicted abundance

of oxidative genes, even surpassing *Aeromonas* and *Rhodococcus*. This functional substitution is consistent with the physiology of *Pseudomonas*, which is known to be efficient in initiating the terminal oxidation of hydrocarbons and anionic surfactants (Macedo et al., 2020a), even though LAS removal remained similar between R1_E and R2_E, probably due to metabolic competition with ethanol.

The most abundant KO in this condition were K00128 and K00121 (Figure 20), respectively, NAD⁺-dependent alcohol dehydrogenase and aldehyde dehydrogenase (Méheust et al., 2020). This fact may indicate the occurrence of primary oxidation reactions from the alkyl chain end to the corresponding carboxylic acid. Despite this activation, ethanol acted as a preferential substrate and diverted the electron flow, restricting the coupling between the initial oxidations and the subsequent degradation pathways, which justifies the absence of a statistical difference in LAS removal between the two systems with ethanol.

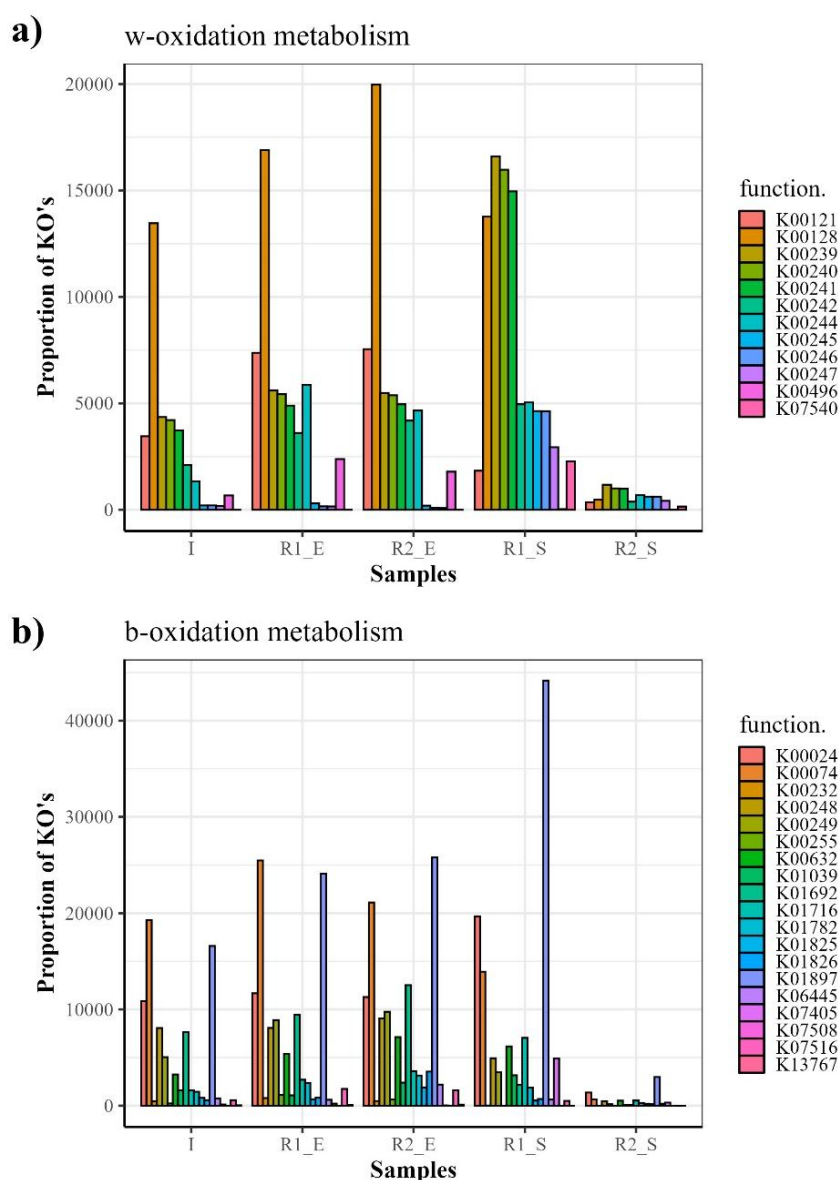
In the systems with sucrose, ω -oxidation showed a change in the most representative KO. There was a reduction in K00128 and a significant increase in K00239, K00240, and K00241, corresponding to acyl-CoA dehydrogenases and enoyl-CoA hydratases, enzymes typical of the processing of β -oxidation intermediates (Méheust et al., 2020; Zhong et al., 2019). This shift in the functional profile suggests a genetic enrichment of the community for pathways associated with the processing of the LAS side chain after initial activation, potentially supporting a more efficient metabolic flow under these conditions.

In microbial terms, *Tolumonas* and *Pleomorphomonas* dominated R1_S, while *Aeromonas* and *Tolumonas* prevailed in R2_S, suggesting that microaeration positively modulated genera with a greater capacity for long-chain oxidation and catabolism of aromatic compounds. This transition, coupled with the greater abundance of oxidative KO in R2_S, explains the observed gain in LAS removal with sucrose, since ω -oxidation generates more hydrophilic intermediate acids, facilitating their subsequent β -oxidation and complete mineralization.

Regarding the metabolic inference related to β -oxidation, the patterns reinforce this interpretation. In the systems with ethanol (R1_E and R2_E), *Pleomorphomonas* and *Pseudomonas* were the main genera, associated with the oxidation of short and intermediate fatty acids of LAS, with emphasis on the KO K00074 (Acetyl-CoA acetyltransferase) and K01897 (3-hydroxyacyl-CoA dehydrogenase).

Figure 20 – Analysis of the main genes in the a) ω -oxidation and b) β -oxidation

pathways.



Source: Prepared by the author.

Despite the expression of these genes, the metabolic shift caused by ethanol and the redox limitation typical of anaerobic systems explain why the functional increase did not translate into greater LAS removal. On the other hand, in systems with sucrose, the scenario was substantially different: *Smithella* and *Aeromonas* were dominant, reflecting a community more adapted to the degradation of long-chain hydrocarbons and partially oxidized aromatic compounds.

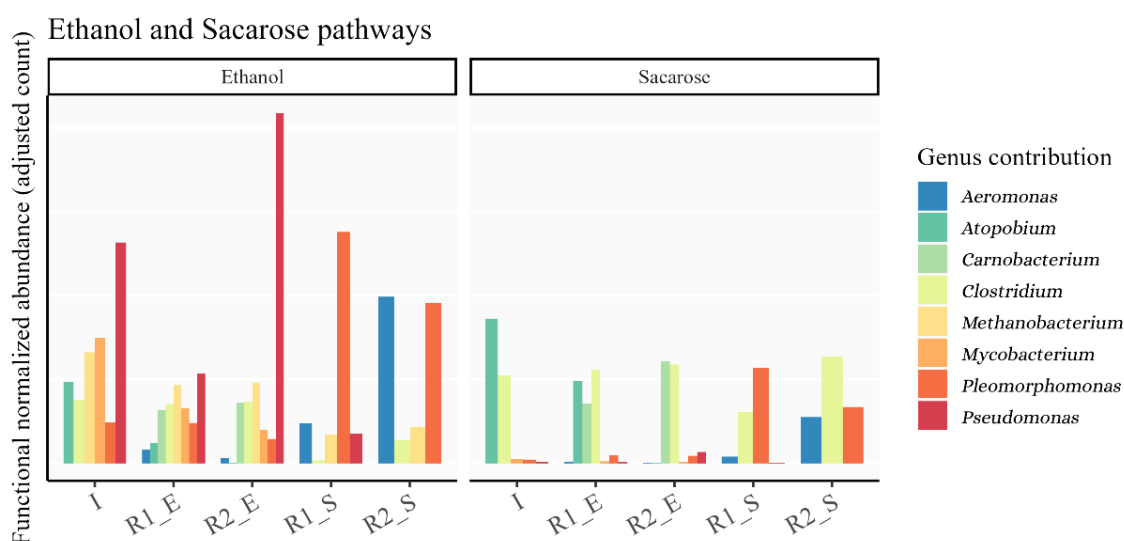
The KO K01897 was especially abundant in R1_S, indicating intense conversion of 3-hydroxyacyl-CoA to β -acetyl-CoA, a key step in the degradative sequence of β -oxidation (Wei et al., 2022). Although R1_S had the highest absolute number of KO,

R2_S maintained greater efficiency in LAS removal, suggesting that microaeration facilitated redox coupling between β - and ω -oxidation, increasing the degradation rate and allowing the mineralization of partially oxidized intermediates.

4.4.4.5 Ethanol and saccharose metabolism

Although at first glance it may seem redundant to investigate the pathways of the added co-substrates themselves (Figure 21), this approach allows us to understand the metabolic role of these compounds in the regulation of oxidative, methanogenic, and surfactant degradation pathways.

Figure 21 – Contribution of the most significant genus to the ethanol and sucrose pathways.



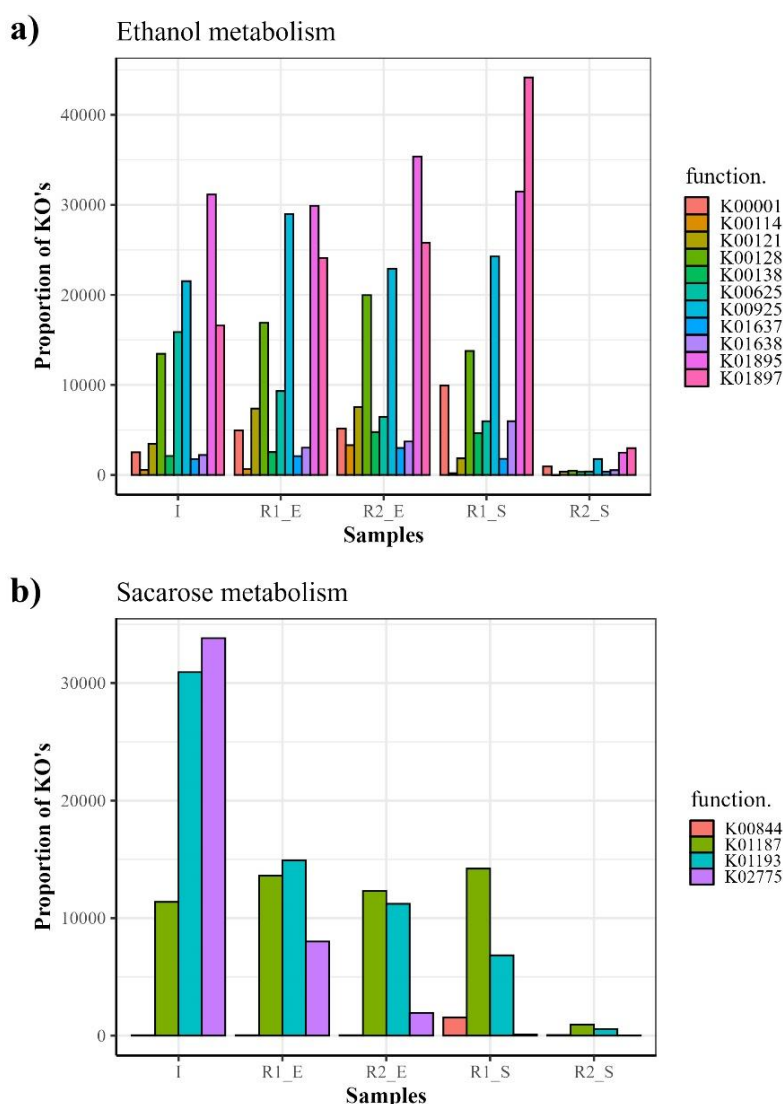
Source: Prepared by the author.

In the ethanol pathway, the reactors supplemented with this co-substrate (R1_E and R2_E) showed a predominance of *Pseudomonas* and *Methanobacterium*, indicating a coexistence between microorganisms with the capacity for partial ethanol oxidation and hydrogenotrophic methanogenic archaea that use the generated byproducts for methane production.

The most expressive set of KO included K01895, acetaldehyde dehydrogenase, responsible for the conversion of acetaldehyde to Acetyl-CoA, and K01897, 3-hydroxyacyl-CoA dehydrogenase, associated with β -oxidation (Figure 22). The higher relative abundance of K01897 suggests that ethanol, being readily metabolized, functioned as a preferred substrate, promoting microbial growth and methanogenic

activity, but limiting the use of LAS as a carbon or electron source.

Figure 22 – Analysis of the main genes active in the a) ethanol and b) sucrose pathways.



Source: Prepared by the author.

In systems operated with sucrose, the ethanol pathway still showed significant evidence, reflecting the metabolic flexibility of microorganisms capable of interconnecting fermentative and oxidative pathways. In this case, *Pleomorphomonas* and *Aeromonas* were the predominant genera (R1_S and R2_S, respectively), and the enzymatic pattern was reversed, with K01895 more abundant than K01897, indicating a greater flux towards the conversion of fermentable sugars into Acetyl-CoA, but with less involvement of oxidative steps of β -oxidation.

R1_S presented the highest absolute number of KO in this ethanol pathway;

however, R2_S, under microaeration, obtained better performance in LAS removal, demonstrating that efficiency depends not only on functional abundance but also on the integration between fermentative and oxidative pathways under controlled redox conditions. Microaeration may have favored the coupling between the partial oxidation of endogenous ethanol (produced by sucrose fermentation) and LAS degradation pathways, maintaining the balance between electron donors and acceptors.

The sucrose pathway, in turn, proved to be determinant for the differentiated behavior of the systems with this co-substrate. In the reactors with ethanol (R1_E and R2_E), despite the absence of sucrose in the medium, there was basal predicted abundance of this pathway, suggesting the presence of microorganisms with broad genetic traits, capable of metabolizing different sugars and oligosaccharides. In these systems, *Clostridium* - was dominant, followed by *Atopobium* in R1_E and *Carnobacterium* in R2_E. These genera are associated with carbohydrate fermentation and the production of organic acids, acting as suppliers of intermediate substrates (acetate, propionate) for methanogenic archaea. The KO K01193 (relating to sucrose phosphorylase) was more abundant in these reactors, indicating a predominance of the direct conversion pathway of sucrose to glucose-1-phosphate, a pathway that saves energy and sustains fermentative metabolism under reducing conditions.

In the systems with effectively added sucrose (R1_S and R2_S), the behavior was different. *Pleomorphomonas* and *Clostridium* dominated in R1_S, while *Clostridium* and *Pleomorphomonas* alternated in R2_S, evidencing a reorganization of the microbial community in response to the new substrate. In these reactors, the K01187 (related to enzyme invertase or β -fructofuranosidase) was the most abundant, catalyzing the initial hydrolysis of sucrose into glucose and fructose, a key step that feeds central pathways such as glycolysis, TCA, and Acetyl-CoA formation. The predominance of K01187 in the sucrose systems suggests more intense fermentative activity, responsible for generating intermediates that support both the oxidative biodegradation of LAS and the maintenance of methanogenic activity. This metabolic coupling justifies the greater LAS removal observed in the sucrose reactors, especially in the microaerated reactor (R2_S), where the balance between fermentation and partial oxidation was more efficient.

4.5. Conclusion

This study concluded that microaeration positively influenced LAS degradation,

particularly when sucrose was used as a co-substrate, modulating microbial structure and functionality distinctly according to the co-substrate used.

In the presence of ethanol, both R1 and R2 presented communities dominated by *Bacillota*, with a prevalence of classic fermentative and methanogenic pathways, and a predominance of *Clostridium* and *Carnobacterium*. Microaeration, although it slightly increased bacterial diversity and favored facultative groups, did not result in a significant increase in LAS removal, as ethanol directed metabolism towards rapid fermentative pathways and preferential consumption of single carbon, limiting the partial oxidation of aromatic compounds. Thus, even with relevant expression of β -oxidation and methanogenesis genes, surfactant degradation remained stable between the two systems, sustained by already adapted anaerobic consortia, in which residual oxygen played a secondary role.

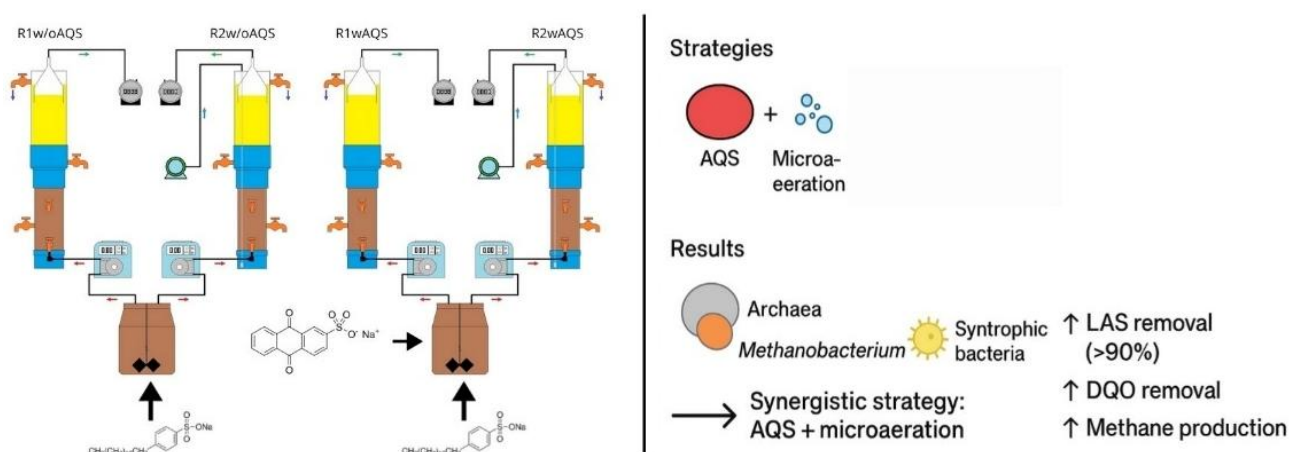
With the substitution of the co-substrate with sucrose, a significant ecological and functional restructuring was observed. The microaerated reactor (R2_S) showed lower diversity, but a more specialized and syntrophic community, composed mainly of *Pseudomonadota*, notably *Pleomorphomonas* and *Desulfovibrio*, whose combined action may have favored the partial oxidation of the benzene ring and the subsequent desulfonation of LAS. This reorganization coincided with the of oxidative pathways (TCA, cleavage of the benzene ring, ω - and β -oxidation) and KO related to desulfurization and sulfur metabolism (K00390), integrated with the maintenance of functional methanogenesis via *Methanobacterium*.

Thus, microaeration and the use of sucrose acted synergistically as complementary selective forces, promoting the coupling between oxidative and anaerobic degradation steps. The result was a less diverse, but more targeted and efficient community, capable of converting recalcitrant intermediates into assimilable compounds and achieving the best overall performance in LAS removal. Future studies should investigate the effects of different microaeration rates, redox mediator supplementation, alternative electron acceptors such as nitrate and nitrite, evaluate the integration of bioaugmentation or co-metabolic strategies, and assess the long-term impacts of microaeration on effluent quality under real-scale operating conditions.

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5. REDOX MEDIATORS AND MICROAERATION BOOSTING THE LINEAR ALKYL BENZENE SULFONATE REMOVAL

This study evaluated the independent and combined effects of the redox mediator anthraquinone-2-sulfonate (AQS) and controlled microaeration on LAS biodegradation in upflow anaerobic sludge blanket (UASB) reactors. Two reactors treating synthetic wastewater containing $15 \text{ mg} \cdot \text{L}^{-1}$ LAS were operated for 90 days: R1, a strictly anaerobic UASB, and R2, a micro-aerated UASB. Each reactor was assessed without (Period I) and with AQS addition ($50 \text{ } \mu\text{M}$, Period II) (Figure 23). Under strictly anaerobic conditions without AQS, LAS removal was low and highly unstable ($32 \pm 16\%$), with pronounced performance fluctuations. The addition of AQS alone significantly improved LAS removal to $65 \pm 4\%$ without affecting methane production, indicating enhanced LAS transformation via electron shuttling rather than changes in methanogenic activity. Microaeration combined with AQS yielded the best overall performance, increasing LAS removal to $77 \pm 1\%$, COD removal to $92.3 \pm 3.8\%$, total methane production to $5.6 \pm 0.7 \text{ L} \cdot \text{day}^{-1}$, and specific methane yield to $0.50 \pm 0.11 \text{ LCH}_4 \cdot \text{gCOD}_{\text{app}}^{-1}$. Microaeration without AQS improved process stability but did not achieve LAS removal comparable to AQS. Microbial analyses showed that AQS promoted the enrichment of hydrogenotrophic methanogens, particularly *Methanobacterium*, and syntrophic taxa such as *midas_g_2229*, while microaeration increased microbial richness. The combined condition exhibited the highest archaeal enrichment and microbial richness. Results demonstrate that AQS and microaeration act through complementary mechanisms, and that their combination effectively overcomes limitations of anaerobic LAS removal.

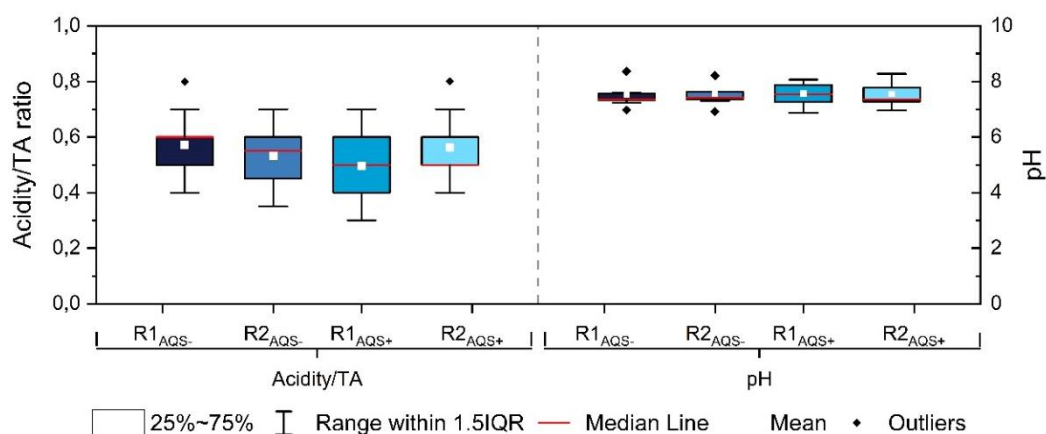
Figure 23 – Graphical Abstract OS III.

Source: Prepared by the author.

5.1. Performance of the UASB reactors

5.1.1. Operational parameters and volumetric biogas production

The evaluation of operational pH and acidity parameters in the UASB reactors, both with and without microaeration, revealed very similar values, regardless of AQS use (Figure 24). The average pH remained between 7.4 and 7.6 throughout the experimental period, with no significant differences between reactors. Acidity/TA ratios ranged from 0.54 ± 0.09 to 0.82 ± 1.06 , and during the stabilization phase, they were further stabilized, with all values falling below the threshold of 0.8 typically associated with process imbalance.

Figure 25 – Box plot of pH and acidity/alkalinity ratio during acclimation and stabilization phases.

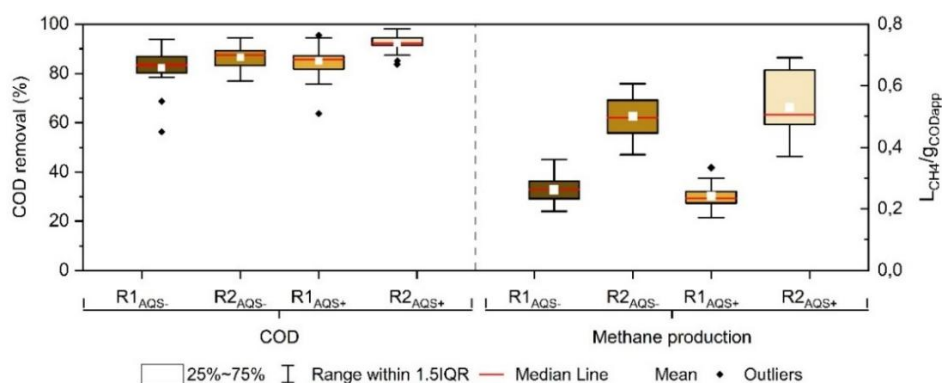
Source: Prepared by the author.

Therefore, the application of AQS and controlled microaeration did not cause acid base disturbances or instability in either pH or Acidity/TA. This suggests that these strategies are compatible with the physicochemical equilibrium required for anaerobic treatment systems and do not compromise reactor stability when applied at moderate doses. In the study by (Do Nascimento et al., 2021b), microaeration ($1 \text{ mL air} \cdot \text{min}^{-1}$) combined with the redox mediator AQDS ($50\text{--}100 \text{ } \mu\text{M}$) also maintained system stability, with the oxidation-reduction potential (ORP) remaining below -200 mV , ensuring conditions suitable for reductive biotransformation.

5.2. Efficiency parameters

The COD removal efficiency and the specific methane yield per unit of Applied organic load are presented in Figure 26. All reactors achieved high COD removal efficiencies, exceeding 80%, indicating effective conversion of organic matter under all operational conditions. In reactors R1_{AQS-} and R1_{AQS+} (anaerobic, with and without AQS), average COD removals were $82.3 \pm 8.0\%$ and $84.9 \pm 7.3\%$, respectively, showing that the addition of AQS alone did not significantly impact the degradation of bulk organic matter ($p > 0.05$).

Reactors R2_{AQS-} and R2_{AQS+}, which received microaeration, presented higher COD removal efficiencies ($p < 0.05$) of $86.5 \pm 4.3\%$ and $92.3 \pm 3.8\%$, respectively. These results suggest that introducing low oxygen doses enhanced the system's oxidative degradation capacity, particularly when combined with AQS in R2_{AQS+}. The elevated performance in R2_{AQS+} may result from synergistic effects between oxygen availability and redox mediation, which collectively stimulate both aerobic and anaerobic metabolic routes, favoring the degradation of more complex or partially oxidized intermediates (Buakaew; Ratanatamskul, 2024; Do Nascimento et al., 2021b).

Figure 24 – Boxplot of Efficiency parameters

Source: Prepared by the author.

However, when COD removal is analyzed alongside methane production, a distinct trend emerges. The specific methane production ($L_{CH_4} \cdot gCOD_{app}$) was significantly lower ($p < 0.05$) in anaerobic reactors (0.19 ± 0.05 in R1_{AQS-} and 0.17 ± 0.04 in R1_{AQS+}) compared to the microaerated systems (0.46 ± 0.08 in R2_{AQS-} and 0.50 ± 0.11 in R2_{AQS+}).

This apparent contradiction can be explained by the fact that while oxygen presence reduced the methane percentage in the biogas, it did not reduce total methane generation relative to organic load. On the contrary, microaeration likely stimulated faster and more complete substrate degradation, leading to greater methane volumes per gram of COD applied, especially in R2_{AQS+}, where the AQS may have facilitated redox reactions essential for the breakdown of intermediate products. Similar effects have been reported in anaerobic reactors treating antibiotics and aromatic compounds using AQS and AQDS, where the redox mediator enhanced electron transfer and accelerated rate-limiting steps (Buakaew; Ratanatamskul, 2024; Dos Santos; Cervantes; Van Lier, 2007).

Furthermore, these results suggest that although a portion of the COD may have been consumed via oxidative pathways or biomass synthesis under microaerobic conditions, a substantial fraction still followed the methanogenic route, particularly in the presence of AQS. The performance of R2_{AQS+} thus highlights the potential of combining microaeration and redox mediation to achieve both high removal efficiency and energy recovery in UASB systems.

Finally, the greater consistency observed in the boxplots for both COD removal and methane yield in R2_{AQS+} supports the reproducibility of this combined strategy. Compared to R1_{AQS-} and R1_{AQS+}, the lower variability in R2_{AQS+} may indicate a more stable and resilient microbial community, better adapted to simultaneous oxygen

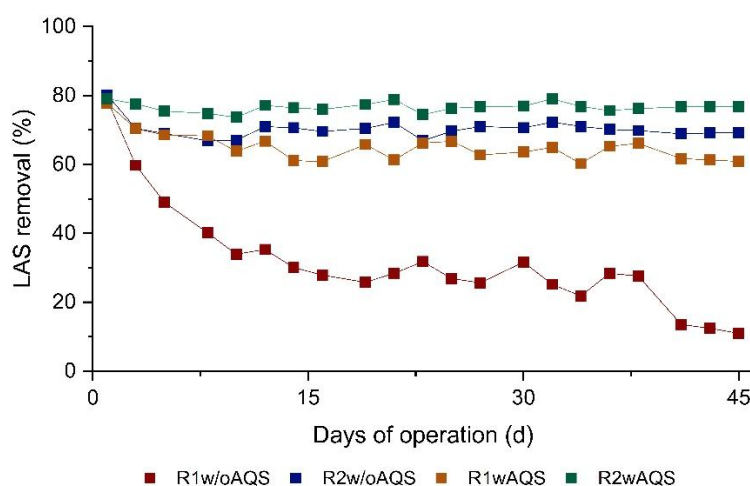
exposure and redox cycling – an aspect further explored in Section 5.3.

5.3. Removal of Linear alkylbenzene sulfonate

5.3.1. Effect of microaeration

In the first days of operation, the four reactors showed average LAS removal efficiencies of around $79 \pm 1\%$, with no statistically significant differences between the systems ($p > 0.05$) (Figure 27). This initial behavior can be attributed to the greater functional diversity of the newly established anaerobic microbiota, as populations are not yet under the selective pressure of sulfonated compounds. At this stage, the lower accumulation of toxic intermediates and the absence of prolonged stress are likely to favor greater activity of consortia capable of degrading xenobiotic compounds such as LAS, even if only partially.

Figure 25 - Temporal variation of LAS removal efficiencies across the experimental phases for reactors



Source: Prepared by the author.

In addition, LAS concentrations had not yet reached critical inhibition levels, which may have allowed secondary or nonspecific metabolic pathways to act in its initial transformation. Among these mechanisms, the action of nonspecific oxidoreductases stands out, which, according to Costa et al. (2024) and Zhou et al. (2022a), can actively participate in the biodegradation of surfactants such as LAS by promoting oxidation and reduction reactions that are not very selective but effective in initially breaking the aromatic structure and aliphatic chain of these compounds. Similarly, Li et al. (2021) observed that UASB reactors showed high efficiency in removing sodium

dodecylbenzene sulfonate (a type of LAS) during the first days of operation, with initial values around 80%, which decreased to less than 20% after 120 days.

Throughout the operation, reactors not subjected to microaeration ($R_{1\text{AQS-}}$ and $R_{1\text{AQS+}}$) exhibited greater operational instability in LAS removal, with more pronounced fluctuations in degradation efficiency and a progressive downward trend, especially in the system without AQS ($R_{1\text{AQS-}}$). $R_{1\text{AQS-}}$ showed the worst performance in LAS removal, with a continuous downward trend until reaching final values close to 11%. This instability may be related to the metabolic limitation imposed by a strictly anaerobic environment in the face of highly recalcitrant compounds, such as LAS. Under these conditions, the degradation process might be limited to primary biodegradation (alkyl chain oxidation), potentially resulting in the accumulation of recalcitrant intermediates such as sulfophenylcarboxylates (SPCs) due to the metabolic difficulty of aromatic ring cleavage under strict methanogenic conditions (Corada-Fernández; González-Mazo; Lara-Martín, 2018). The lack of microaeration also compromises the development of niches for facultative or microaerophilic microorganisms (Castro; Azevedo; Souza, 2023; Do Nascimento et al., 2021a), which could complement surfactant degradation through partial oxidative processes, thereby contributing to greater system stability.

The systems subjected to microaeration showed significantly higher and more stable LAS removals over time than reactors operated under strictly anaerobic conditions ($p < 0.001$). In particular, the $R_{2\text{AQS-}}$ reactor, microaerated and without a redox mediator, achieved an average removal of $70 \pm 3\%$, while the $R_{1\text{AQS-}}$ reactor, operated without microaeration and without AQS, showed lower removal, with an average of $32 \pm 16\%$. These results confirm that the presence of oxygen, even at very low concentrations, is decisive for the degradation of surfactants such as LAS, corroborating the literature that points to the aerobic pathway as the main transformation route of this compound (Costa et al., 2024).

The controlled introduction of oxygen into the system favors the activity of strict and facultative aerobic microorganisms, which can express oxygenases, especially monooxygenases, that catalyze the initial hydroxylation of the aromatic ring of LAS (Fu et al., 2023a; Xu et al., 2023). This reaction transforms the original compound into more hydrophilic and reactive derivatives, paving the way for the cleavage of the aromatic ring and, subsequently, for the complete or partial mineralization of the molecule. According to (Zhou et al., 2022a), monooxygenation requires molecular oxygen as a terminal electron acceptor, which explains the significant improvement in LAS removal under

microaeration.

Furthermore, the partial oxidation promoted under these conditions yields less toxic, more biodegradable intermediates, thereby reducing the inhibitory impact of LAS on biomass.

The introduction of microaeration into anaerobic systems has been reported to enhance the biodegradation of surfactants and other difficult-to-biodegrade compounds. (Cheng et al., 2018) reduced the surfactant concentration from $9000 \text{ mg} \cdot \text{L}^{-1}$ to $2000 \text{ mg} \cdot \text{L}^{-1}$ without foam formation or significant VFAs mineralization. (Carvalho et al., 2020) achieved complete removal of the azo dye Direct Black 22 with microaeration in the UASB reactor.

The action of microaerobic pathways also contributes to maintaining a more dynamic redox environment, in which fermentative, reducing, and oxidative processes coexist, increasing metabolic diversity and, consequently, the system's resilience in the presence of recalcitrant compounds (Fu et al., 2023a; Nascimento et al., 2021). Therefore, microaeration promotes not only greater efficiency but also greater stability in LAS removal, as evidenced by the low variability in results obtained in $R2_{\text{AQS-}}$.

5.3.2. Impact of anthraquinone-2-sulfonate (AQS) supplementation

The addition of the redox mediator anthraquinone-2-sulfonate (AQS) resulted in significant increases in LAS removal in both systems, demonstrating the fundamental role of this compound in enhancing electron transfer and overcoming limiting steps in LAS biodegradation. Despite this, the microaerated reactor with AQS ($R2_{\text{AQS+}}$) showed the best overall performance, with an average removal of $77 \pm 1\%$, significantly higher than that observed in the reactor also doped with AQS, but operated without microaeration ($R1_{\text{AQS+}}$), which obtained $65 \pm 4\%$ ($p < 0.001$).

AQS functions as an extracellular redox mediator, promoting electron transfer between microorganisms and substrates and yielding very satisfactory results in systems treating highly recalcitrant compounds, such as LAS (Ren et al., 2022; Song et al., 2020). Extending its application beyond surfactants, Li et al. (2022) reported that the use of AQS as a redox mediator enabled the removal of approximately 99% of antimony (Sb) from sulfur based industrial wastewater. The beneficial effect of quinone-based redox mediators is not restricted to inorganic or surfactant pollutants. For instance, Nascimento et al. (2021) observed an increase in antibiotic removal from 6% to 75% upon the addition of such mediators. In line with these findings, Tang et al. (2024) also reported an

improvement in the denitrification rate in the presence of quinone-type redox mediators.

In this context, AQS is highly stable and can alternate between its oxidized (quinone) and reduced (hydroquinone) forms, facilitating redox reactions in conditions where microorganisms have limited direct electron transfer (Ren et al., 2022).

This mechanism is particularly relevant when evaluating the synergistic effect of the combination of microaeration and AQS, as in reactor R2_{AQS+}, which enhanced the beneficial effects of both stimuli. The simultaneous presence of low-concentration oxygen and the redox mediator may have created a more versatile redox environment, enabling both oxygen-dependent enzymes (such as monooxygenases) and reactions mediated by extracellular redox compounds to occur in an integrated manner. This synergy may have favored the activation of parallel or complementary metabolic pathways, promoting more efficient degradation of LAS and avoiding the accumulation of toxic or refractory intermediates.

Although AQS promoted significant gains in LAS removal in both systems, its effect was more pronounced in the conventional UASB reactor operated without microaeration. In R1_{AQS+}, the presence of the mediator resulted in a removal efficiency about twice that of the control reactor without AQS (R1_{AQS-}), increasing the average from $32 \pm 16\%$ to $65 \pm 4\%$. On the other hand, in the system with microaeration (R2_{AQS+}), the increase provided by the AQS was proportionally smaller, reaching only about 10% compared to the microaerated reactor without mediator (R2_{AQS-}), which achieved $70 \pm 3\%$. These results indicate that although the AQS significantly intensified LAS biodegradation, especially under strictly anaerobic conditions, it was not sufficient to match the removal levels achieved by systems with oxygen supply ($p=0.017$).

The difference observed between the systems reinforces the limitation imposed by the absence of oxygen in strictly anaerobic environments, which restricts the action of oxidative pathways essential for LAS degradation. Although the AQS may have increased electron transfer and promoted partial transformation of the molecule, the opening of the aromatic ring and desulfonation, which are key steps in its mineralization, remain strongly dependent on reactions that require molecular oxygen. Thus, even with the addition of the mediator, the performance of the R1_{AQS+} reactor did not match that of the microaerated system without AQS (R2_{AQS-}), whose greater LAS removal highlights the central role of microaeration in enabling more effective biochemical pathways for recalcitrant compounds such as sulfonated surfactants.

Do Nascimento et al. (2021b) also observed significant gains in antibiotic removal

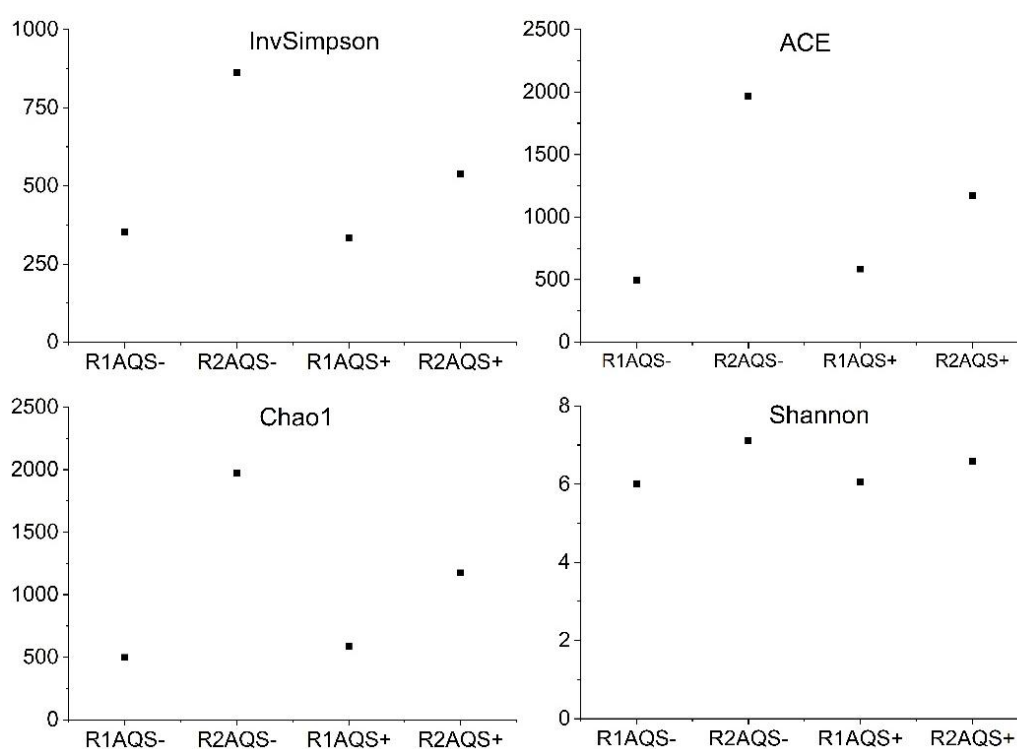
when combining AQS with microaeration in UASB reactors. However, the authors point out that, from a technical standpoint, diluting biogas and the associated costs may not justify its use.

In summary, the absence of microaeration prevented the formation of intermediate redox zones, which could facilitate the partial oxidation or desulfonation of LAS. Even with the presence of AQS, which favored mediated electron-transfer pathways, essential for accelerating the rate-limiting steps of biodegradation, the absence of microaeration prevented the formation of intermediate redox zones. Therefore, although AQS represents an important technological resource for increasing the efficiency of anaerobic reactors, its effect is limited when isolated from a more favorable redox environment. The persistence of LAS in anaerobic systems, even in the presence of redox mediators, underscores the need for integrated strategies, such as combining microaeration with AQS addition, to treat recalcitrant anionic surfactants in UASB reactors efficiently.

5.4. Microbial community

5.4.1. Microbial community richness and diversity

Microbial richness and diversity were assessed using four indices: Chao1 and ACE (estimators of species richness), and Shannon and inverse Simpson (diversity indices accounting for richness and evenness). As shown in Figure 28, substantial differences were observed among the treatments.

Figure 26 – Ecological indices of richness and diversity.

Source: Prepared by the author.

Reactors R2_{AQS-} and R2_{AQS+}, which received microaeration (with and without AQS), exhibited markedly higher species richness than their strictly anaerobic counterparts (R1_{AQS} and R1_{AQS+}). The Chao1 index reached 1969 in R2_{AQS-} and 1174 in R2_{AQS+}, compared to only 498 and 586 in R1_{AQS-} and R1_{AQS+}, respectively. A similar trend was observed in the ACE index, reinforcing the finding that oxygen availability increased the number of detectable taxa. These results align with previous studies suggesting that microaeration promotes the coexistence of both facultative and obligate anaerobes, expanding ecological niches within anaerobic environments (Buarque et al., 2019; Do Nascimento et al., 2021b).

The application of AQS alone (R1_{AQS+}) slightly increased the richness values, with Chao1 and ACE slightly higher than those of R1_{AQS-}. A similar behavior was observed by (Li et al., 2022). This suggests that, under strictly anaerobic conditions, the addition of AQS at the tested concentration (50 μ M) was insufficient to promote a more diverse microbial community. (De Barros et al., 2018) analyzed the impact of microaeration and AQDS on azo dye reduction and observed a reduction in microbial richness and diversity when the redox mediator was added to the treatment.

Regarding microbial diversity, while Shannon indices showed similar patterns,

the InvSimpson values were consistently higher under microaeration. For reactor R2, microbial diversity was higher without AQS (Shannon = 7; InvSimpson = 861) than with AQS (Shannon = 7; InvSimpson = 537). In contrast, reactor R1 showed lower diversity in both conditions (R1_{AQS-}: Shannon = 6; InvSimpson = 351; R1_{AQS+}: Shannon = 6; InvSimpson = 334). These findings indicate that microaeration not only increased richness but also contributed to a more even distribution of taxa, reducing the dominance of a few specialized groups and favoring more balanced microbial communities.

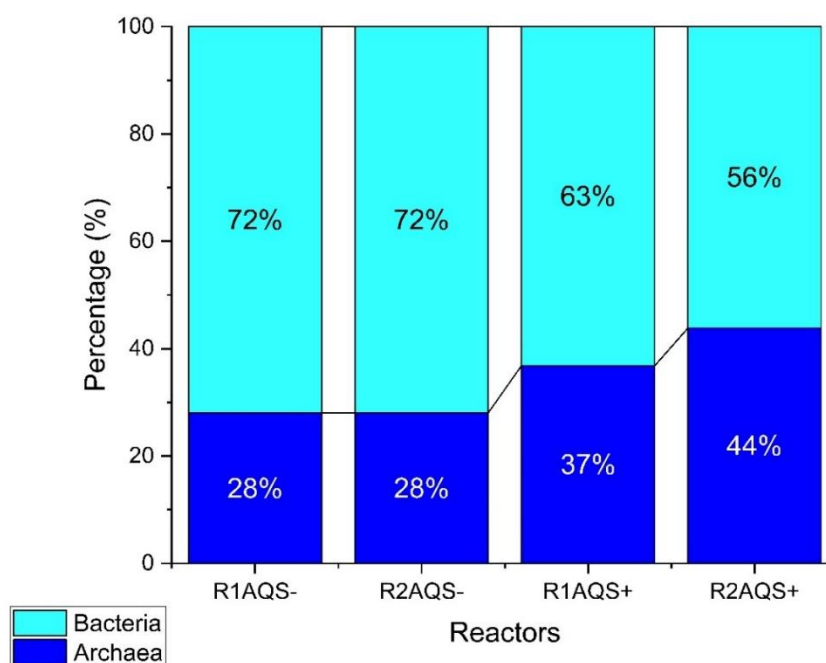
This may demonstrate that the greater removal of LAS in the presence of AQS is not primarily linked to microbiological adaptation, but thanks to a greater ease of electron exchange between the substrate and the contaminant, as already mentioned in other studies using quinones for the biodegradation of sulfonated compounds (Do Nascimento et al., 2021b; Li et al., 2022)

5.4.2. Microbial community structure

The microbial community analysis revealed a predominance of Bacteria over Archaea in all reactors, with the highest bacterial proportions observed in R1_{AQS-} (72.0%) and R2_{AQS-} (72.0%) (Figure 29). These results are typical of anaerobic systems, where Bacteria dominate the initial degradation stages, including hydrolysis and acidogenesis, while Archaea are primarily responsible for methanogenesis. The similar proportions in these two reactors suggest that microaeration alone did not significantly impact domain-level community structure.

In contrast, reactors R1_{AQS+} and R2_{AQS+} (operated with AQS) exhibited a marked increase in archaeal abundance, reaching 36.9% and 43.9%, respectively. This enrichment may reflect a favorable environment for methanogenic Archaea, facilitated by improved electron-transfer conditions provided by the redox mediator. also observed a higher prevalence of Archaea than Bacteria when the AQDS was used.

Taxonomic analyses revealed distinct microbial communities across treatments, reflecting the effects of AQS addition and microaeration on community structure (Figure 30). At the phylum level, *Firmicutes* and *Actinobacteriota* were predominant in R1_{AQS-}, while *Euryarchaeota* dominated in R2_{AQS-}, R1_{AQS+}, and especially R2_{AQS+}, reaching 40.0%. This trend suggests stimulation of archaeal populations in the presence of AQS, particularly under combined microaerobic conditions (R2_{AQS+}), consistent with the higher archaeal proportions observed at the domain level.

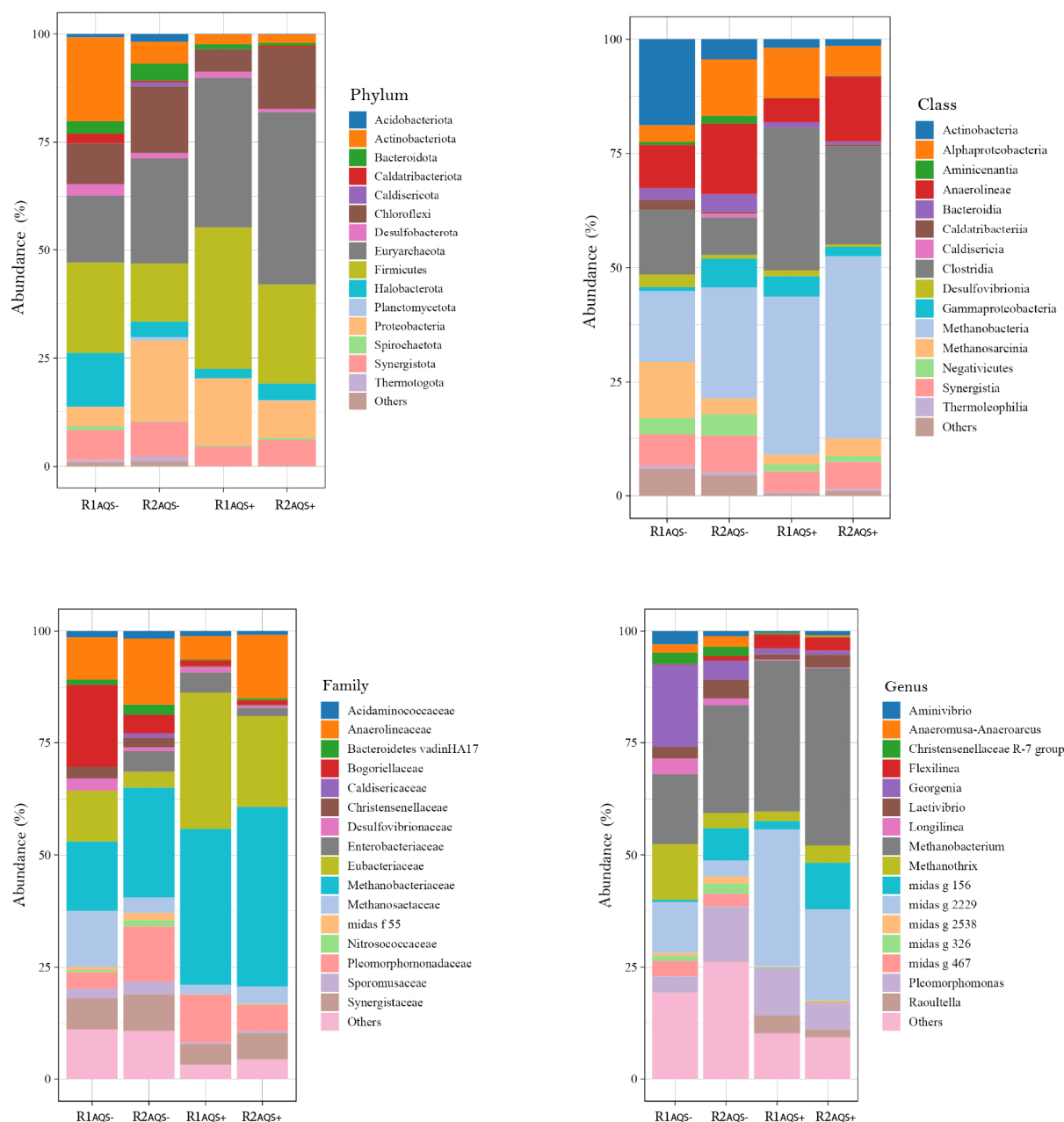
Figure 27 – Taxonomic distribution of DNA sequence.

Source: Prepared by the author.

The enrichment of *Firmicutes* (e.g., *Clostridia*) and *Actinobacteriota* in R1_{AQS+} and R2_{AQS+} supports the hypothesis that fermentative and syntrophic bacteria were stimulated by AQS addition. These groups are known to degrade aromatic and sulfonated compounds under anaerobic conditions, particularly when aided by redox mediators (Do Nascimento et al., 2021b; Van der Zee; Cervantes, 2009). Specifically, *Firmicutes* may participate in the initial breakdown of LAS or its intermediate products, while *Actinobacteria* may contribute to surfactant adsorption and partial transformation.

At the class level, *Methanobacteria*, a strictly methanogenic group, increased progressively from R1_{AQS-} (15.5%) to R2_{AQS+} (40.0%). R1_{AQS-} was uniquely characterized by the presence of *Actinobacteria* (18.8%), a class associated with organic matter hydrolysis. In contrast, *Clostridia* and *Anaerolinea*, typical of syntrophic and fermentative metabolism, were more prominent in R1_{AQS+} and R2_{AQS+}, suggesting enhanced electron transfer and degradation of complex compounds in reactors exposed to AQS and/or microaeration.

Figure 28 – Variation of bacterial community structure at the phylum, class, family, and genus level.



Source: Prepared by the author.

At the family level, *Methanobacteriaceae* was the dominant archaeal group across all reactors, with the highest relative abundance in R2AQ5+ (40.0%). This was followed by *Eubacteriaceae* and *Anaerolineaceae*, families involved in syntrophic acetate oxidation and carbohydrate fermentation. Notably, *Bogoriellaceae*, which is dominant only in R1AQ5- (18.4%), is typically found in aerobic or facultative environments, suggesting a residual influence from the initial inoculum or selective pressure due to LAS in anaerobic

conditions.

At the genus level, *Methanobacterium*, a hydrogenotrophic methanogen, showed a marked increase in reactors with AQS (R1_{AQS+} and R2_{AQS+}), confirming its sensitivity to enhanced electron transfer conditions. These hydrogenotrophic methanogens play a critical role in syntrophic associations by consuming H₂ produced during the fermentation of complex organics, thereby maintaining favorable redox conditions for the degradation of recalcitrant compounds (Li et al., 2022). Their high abundance likely facilitated the regeneration of reduced AQS (AH₂QS), enabling continuous redox cycling and enhancing LAS biodegradation via electron-shuttling mechanisms. The presence of *Methanothrix* (formerly *Methanosaeta*) in R1 also suggests the coexistence of acetoclastic methanogenesis pathways, albeit with lower impact on LAS transformation.

The dominance of *midas* g_2229 in R1_{AQS+} and R2_{AQS+}, a yet-uncultured taxon affiliated with *Eubacteriaceae*, suggests its potential role in LAS degradation or syntrophic interactions. The genus *Georgenia*, which, although potentially involved in organic matter degradation, does not appear to contribute directly to LAS transformation pathways. This genus was prominent in R1_{AQS-} but absent in other treatments, reflecting a more oxygen sensitive or less competitive population under altered redox conditions. The detection of *Anaerolineaceae* and *midas* g_2229 (an unclassified syntrophic lineage) in microaeration treated reactors without AQS supplementation indicates a more robust syntrophic network. These taxa are typically involved in the degradation of long-chain hydrocarbons and surfactants in anaerobic systems and may have worked synergistically with methanogens to mineralize LAS or its transformation intermediates (Motteran; Varesche; Lara-Martin, 2022). The balance between fermentative bacteria and hydrogenotrophic methanogens likely ensured efficient electron flow, reduced accumulation of inhibitory intermediates, and improved process stability.

Overall, these taxonomic shifts highlight the selective enrichment of syntrophic and methanogenic groups in response to AQS and microaeration, particularly in R2_{AQS+}, where metabolic cooperation and archaeal dominance were most evident. This microbial configuration likely contributed to the superior process performance observed in terms of LAS removal, methane production, and COD degradation. Altogether, the microbial community structure of R1_{AQS+} and R2_{AQS+} suggests a synergistic interaction between fermentative bacteria, syntrophs, and hydrogenotrophic methanogens, mediated by AQS and enhanced by microaeration in R2_{AQS+}. These interactions created favorable conditions for both LAS removal and methane production, explaining the superior performance of

these reactors compared to the controls.

5.5. Impact of anthraquinone-2-sulfonate (AQS) supplementation and microaeration

The enhanced LAS removal in the AQS-amended reactors ($R1_{AQS+}$ and $R2_{AQS+}$) is consistent with the role of quinone-based redox mediators in accelerating the transformation of recalcitrant contaminants. In practical terms, introducing AQS creates an electron shuttling pathway that complements microbial metabolism and promotes the breakdown of surfactants such as LAS, as has already been reported for pharmaceuticals, dyes, and other personal-care-related compounds. When microaeration is added ($R2_{AQS+}$), this effect becomes even more relevant from a technological standpoint, as low, well-controlled oxygen inputs not only prevent methanogenesis inhibition but also support enzymatic activation steps that require minimal oxygen. This microaerobic window can simultaneously stimulate the oxidative activation of aromatic moieties from LAS and enhance the subsequent anaerobic reductive steps mediated by AQS. The result is a process capable of both improving LAS degradation and enabling partial biogas purification by oxidizing trace reduced sulfur compounds, while also attenuating volatile compounds associated with personal-care products.

From an operational perspective, however, microaeration demands strict control. Oxygen must be dosed at levels sufficient for enzyme-level activation but low enough to avoid its escape to the headspace, where it would compromise biogas combustion safety by altering the flammability range. Maintaining this balance is challenging because real-time dissolved oxygen monitoring in anaerobic systems remains unreliable, and commercial DO sensors often exhibit poor sensitivity at the microaerobic range required for mediators and surfactant transformation. Thus, control strategies based on the system's redox potential become particularly attractive. Because AQS actively participates in redox cycling, monitoring the reactor's redox potential provides a more mechanistic and responsive basis for adjusting aeration, avoiding oxygen accumulation, and optimizing mediator performance.

The regeneration of the redox mediator (AQS) is a critical step for maintaining its catalytic efficiency. In the microaerated reactor ($R2$), the residual oxygen acts as the primary electron acceptor for the reoxidation of anthrahydroquinone (AH_2QS) back to AQS, a process that can occur both abiotically or mediated by facultative microorganisms. In contrast, under strictly anaerobic conditions ($R1$), the reoxidation of

the mediator likely relies on alternative electron acceptors present in the matrix, such as sulfate. The synergistic effect observed between microaeration and AQS suggests that oxygen facilitates a faster turnover of the redox cycle, preventing electron transfer limitations that are more prevalent in purely methanogenic environments (Do Nascimento et al., 2021b; Van der Zee; Cervantes, 2009).

Despite these advantages, the continuous addition of soluble AQS increases operational costs and generates concerns for downstream treatment, especially if residual mediator exits with the effluent. For full-scale applications, this limitation has driven interest in immobilized mediator systems. Activated carbon, biochar, and engineered nanomaterials containing quinone-rich functional groups can retain the mediator within the reactor, provide long-term electron-shuttling capacity, and substantially reduce the demand for repeated dosing (Zhang et al., 2020). Such materials also align with practical anaerobic treatment constraints by simplifying operation and lowering chemical consumption while preserving the enhanced LAS degradation observed in the present study.

Overall, integrating AQS with microaeration offers a promising, operationally relevant approach to improving the removal of LAS and other personal-care-related pollutants while simultaneously enhancing biogas quality. The feasibility of this strategy, however, will depend on advances in microaeration control, redox-based monitoring, and cost-effective immobilization of redox mediators for long-term application.

5.6. Conclusion

This study demonstrated that the use of anthraquinone-2-sulfonate (AQS) as a redox mediator, both alone and in combination with microaeration, can enhance the performance of UASB reactors treating LAS-containing wastewater and treated at low HRTs. While the application of AQS under strictly anaerobic conditions did not significantly alter reactor stability or organic matter removal, it promoted the enrichment of methanogenic Archaea and selected syntrophic bacterial groups, suggesting the development of improved redox driven biodegradation pathways.

The combination of AQS and microaeration (R2AQS+) yielded the highest LAS removal, COD degradation, and methane production per unit of applied organic load, indicating a synergistic effect between redox mediation and controlled oxygenation. This condition also promoted a more diverse and archaeal-enriched microbial community, characterized by the dominance of *Methanobacterium* and *midas_g_2229*, potentially

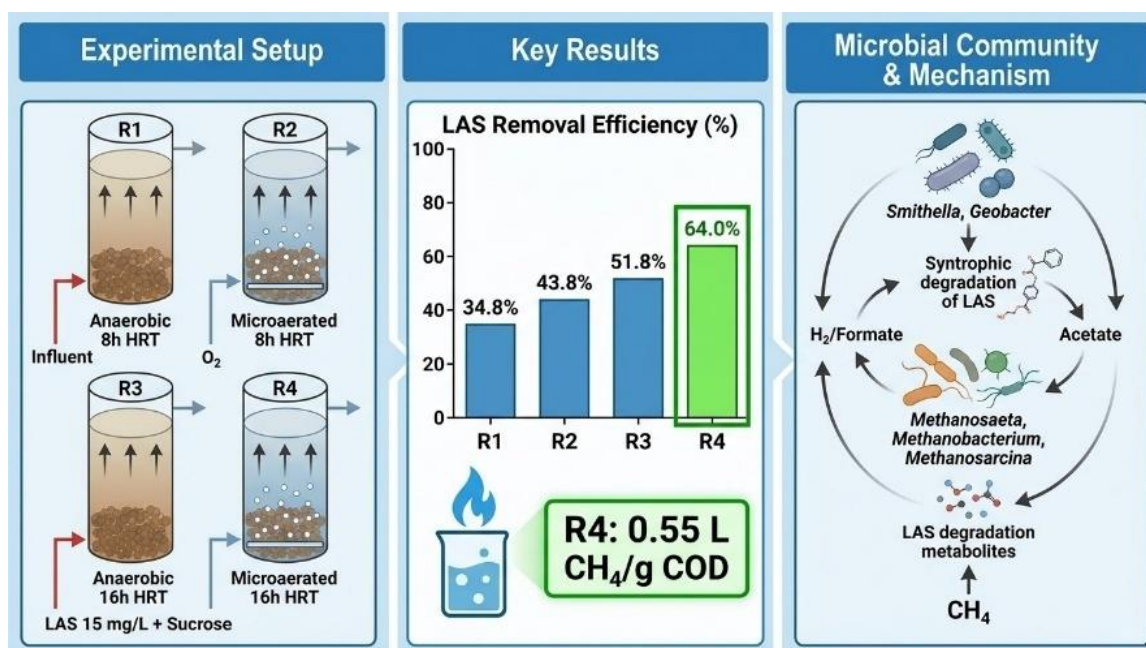
associated with enhanced LAS degradation under syntrophic conditions.

These findings highlight the potential of integrating redox-active compounds and low-intensity aeration as a viable strategy to improve anaerobic treatment of surfactant-rich wastewaters. Future studies should investigate the long-term stability of such systems, assess the fate of intermediate metabolites, and explore the functional roles of key microbial taxa identified, particularly through metagenomic or transcriptomic approaches.

The full version of this paper is under review in Bioresource Technology (2026) under the same name as this subsection.

6. CAN MICROAERATION AND HYDRAULIC RETENTION TIME STEER ANAEROBIC MICROBIOMES TOWARD EFFICIENT LAS DEGRADATION?

This study investigated the synergy between Hydraulic Retention Time (HRT) and controlled microaeration as a strategy to intensify LAS degradation and energy recovery in UASB reactors. Four configurations were evaluated: R1 (anaerobic, 8-h HRT), R2 (microaerated, 8-h HRT), R3 (anaerobic, 16-h HRT), and R4 (microaerated, 16-h HRT) (Figure 31). Results demonstrated that while doubling the HRT from 8 to 16 h improved COD removal from 65.7% to 87.4%, microaeration was the decisive driver for surfactant biotransformation. R4 achieved the highest LAS removal ($64.0 \pm 6.2\%$), significantly outperforming the strictly anaerobic R3 ($51.8 \pm 7.7\%$). Mechanistically, the 16-h HRT provided a critical "kinetic window" for oxygen-dependent enzymes (mono- and dioxygenases) to catalyze ω -oxidation and aromatic ring cleavage, avoiding the accumulation of toxic intermediates observed at shorter HRTs. Notably, R4 exhibited a remarkable specific methane yield of $0.55 \pm 0.06 \text{ L}_{\text{CH}_4} \cdot \text{gCOD}_{\text{app}}^{-1}$, doubling the efficiency of the other reactors. Microbial analysis confirmed that microaeration expanded redox niches, fostering a diverse consortium where oxidative bacteria (e.g., *Smithella*, *Thauera*) and acetoclastic methanogens (*Methanosaeta*) coexisted, in addition to providing other pathways for degradation.. These findings indicate that microaeration, coupled with optimized HRT, effectively overcomes the thermodynamic and kinetic barriers of LAS degradation, positioning it as a robust tool for process intensification in wastewater treatment plants.

Figure 29 – Graphical abstract of OS IV.

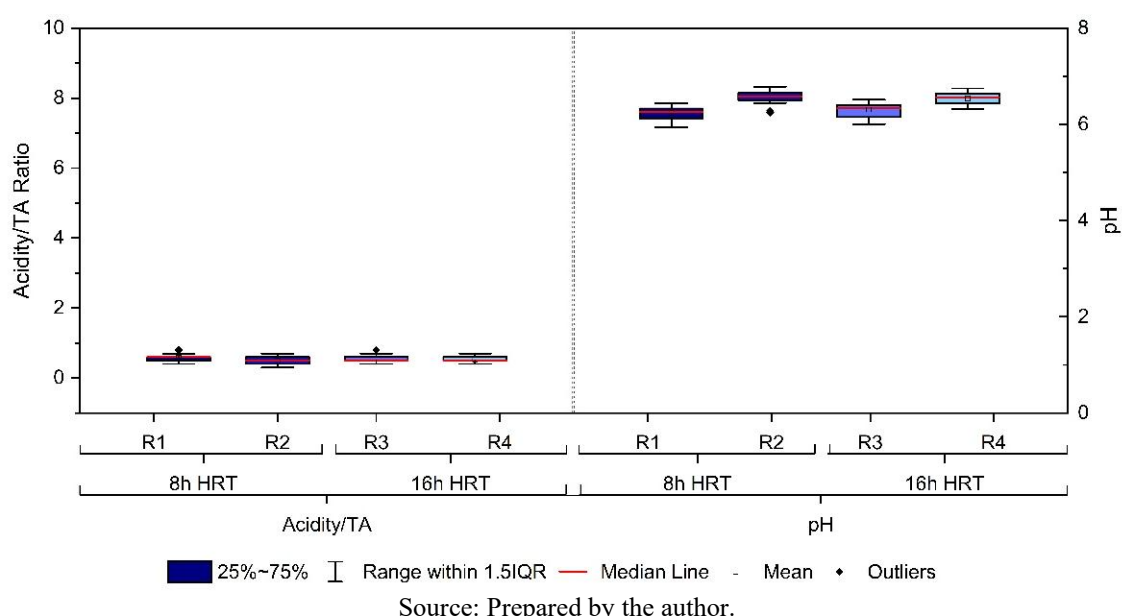
Source: Prepared by the author.

6.1. Performance of the UASB reactors

6.1.1. Operational parameters and volumetric biogas production

The monitoring of pH and Acidity/TA ratios showed no statistically significant differences between the reactors ($p > 0.05$), indicating that the system maintained a stable buffering capacity regardless of the HRT or the redox environment (Figure 32). All reactors operated within an alkaline pH range (7.6–8.0), which is optimal for methanogenic activity.

The Acidity/TA ratios remained consistently above the 0.3 threshold (0.5–0.6) across all treatments. This absence of statistical variance suggests that the accumulation of volatile fatty acids (VFAs) was a common characteristic of the influent (sucrose + LAS) rather than a result of operational changes. The stability of these parameters proves that neither the high organic load in the 8h HRT nor the oxygen supply in the microaerated systems (R2 and R4) compromised the global metabolic balance, allowing the subsequent evaluation of LAS removal under a stabilized anaerobic-microaerophilic environment.

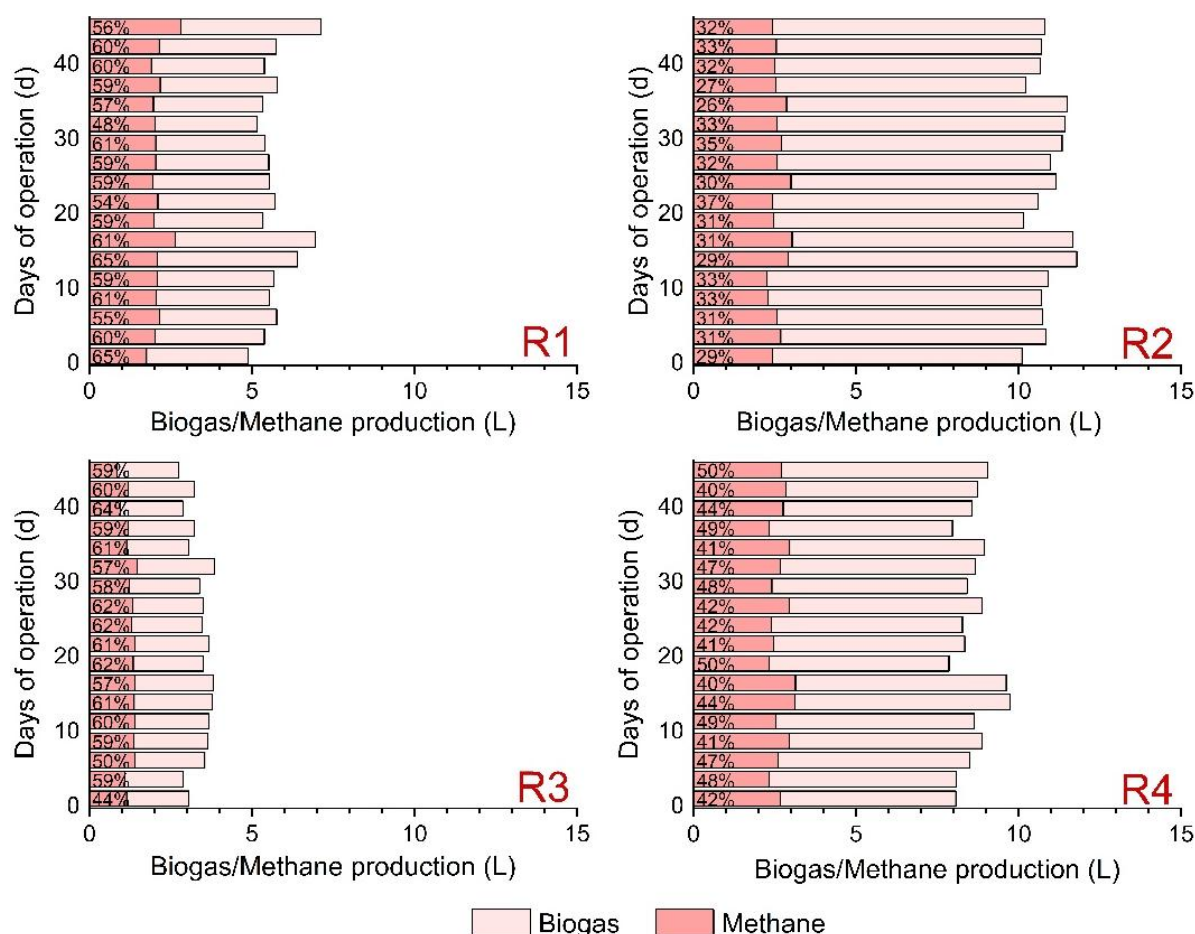
Figure 30 – Box plot of pH and acidity/alkalinity ratio.

The biogas dynamics reflected the expected trade-off between organic loading and the redox environment (Figure 33). In strictly anaerobic reactors, the 8h HRT (R1) yielded a higher volumetric methane production ($3.6 \pm 0.4 \text{ L} \cdot \text{d}^{-1}$) compared to the 16h HRT (R3 = $2.1 \pm 0.2 \text{ L} \cdot \text{d}^{-1}$). This result is directly attributed to the higher Organic Loading Rate (OLR) applied at the shorter HRT, which provided a greater total amount of organic matter for methanogenesis per unit of time. Conversely, microaerated systems (R2 and R4) showed significantly higher total biogas volumes (8.6 and $6.0 \text{ L} \cdot \text{d}^{-1}$, respectively), primarily due to the introduction of N_2 and residual O_2 into the gas phase.

HRT was a critical factor for both methane purity and productivity. R4 (16h) achieved $44.6 \pm 3.7\% \text{ CH}_4$, nearly reaching the 45% energy recovery threshold (Cruz-Salomón et al., 2017), whereas R2 (8h) dropped to $31.5 \pm 2.5\%$ due to biogas dilution at shorter HRTs. This gas quality is comparable to that observed by (Delforno et al., 2019a), who treated anionic surfactants in a combined system and noted that the oxygen-biogas interface requires precise HRT control to avoid excessive CO_2 and N_2 enrichment. Despite this, absolute methane productivity in microaerated systems (R2: 2.6 ; R4: $2.7 \text{ L} \cdot \text{d}^{-1}$) significantly exceeded that of the 16h anaerobic control (R3: $1.2 \text{ L} \cdot \text{d}^{-1}$). These results surpass the yields reported by Okada et al. (2013e), who found that anaerobic reactors treating high LAS concentrations (up to $40 \text{ mg} \cdot \text{L}^{-1}$) exhibited significant methanogenic inhibition and lower volumetric production. The higher productivity in R4 suggests that microaeration effectively bypassed the toxic effects of LAS on archaea, a

benefit also highlighted Zhou et al. (2022b) in systems where micro-oxygenation enhanced the mineralization of sulfonated aromatic compounds without compromising the final methane output.

Figure 31 – Biogas and methane production from reactors throughout the experiment.



Source: Prepared by the author.

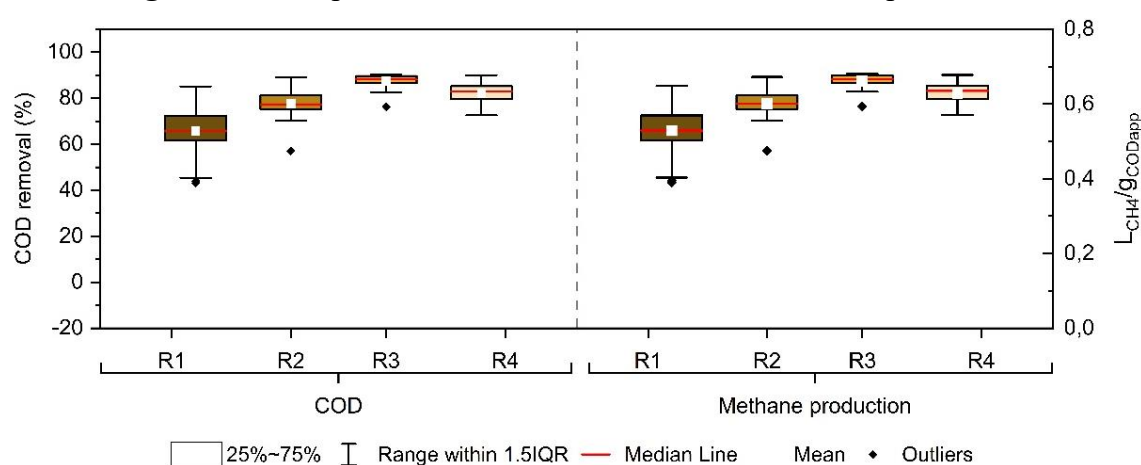
6.1.2. Efficiency Parameters

COD removal efficiency across all experimental systems ranged from $65.7 \pm 12.3\%$ to $87.4 \pm 3.4\%$, indicating that HRT is the primary driver of organic matter stabilization. In both strictly anaerobic and microaerated regimes, doubling the residence time from 8h (R1 and R2) to 16h (R3 and R4) resulted in the highest and most consistent removal rates (Figure 34). This trend indicates that the 8h HRT imposes kinetic limitations on the consortia, likely exacerbated by LAS inhibition, which hinders the full stabilization of the sucrose-based influent regardless of the aeration status.

Microaeration served as an effective oxidative boost, particularly at the shorter HRT. R2 (8h, microaerated) achieved $77.6 \pm 6.8\%$, significantly outperforming its

anaerobic counterpart (R1) ($p < 0.05$). This improvement can be attributed to the stimulation of facultative microorganisms with faster kinetic rates for oxidizing organic intermediates (Nascimento et al., 2021). At 16h, microaeration did not significantly impact the degradation of bulk organic matter, while R4 (82.1%) and R3 (87.4%) had similar removal efficiencies ($p > 0.05$). These results suggest that while a 16h HRT provides sufficient contact time for near-maximal anaerobic stabilization, microaeration is a vital strategy for maintaining system functionality and intensifying the process under higher volumetric loading rates.

Figure 32 – Boxplot of COD removal and relative methane production.



Source: Prepared by the author.

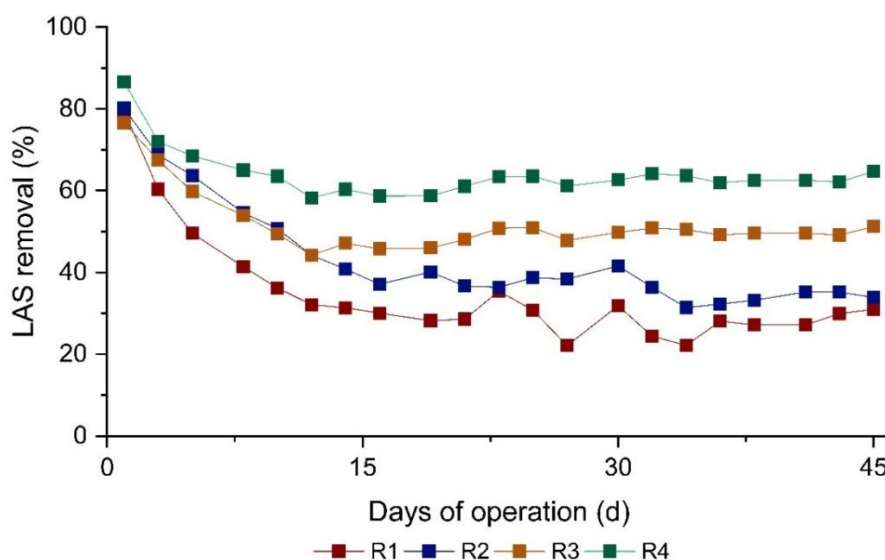
The specific methane yield revealed a significant synergy between microaeration and the 16h HRT. While the strictly anaerobic reactors (R1 and R3) and the 8h microaerated reactor (R2) showed yields between 0.2 and 0.3 $L_{CH_4} \cdot g_{COD_{app}}^{-1}$, R4 (16h, microaerated) achieved a remarkable $0.6 \pm 0.06 L_{CH_4} \cdot g_{COD_{app}}^{-1}$. This performance significantly exceeds the yields reported by Duarte et al. (Duarte et al., 2010), who observed specific methane yields around 0.2 $L_{CH_4} \cdot g_{COD_{app}}^{-1}$ in UASB reactors treating LAS under strictly anaerobic conditions, where surfactant toxicity limited methanogenic efficiency. The doubling of the methane yield compared to the anaerobic control (R3) demonstrates that controlled oxygenation acts as a process intensifier. This trend is supported by Delforno et al. (2012), who reported that the introduction of oxygen-mediated stages in the treatment of anionic surfactants led to more robust aromatic ring degradation, thereby increasing the availability of acetate for methanogens and boosting specific methane production compared to purely anaerobic benchmarks. By overcoming

the typical kinetic barriers of surfactant-rich wastewater, R4 maximized the conversion of the applied organic load into energy.

The exceptionally high performance in R4 suggests that microaeration accelerates rate-limiting steps by facilitating the hydrolysis of complex compounds and the conversion of long-chain fatty acids into acetic acid (Chen et al., 2020). The 16h HRT provided the necessary kinetic window for the biomass to fully exploit these oxidative-reductive routes, potentially mobilizing organic fractions from LAS degradation that would remain recalcitrant in strictly anaerobic or short-HRT systems. Thus, the "oxidative boost" provided by microaeration does not suppress methanogenesis; instead, it optimizes the electron flow toward methane production, resulting in a more efficient and resilient bioprocess.

6.2. Removal of Linear alkylbenzene sulfonate

The removal of LAS was synergistically governed by the redox environment and the HRT, with efficiencies ranging from 34.8% (R1) to 64.0% (R4) (Figure 35). During the early operational phase, all four reactors achieved average LAS removal efficiencies exceeding 70%, with no statistically significant differences between the systems ($p > 0.05$). This performance is linked to the high functional diversity of the incipient anaerobic microbiota, which had not yet been subjected to the selective pressure of sulfonated compounds. At this stage, the likely minimal accumulation of toxic intermediates, combined with the absence of chronic stress, favored the activity of consortia capable of at least partially degrading xenobiotics. Furthermore, because LAS concentrations remained below inhibitory thresholds, microbial activity was not significantly suppressed, allowing broad and non-specific metabolic pathways to participate in its initial co-metabolic transformation.

Figure 33 – LAS removal during the experiment.

Source: Prepared by the author.

The chemical nature of the pollutant dictates the shift from initial transformation to long-term stability. Specifically, the highly stable sulfonated aromatic ring and the hydrophobic alkyl chain of LAS impose severe thermodynamic constraints under strictly anaerobic conditions (R1 and R3). In the absence of molecular oxygen, benzene ring cleavage is restricted to the multi-step benzoyl-CoA pathway. This slow reductive route is frequently hindered by the molecule's inherent toxicity (Fu et al., 2023), thus limiting the overall rate of organic matter stabilization.

In strictly anaerobic systems, doubling the HRT from 8h (R1) to 16h (R3) increased LAS removal from 34.8% to 51.8%. This performance gain confirms that the structural recalcitrance of the sulfonated aromatic group requires an extended 'kinetic window' to accommodate the slow metabolic adaptation of anaerobic consortia (Corada-Fernández; González-Mazo; Lara-Martín, 2018). The limitation observed at 8h aligns with the findings of (Carneiro et al., 2024), who reported that short residence times (e.g., 6h) in anaerobic stages are often insufficient for the effective biotransformation of complex aromatic rings, resulting in negligible removal of recalcitrant micropollutants. In these systems, cleavage of the benzene ring under strictly reductive conditions is the rate-limiting step. Therefore, the 16h HRT likely provided the minimum contact time required for enzymatic attack on the LAS molecule, reducing mass transfer and reaction rate constraints that commonly limit degradation in high-rate anaerobic systems.

The introduction of microaeration induced a fundamental mechanistic shift,

achieving peak efficiency in R4 ($64.0 \pm 6.2\%$). While oxygen availability might have activated monooxygenases and dioxygenases in both microaerated reactors, the 16-h HRT in R4 might have provided the required kinetic window for these enzymes to catalyze the initial hydroxylation of the benzene ring and the terminal carbon of the alkyl chain (ω -oxidation). This oxidative attack reduces the molecule's hydrophobicity, transforming it into sulfophenylcarboxylates (SPCs), which are more hydrophilic and less toxic to methanogens (Liu et al., 2024a; Zhou et al., 2022c). Consequently, the extended residence time in R4 favored higher LAS removal efficiency. Although intermediate metabolites were not directly quantified in this study, previous findings by other authors suggest that under microaerated conditions, oxidative enzymes can initiate LAS transformation into more hydrophilic compounds, such as sulfophenylcarboxylates (Liu et al., 2024a; Zhou et al., 2022c). Therefore, it is plausible that similar mechanisms contributed to the enhanced removal observed in R4, whereas the shorter HRT in R2 may have limited the progression of these oxidative steps.

In contrast, the 8-h HRT reactors (R1 and R2) exhibited significant instability and lower removal efficiencies. In R1, the short HRT likely led to excessive LAS adsorption onto the sludge granules. This surfactant accumulation increases cell membrane permeability and inhibits slow-growing methanogens (Braga et al., 2015). While microaeration in R2 improved removal (43.8%) compared to R1, the 8-h period was insufficient to exploit the oxidative-reductive synergy fully. The "oxidative boost" in R2 was likely limited to partial side-chain oxidation. In contrast, the 16-h HRT in R4 provided the required window for aromatic ring cleavage, which is the rate-limiting step in LAS mineralization (Costa et al., 2024).

Finally, the results demonstrate that, while both strategies improve LAS degradation, their combined effect is synergistic. Extending the HRT from 8 to 16 h proved to be a more significant driver for LAS removal than microaeration alone, as it overcomes the fundamental kinetic limitations of anaerobic consortia. However, the integration of microaeration at a 16-h HRT (R4) yielded the highest performance, intensifying removal beyond the levels achieved by either strategy in isolation. This synergy may be explained by a dual-stage mechanism: the microaerated environment may facilitate the initial, energy-intensive oxidative attack (ring hydroxylation) via oxygenases, while the extended 16-h HRT may ensure that the resulting intermediates do not "wash out". Instead, these metabolites may be effectively integrated into the syntrophic anaerobic matrix for complete mineralization. This configuration optimizes an

“oxidative-reductive” metabolic niche (Do Nascimento et al., 2021a), suggesting that the maximum degradation potential of recalcitrant surfactants may be reached when thermodynamic barriers (overcome by O₂) and kinetic constraints (overcome by HRT) are addressed simultaneously.

6.3. Microbial dynamics during the removal of LAS

6.3.1. Microbial community richness and diversity

The analysis of richness and diversity indices reveals a clear influence of microaeration on microbial community complexity and structural stability within the UASB systems (Table 1).

Table 1 – Valid sequences and alpha-diversity indices of each sample.

Samples	Valid Sequences	Chao1	ACE	Shannon	Inv_Simpson
Inoculum	87014	589.6	591.8	4.1	26.5
R1	72655	396.1	397.4	3.5	13.4
R2	75642	719.6	722.9	4.2	28.3
R3	89748	426.1	427.2	3.6	15.6
R4	76789	533.4	536.2	4.1	30.1

Source: Prepared by the author.

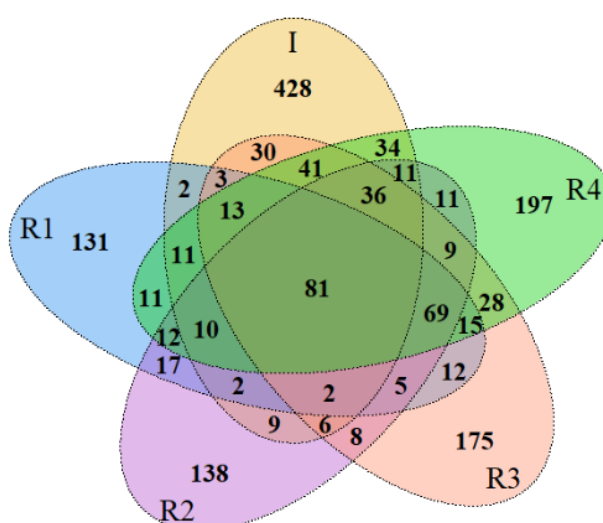
According to Chao1 and ACE indices, reactors operated under microaerated conditions exhibited higher microbial richness than their conventional counterparts, indicating that low oxygen availability promoted the establishment of a broader range of taxa. Similarly, Shannon and Simpson indices demonstrated that microaerated systems sustained higher diversity, reflecting a more even distribution of taxa and reduced dominance effects. This pattern suggests that microaeration alleviated competitive exclusion by creating redox gradients that enabled the coexistence of facultative, strictly anaerobic, and oxygen-tolerant microorganisms, thereby increasing functional redundancy and metabolic versatility.

The inoculum exhibited intermediate richness and diversity, lower than those observed in the microaerated reactors but higher than in the conventional UASB systems. In contrast, its diversity indices were comparable to the microaerated condition, indicating a relatively balanced community. Upon reactor operation, conventional UASB conditions, particularly at short HRT, exerted strong selective pressure, reducing richness and favoring fast-growing fermentative taxa. In contrast, microaeration mitigated this

effect by expanding redox niches and sustaining a broader range of functionally relevant microorganisms, including syntrophic bacteria and methanogens.

This structuring effect is further supported by the Venn diagram analysis, which demonstrates pronounced differentiation among communities (Figure 36). The inoculum contained a large number of unique ASVs, reflecting its heterogeneous origin. At the same time, all reactors developed distinct assemblages with comparable numbers of exclusive ASVs, indicating rapid community reorganization driven by operational conditions. The progressive increase in unique ASVs from R1 to R4 highlights the combined influence of longer HRT and microaeration in expanding ecological niche space and enabling the establishment of specialized populations associated with LAS transformation and methanogenic activity.

Figure 34 – Vennon diagram for each system operated.



Source: Prepared by the author.

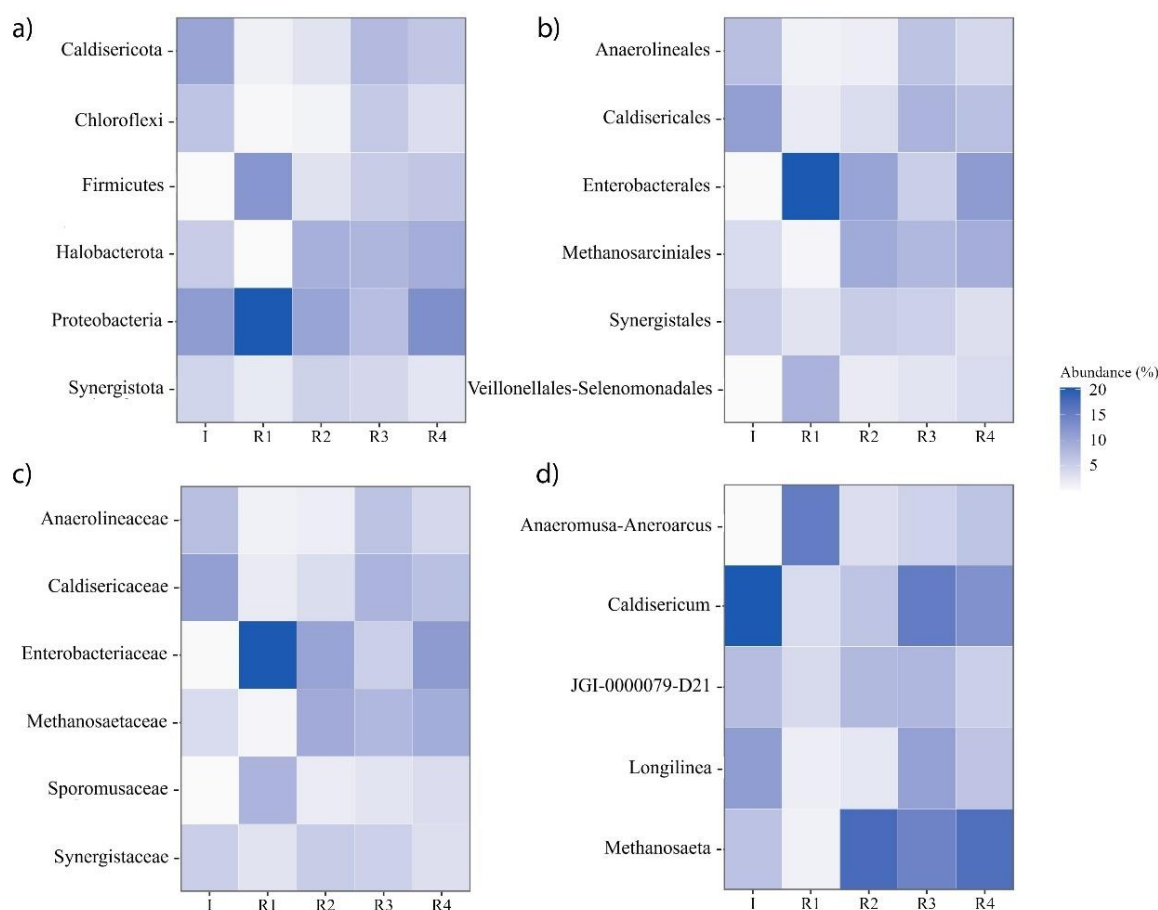
The limited number of ASVs shared among all systems underscores the strong selective pressure imposed by reactor operation. It indicates that LAS degradation depended on reactor-specific microbial assemblies rather than on a conserved core microbiome. In microaerated reactors, a higher proportion of exclusive ASVs reflects greater functional diversification and community resilience, consistent with their superior performance in LAS removal. Overall, these results demonstrate that microaeration, reinforced by longer HRT, promoted a more complex and functionally versatile microbial community, thereby enhancing the system capacity for efficient LAS transformation.

6.3.2. Microbial community structure

The microbial community structure clearly reflected the combined effects of hydraulic retention time and microaeration (Figure 37).

At the phylum level, *Proteobacteria* dominated all systems, which is aligned with their central role in the anaerobic transformation of complex organic compounds, including surfactants, through fermentative and facultative pathways (De Barros et al., 2018; Zhu et al., 2020b). Relative to the inoculum, a reduction in *Caldisericota* and *Chloroflexi* was observed, particularly under the 8-h HRT, indicating that short contact times imposed strong selective pressure against slow-growing syntrophic and filamentous taxa involved in biomass structuring and long-chain organic matter conversion.

Figure 35 – Structure of the microbial community in terms of phylum, order, family, and genus.



Source: Prepared by the author.

Comparison between the conventional UASB reactors operated at 8 h (R1) and 16 h HRT (R3) highlights the role of residence time in shaping metabolic specialization

and syntrophic stability. In R1, enrichment of *Firmicutes* alongside *Proteobacteria* may have favored fast fermentative metabolism, with dominance of *Enterobacterales*, *Veillonellales*, and *Selenomonadales*, and families associated with acidogenesis and the rapid conversion of readily biodegradable substrates into short-chain fatty acids and alcohols (Nascimento et al., 2021; Zígolo; Irazusta; Rajal, 2020).

The stimulation of *Anaeromusa*–*Anaeroarcus*, absent in the inoculum, further reflects selection for rapid fermentative pathways. Although such organisms may contribute to partial LAS transformation via co-metabolism and desulfonation, their metabolic orientation favors rapid carbon turnover rather than complete mineralization or stable syntrophic interactions with methanogens (Costa et al., 2024; Terreros-Mecalco et al., 2024b). This configuration explains the lower LAS removal and moderate methane production in R1, along with inhibition of *Methanosaetaceae*, leading to sustained methane formation via less efficient fermentative and incomplete oxidation pathways.

In contrast, R3 exhibited a community structure indicative of enhanced process stability and syntrophic integration. The persistence of *Caldisericota* and enrichment of *Anaerolineales* reflect improved adaptation to slow hydrolysis, biomass structuring, and interspecies metabolite exchange. At the genus level, the co-occurrence of *Caldisericum*, *Methanosaeta*, and *Longilinea* indicates a tighter integration among fermentative, acetogenic, and acetoclastic methanogenic pathways. The longer HRT reduced selective pressure, allowing *Methanosaetaceae* to establish and sustain activity, which is particularly relevant for LAS degradation, where acetate is produced gradually through complex transformation pathways. Despite lower methane production than in R1, the higher LAS removal in R3 indicates that extended contact time promoted more complete and structured transformation pathways.

Microaeration further intensified community differentiation, particularly under extended HRT. At 8h HRT, R2 exhibited a mixed assemblage dominated by *Proteobacteria* and *Halobacterota*, with coexistence of facultative heterotrophs and metabolically flexible methanogens, including *Methanosarciniales*. This structure suggests that microaeration created redox heterogeneity within the sludge bed, enabling oxidative activation of LAS while sustaining methane production despite short residence time. However, partial inhibition of strictly anaerobic syntrophs indicates that deeper mineralization steps remained constrained under these conditions (Carosia et al., 2014; Do Nascimento et al., 2021c; Luo et al., 2023).

In R4, extending the HRT to 16h under microaeration resulted in a markedly more

favorable configuration. Stable enrichment of *Methanosarciniales* and *Methanosaetaceae*, together with growth of *Anaerolineales*, reflects a balanced stratification in which oxidative activation of LAS was enhanced without disrupting anaerobic syntrophic niches. High abundances of *Methanosaeta* and *Caldisericum* indicate efficient integration between fermentation, acetogenesis, and acetoclastic methanogenesis, explaining the superior LAS removal and highest methane production observed in this reactor.

Overall, comparisons within each HRT confirm that microaeration consistently promoted microbial communities better suited for LAS transformation. At the same time, extended residence time amplified this effect by allowing syntrophic and methanogenic networks to consolidate. Among all configurations, R4 represents the most favorable strategy, where controlled oxygen input and sufficient HRT steered the microbiome toward integrated metabolic pathways that optimized both LAS removal and energy recovery.

6.3.3. Metabolic pathway analysis and functional implications

The distribution of metabolic pathways across the reactors reflected the selective pressures imposed by hydraulic retention time and microaeration and was fully consistent with the microbial community structures previously described (Figures 38 - 41).

In the conventional UASB operated at 8h HRT, the dominance of sucrose metabolism, the TCA pathway, ω -oxidation, and sulfur metabolism indicate a system primarily oriented toward rapid fermentative activity and incomplete oxidation processes.

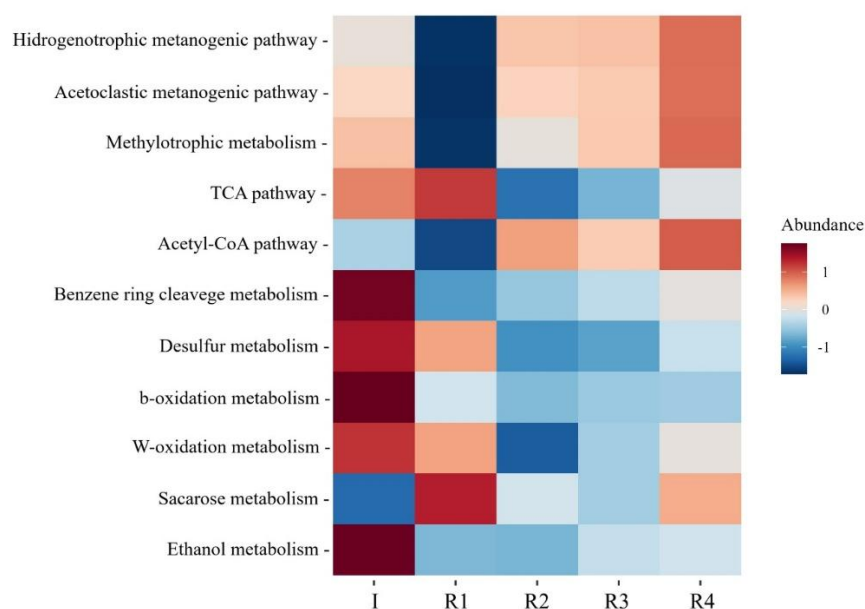
This functional profile is consistent with the enrichment of *Proteobacteria* and *Firmicutes*-related taxa, particularly *Enterobacteriaceae*, *Sporomusaceae*, and *Anaeromusa–Anaeroarcus*, which favor rapid substrate turnover but provide limited metabolic continuity for the complete degradation of complex molecules such as LAS (Carosia et al., 2014).

Although ω -oxidation pathways driven by *Anaeromusa–Anaeroarcus* and *Rhodococcus* enabled initial activation of the alkyl chains, the lack of sustained downstream processing restricted the extent of LAS transformation. In parallel, the strong predicted abundance of sulfur metabolism, mainly associated with *Desulfovibrio* and supported by *Thauera* and *Mycobacterium*, indicates that desulfonation was active but competed with methanogenic processes for reducing equivalents. As a result, LAS removal in R1 remained limited.

In the microaerated UASB operated at 8h HRT, the metabolic profile shifted toward pathways associated with improved energy conservation, with dominance of the Acetyl-CoA pathway and both hydrogenotrophic and acetoclastic methanogenesis. This configuration reflects a more organized metabolic sequence in which fermentative and acetogenic reactions were efficiently aligned with methane formation. *Methanosaeta* emerged as a central genus across all methanogenic routes, supported by *Methanobacterium* and *Methanospirillum*, which together facilitated effective hydrogen consumption and acetate utilization.

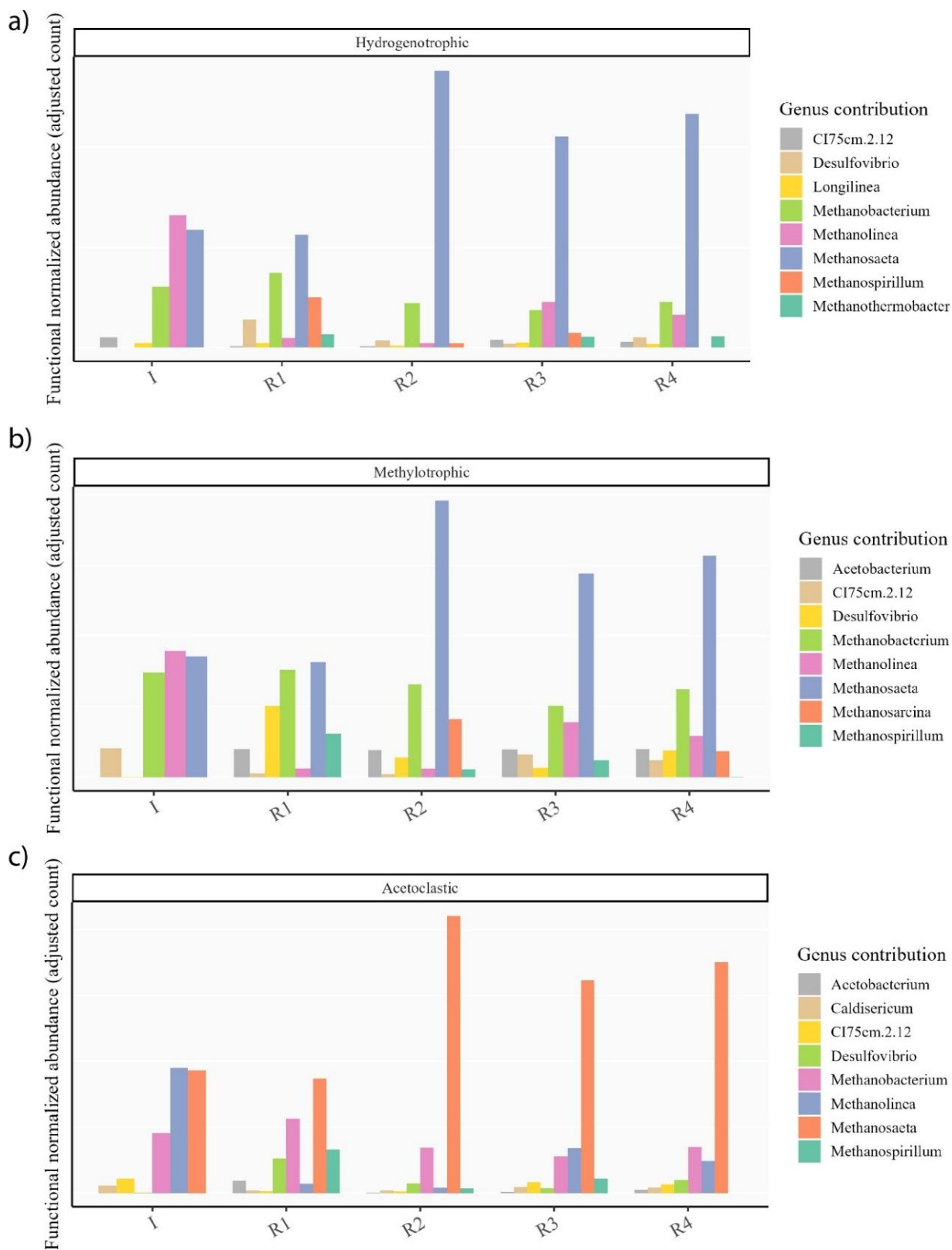
Functional inference indicated that microaeration was associated with an increased genetic potential for ω -oxidation pathways involving *Longilinea*, *Desulfovibrio*, and *Anaeromusa-Anaeroarcus*, suggesting a possible enhancement in alkyl chain activation. Predicted β -oxidation pathways linked to *Pseudomonas* and *Acinetobacter* further indicate a potential for fatty acid intermediate processing. In addition, the inferred participation of *Thauera* and *Methanosaeta* in aromatic compound metabolism suggests a capacity for benzene ring destabilization. However, as these results are based on gene prediction rather than transcriptomic, proteomic, or metabolomic evidence, they represent potential metabolic capabilities that may help explain the higher LAS removal observed in R2 compared with the conventional reactor at the same HRT.

Figure 36 – Z-score of the relative abundance of metabolic pathways involved in the biological degradation process of LAS.



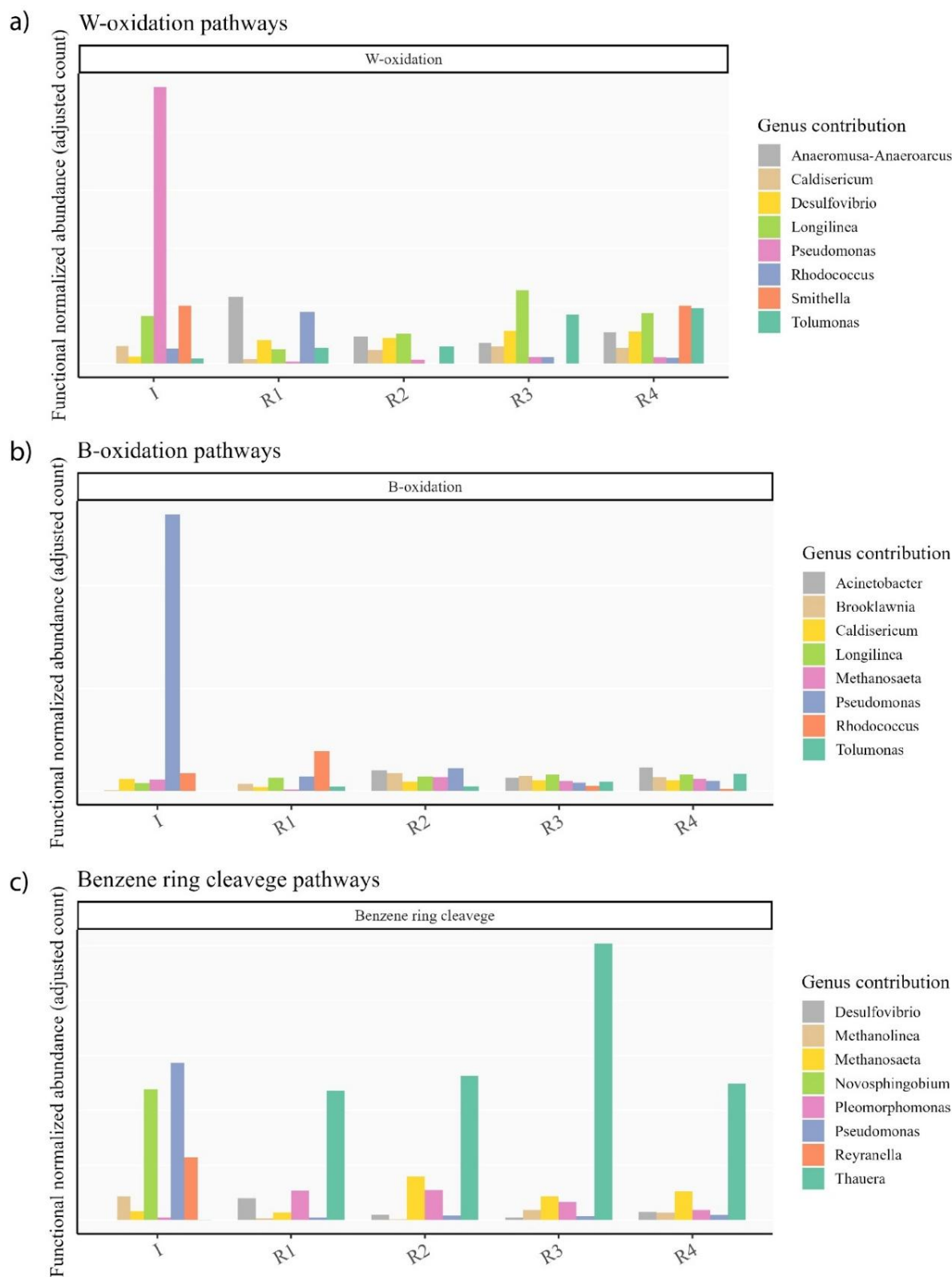
Source: Prepared by the author.

Figure 37 – Contribution of the most significant genus in methanogenic pathways: a) Hydrogenotrophic, b) Methyloctrophic and c) Acetoclastic.

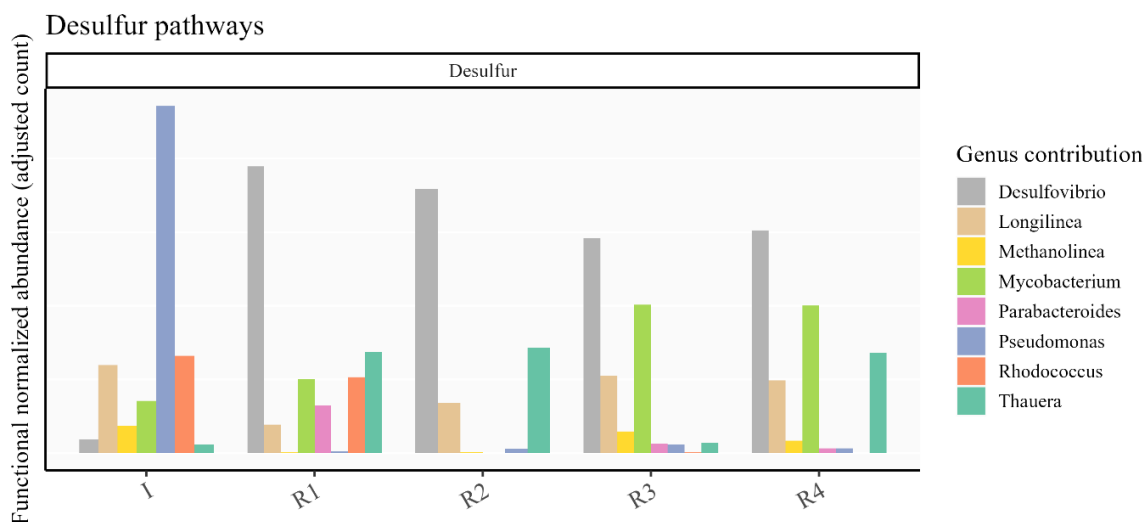


Source: Prepared by the author.

Figure 38 – Contribution of the most significant genus to the a) ω -oxidation, b) β -oxidation and c) benzene ring cleavage metabolism pathways.



Source: Prepared by the author.

Figure 39 – Contribution of the most significant genus to desulfur metabolism.

Source: Prepared by the author.

At 16-h HRT in the conventional UASB, functional prediction indicated enrichment of methanogenic pathways, including hydrogenotrophic, acetoclastic, and methylotrophic routes. The extended retention time was associated with a genetic profile consistent with a metabolically coordinated anaerobic network in which *Methanosaeta*, *Methanobacterium*, and *Methanolinea* potentially contributed to hydrogen and acetate turnover. Predicted ω -oxidation and sulfur metabolism pathways linked to *Longilinea* and *Desulfovibrio* suggest a genetic potential for alkyl chain activation and desulfonation under more stable conditions. Similarly, inferred β -oxidation pathways involving *Longilinea*, *Acinetobacter*, and *Brooklawnia* indicate a possible capacity for gradual intermediate conversion, while predicted aromatic compound metabolism associated with *Thauera* and *Methanosaeta* suggests potential involvement in aromatic ring destabilization. These inferred metabolic capacities may explain the higher LAS removal in R3 compared to R1, although methane production likely remained constrained by kinetic limitations typical of longer HRT and acetoclastic dominance.

The microaerated UASB operated at 16-h HRT exhibited the most functionally diverse predicted metabolic profile. Functional inference indicated enrichment of the Acetyl-CoA pathway alongside genes associated with hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis, suggesting a broad metabolic potential (Lara-Martín et al., 2010a; Motteran et al., 2018b). *Methanosaeta*, supported by *Methanobacterium* and *Methanolinea*, was associated with predicted pathways related to acetate and hydrogen utilization. Predicted ω -oxidation pathways linked to *Smithella*, *Tolomonas*, and

Longilinea suggest a genetic capacity for alkyl chain activation, while inferred β -oxidation pathways associated with *Longilinea* and *Acinetobacter* indicate potential for intermediate processing.

In parallel, functional prediction suggested genetic potential for aromatic compound transformation (*Thauera* and *Methanosaeta*) and sulfur metabolism (*Desulfovibrio*). Together, these predicted capacities may help explain the superior LAS removal and stable methane production observed in R4.

Overall, the functional pathway prediction suggests that LAS removal in UASB systems may be associated with the degree of potential integration among fermentation, oxidation, desulfonation, and methanogenesis pathways. Short HRT was associated with a predicted metabolic profile consistent with rapid but potentially incomplete transformation routes, whereas longer HRT correlated with a functional signature indicative of more stabilized methanogenic pathways and greater potential for intermediate conversion. Microaeration was linked to an increased predicted genetic potential for early oxidative steps and improved redox balance, particularly when combined with longer HRT.

6.4. Conclusion

High-throughput sequencing showed that R4 (64% LAS removal) was associated with a distinct microbial community structure. The enrichment of facultative and microaerophilic genera, such as *Thauera* and *Desulfovibrio*, together with functional predictions indicating oxygenase-related genes, suggests a potential for enhanced initial oxidative steps in LAS transformation. However, these inferences reflect predicted genetic potential rather than confirmed pathway expression or enzymatic activity, but further in-depth studies are needed.

The 16-h HRT was associated with improved LAS removal, higher COD stabilization (up to 87%), and more stable methane composition (~45%), likely due to improved biomass retention and reduced washout of slower-growing populations. Although intermediate metabolites were not measured, the observed performance suggests improved coordination between oxidative and methanogenic groups in the microaerated system. The dominance of *Methanosaeta* indicates the establishment of a stable acetoclastic community under these conditions. The increased abundance of Firmicutes and Proteobacteria in the microaerated reactors further supports the role of these phyla in handling complex organic loads via diverse metabolic pathways.

In contrast, the strictly anaerobic reactors (R1 and R3) showed lower LAS removal and were dominated by fermentative groups, whose functional prediction suggests differences in potential metabolic pathways.

Overall, combining microaeration with optimized HRT was associated with enhanced LAS removal and process stability. The data support the role of operational conditions in shaping microbial communities with broader metabolic potential, although the exact biochemical mechanisms underlying LAS transformation remain to be experimentally validated.

7. FINAL CONSIDERATIONS

This thesis establishes a new operational framework for treating Linear Alkylbenzene Sulfonate (LAS) in high-rate anaerobic reactors. By integrating microaeration, redox mediation, and hydraulic optimization, this work moves beyond the traditional view of LAS as a strictly recalcitrant inhibitor, demonstrating that its mineralization is technically feasible through the strategic management of the reactor's redox environment.

The investigation of co-substrate quality in OS I and II reveals that the success of microaeration is inherently tied to the system's energetic flux. The transition from ethanol to sucrose as a co-metabolite demonstrates that favoring syntrophic pathways, specifically those involving *Pleomorphomonas* and *Desulfovibrio*, is essential to trigger oxidative aromatic ring cleavage. This suggests that for WWTPs treating surfactant-rich effluents, prioritizing carbon sources that promote complex syntrophy helps maintain the microaerobic "bridge" and avoids the limitations of simple fermentation.

In OS III, the introduction of Anthraquinone-2-sulfonate (AQS) catalyzes process intensification. The synergy between chemical redox shuttling and micro-oxygenation directly addresses the kinetic bottlenecks of extracellular electron transfer, offering a practical route for stabilizing reactors under surfactant-induced stress. By using low concentrations of redox mediators, it is possible to maximize methane production while minimizing the need for high-intensity aeration, thereby preserving the energetic advantages inherent to anaerobic systems.

Furthermore, OS IV redefines the necessary "kinetic window" for LAS biotransformation, identifying a 16h HRT as a critical threshold for operational stability. This finding provides a necessary counterpoint to the trend of excessively short HRTs (6–8 h) in UASB reactors, which often lead to process failure. Establishing this broader operational range prevents the washout of slow-growing oxidative genera, such as *Thauera*, thereby maintaining metabolic capacity for aromatic ring destabilization alongside high volumetric methane productivity.

Taken together, these findings challenge the conventional boundary between anaerobic and aerobic treatment. This work demonstrates that microaeration acts as a metabolic catalyst, reducing the thermodynamic barriers for aromatic ring cleavage, the rate-limiting step in LAS biodegradation. By achieving removal efficiencies of up to ~80%, this research positions the microaerobic UASB as a high-performance technology capable of handling emerging contaminants while producing a high-quality biogas (methane content near 45–77%).

Beyond advancing fundamental understanding, this work offers practical implications

for the design and operation of wastewater treatment plants (WWTPs). The demonstrated feasibility of achieving high LAS removal (up to ~80%) in high-rate reactors at moderate HRTs highlights a clear pathway toward more robust, efficient treatment of industrial and domestic effluents. By integrating micro-oxygenation with redox mediation and nutrient management, UASB technology is positioned as a flexible, high-performance solution capable of addressing the challenges posed by emerging contaminants.

This work underscores the need for future research using metagenomic and transcriptomic approaches further to explore the functional roles of the identified taxa. Long-term studies evaluating the fate of intermediate metabolites and the economic feasibility of microaeration in full-scale applications will be essential to translate these findings into sustainable engineering practices. Overall, this work contributes to a new paradigm in anaerobic digestion, in which controlled redox intensification converts recalcitrant surfactants into treatable substrates.

In conclusion, this thesis advances the field by providing a multidimensional intensification strategy. It offers engineers and operators a "toolbox" of co-substrates, redox mediators, and hydraulic setpoints that turn recalcitrant surfactants into manageable substrates. This transition from strictly anaerobic to redox-modulated systems represents a sustainable pathway for upgrading existing WWTPs, aligning the removal of emerging contaminants with the principles of the circular bioeconomy and energy efficiency.

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