Electroactive biofilters outperform inert biofilters for treating surfactant-polluted wastewater by means of selecting a low-growth yield microbial community

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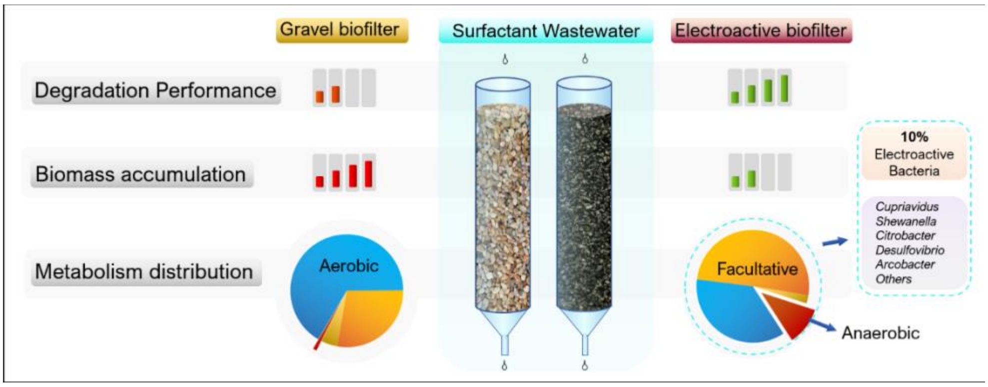
Keywords:

Surfactants
Biodegradation
Biomass accumulation
Electroactive microorganisms
Wastewater treatment



Abstract

Electrobioremediation is one of the most innovative disciplines for treating organic pollutants and it is based on the ability of electroactive bacteria to exchange electrons with electroconductive materials. Electroactive biofilters have been demonstrated to be efficient for treating urban wastewater with a low footprint; however, their application can be expanded for treating industrial wastewater containing significant concentrations (2.4 $\%_{vol}$) of commercial surfactants (containing lauryl sulfate, lauryl ether sulfate, cocamydopropyl betaine, and dodecylbenzene sulfonate, among others). Our electroactive biofilter outperformed a conventional inert biofilter made of gravel for all tested conditions, reaching removal rates as high as 4.5 kg COD/m^3_{bed}·day and withstood Organic Loading Rates as high as 9 Kg COD/m^3·d without significantly affecting removal efficiency. The biomass accumulation reduced available bed volume in the electroactive biofilter just by 39%, while the gravel biofilter decreased by 80%. Regarding microbial communities, anaerobic and electroactive bacteria represented a substantial proportion of the total population in the electroactive biofilter. *Pseudomonas* was the dominant genus, while *Cupriavidus*, *Shewanella*, *Citrobacter*, *Desulfovibrio*, and *Arcobacter* were potential

electroactive strains found in relevant proportions. The microbial community's composition might be the key to understanding how high removal rates can coexist with limited biomass production, making electroactive biofilters a promising strategy to overcome classical biofilter limitations.

1 Introduction

Surfactant pollution in aquatic environments has been of concern since its spread as a household commodity. In the past century, the authorities detected that the early market-available surfactants generated significant foaming problems in the sewage and wastewater treatment plants and were found to have limited biodegradability. Common detergent products mainly combine anionic and nonionic surfactants to enhance their properties. Anionics accounted for around 70% of global surfactant production (Liwarska-Bizukojc et al., 2005). However, they are being partially replaced in formulations by amphoteric surfactants, which are more stable, less toxic and irritating, and more biodegradable (Sarkar et al., 2021).

Surfactants are indeed abundant in urban wastewater and apart from the possible direct discharges without treatment, the main route for surfactants to reach the environment is wastewater treatment plants (WWTP) operating under distress, episodes of sludge reuse, or discharge in landfills. Surfactants might cause disturbances in primary wastewater treatment, but in the secondary treatment, over 90% of the most common surfactants are removed (Arora et al., 2022; Camacho-Muñoz et al., 2014; Ivanković & Hrenović, 2010). Surfactant concentrations that generate foam significantly disturb the operation of WWTPs, leading to poor-quality effluents (Berna et al., 1991).

The decreased surfactant concentration observed in WWTP can be attributed to sorption on sludge and biodegradation (Schinkel et al., 2022). Surfactants tend to attach to anaerobic sludge particles where biodegradation may occur, although those seem more effectively degraded by aerobic biofilm-forming organisms (Ying, 2006). Most surfactants in household detergents are biodegradable under aerobic conditions, but only a few are degradable when oxygen is limited (Merrettig-Bruns & Jelen, 2009). This oxygen dependence was confirmed when linear alkyl-benzene sulfonate's (LAS) presence in anaerobic sludge was between 1 to 2 orders of magnitude higher than in aerobically treated sludge as a fraction of dry mass (Jensen, 1999).

Biodegradation pathways for the most common surfactants have already been determined. Sodium dodecyl-benzene sulfonate (SDBS) breakdown involves the cleaving and degrading of the alkyl chain, the benzene ring, and a sulfonate ring that eventually transforms into SO_4^{2-}. The degradation of the alkyl chain starts with the ω-oxidation of the terminal methyl group. As for de-sulfonation, three mechanisms have been proposed: hydroxylative de-sulfonation, monooxygenase catalysis, and reductive de-sulfonation. After that, the sulfite can be oxidized to sulfate. Eventually, these cleavages result in phenylacetic or benzoic acids. Finally, these acids can be transformed into fumaric and acetoacetic acids and converted to catechol (Scott & Jones, 2000). For sodium lauryl ether sulfonate (SLES), the first step for degradation can be ether cleavage or ester cleavage, releasing intermediates further decomposed by β-oxidation until mineralization (Paulo et al., 2017).

Over the last decades, several studies have tried to provide solutions for the treatment of surfactant wastewater under different disciplines: electrochemical treatment (Panizza et al., 2005), Fenton oxidation (X. J. Wang et al., 2008), adsorption (Kim et al., 2019), and coagulation combined with biological treatment (Aloui et al., 2009), among others. Regarding more sustainable and natural solutions, artificial/constructed wetlands have been proposed to treat surfactant-containing wastewater (Justin et al., 2009; Šíma & Holcová, 2011).

In this sustainability scenario, a recent strategy, named electrobioremediation, bridges the gap between high-efficiency treatments and passive bio-based treatments under a natural and sustainable scheme (X. Wang et al., 2020). In fact, electrobioremediation is based on the singular capabilities of electroactive microorganisms to couple the oxidation of organic matter with the respiration of electroconductive materials (electrodes). This coupling minimizes the respiration bottleneck in anaerobic environments, where the availability of the terminal electron acceptor (TEA) like O_2 is limited. This disruptive discovery (Bond & Lovley, 2003) led to the development and rapid expansion of microbial electrochemistry, which continues to show promising applications in environmental protection and recovery and, more specifically, in water treatment strategies.

Recently, a study was carried out for the simultaneous energy recovery and treatment of real carwash wastewater (Radeef & Ismail, 2021). Surfactants have also been studied as additives in bioelectrochemical reactors like Microbial Fuel Cells, where their presence enhanced the bioavailability of hydrophobic substances (Hwang et al., 2019), improved biofilm formation by interacting with the surface of the electrode (Zhang et al., 2017), and seemed to produce a synergistic effect with electron shuttles (Pham et al., 2008).

Originally the concept electroactive biofilter resulted from the process integration of microbial electrochemistry into a biofilter-based natural concept as treatment wetlands, where a natural ecosystem operates in equilibrium to remove organic matter with no energy requirements. Indeed, replacement of inert bed with electroconductive material enables the connection of differing redox environments and the selection of electroactive microorganisms (EAM). The new configuration included plants and it was named METland® (Aguirre-Sierra et al., 2016). Indeed, a redox gradient is formed by creating this electrical connection along the bed, allowing the flow of electrons from the anaerobic locations towards the oxidative areas (O_2 rich), thus, providing EAM with an unlimited sink of electrons. Initially these systems were operated under the microbial electrochemical snorkel (MES) configuration, where the circuit assembly sacrifices current production to enhance catabolic activity (Aguirre-Sierra et al., 2016). As this open circuit alleviates TEA limitation, pollutant removal is maximized. METland® originally operated under flooded conditions, and the electron flux was confirmed and measured using a custom-made electric potential sensor (Peñacoba-Antona et al., 2022a; Prado et al., 2020, 2022; Ramírez-Vargas et al., 2019). A METland® can also be operated in a downflow non-flooded mode (Aguirre-Sierra et al., 2020), taking advantage of a passive supply of air, where the aerobic and anaerobic environments (outer and inner layers of biofilm, respectively) generate redox gradients at micro-scale in the stratified layers of the biofilm. In fact, biofilms play a key role in bioelectrochemical processes since higher cellular densities account for more cellular proximity and contact, increasing electron transfer possibilities (Yang et al., 2012). METland have been demonstrated at full scale using Life Cycle Assessment as validation tool (Peñacoba-Antona et al., 2021).

This study aims to validate lab-scale electroactive biofilters for treating wastewater containing anionic and amphoteric surfactants, including operational and performance factors. In spite of the harsh environment of surfactant-polluted wastewater, the microbial community was characterized to reveal how high biodegradation rates can coexist with limited biomass production.

2 Materials & Methods

2.1 Wastewater composition.

Synthetic wastewater was prepared using a salt medium and a mixture of commercial detergent products. The salt medium composition was: 0.4 g/L of sodium bicarbonate ($NaHCO_3$), 0.04 g/L of ammonium chloride (NH_4Cl), 0.04 g/L of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 0.03 g/L sodium dihydrogen phosphate (NaH_2PO_4), 0.02 g/L potassium nitrate (KNO_3), 0.02 g/L magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 0.02 g/L of potassium chloride (KCl) and 0.02 g/L of iron sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) (5% weighting error).

The concentration of the detergent mixture was dependent on the experiment, but 2.4% by volume on water was the concentration typically used (ca. 5100±200 ppm of chemical oxygen demand (COD)). The proportion of commercial detergent products in the mixture was selected to emulate a hypothetical composition of a detergent production factory wastewater. This concentration was kept constant for all experiments: 50% dishwasher soap (Carrefour Expert Lemon dishwasher, Carrefour, France), 20% laundry detergent (Carrefour Expert Optimal Clean, Carrefour, France), 20% toilet detergent (Eco Planet Eucalyptus WC detergent, Carrefour, France), and 10% glass cleaning detergent (Carrefour generic glass cleaner, Carrefour, France). The surfactant components mentioned in the labels are sodium lauryl ether sulfate (SLES), Cocamidopropyl betaine (CAPB), sodium C14-16 olefin sulfonate, and methyl alkyl benzene sulfonate (ME-DBS). It also contains alcohol ethoxylated surfactants and additives such as methylchloroisothiazolinone, methylisothiazolinone, and phenoxyethanol.

The real surfactant wastewater was prepared using the same salt medium and dissolving a concentrated mixture of residual detergents provided by a detergent factory that produces similar detergent products as those mentioned before.

For the experiments with single surfactants, the products used were: sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), and Cocamidopropyl betaine (CAPB) (Guinama S.L., Spain); sodium dodecylbenzene sulphonate (SDBS), Tween 80, and Triton X-100 (Sigma Aldrich, USA).

2.2 Analytical Methods

COD was determined using a cuvette test from (LCK Hach), digested in an HT200S (Hach Lange, USA) thermostatic digestor, and measured using a DR 3900 spectrophotometer (Hach Lange, USA). The conductivity was measured with a conductometer GLP 31 (Crison, Spain), and the pH was measured with the pH-meter Basic 20+ (Crison, Spain). Sulfate was measured using a Metrhom Compact IC model 930 ionic chromatograph.

The specific chemical identifications were performed with an Agilent Technologies 1200 HPLC (Agilent Technologies, USA), coupled to an Agilent Technologies 6230 MS-TOF (Agilent Technologies, USA), using an Acclaim Surfactant Plus column (150mm x 3mm x3 µm) (Thermo Fisher, USA). The mobile phase was ammonium acetate 0,1M at ph 5 (A) and acetonitrile (B), run at 0.6ml/min, 25 ºC, and 5µL sample injection. Further details can be found in supplementary section II table 7.

2.3 Experiments and Set-ups

2.3.1 Impact of material nature on biomass formation and hydraulic conductivity

A comparative experiment was made using two identical glass reactors consisting of single cylinder pieces 50 cm long and 6 cm of internal diameter, narrowing down at the bottom to 0.8 cm. Each one was filled with 0.8L of electroconductive carbonaceous material (METFILTER SL, Spain) or gravel, with a 6-8 mm granulometry, and inoculated with a microbial consortium available in our research group. This specific set-up was designed to maximize granule comparability and allow gravity drainage trials. The same detergent-based synthetic wastewater was continuously fed to both systems (1.6 L/d), following a sequence i) fresh media for 5 days and ii) 2 days reusing the effluent. Initially, there was no biomass in the system, and its development was evaluated periodically over 72 days of operation.

Two different experiments were carried out to evaluate the impact of biomass growth on the biofilters' hydraulic characteristics.

Residence time distribution using a tracer:

Pulse tracer experiments were conducted to determine the residence time distribution using potassium bromide (KBr) as a tracer. Bromide was selected as a tracer mainly because: i) given its negative charge, it was expected to behave similarly to anionic surfactants; ii) it has little interaction with microbial life.

A tracer solution was pulsed for 5 minutes, after which the columns were returned to regular operation. The flow rate and the surfactant content were not altered during this period. Samples were collected periodically during the experiment, which lasted around 120 minutes.

The residence time distribution (RTD) is represented such that only the recovered tracer is considered the area below the curve, not considering here losses on recovery.

$$\int_0^{\infty} \boldsymbol{E}\, dt = 1$$

The residence-time distribution function (*E(t)*) is obtained from the normalization of the concentration curve, which is determined by the changing effluent tracer concentrations over time.

$$\boldsymbol{E}(t) = \frac{C(t)}{\int_0^{\infty} C(t)\, dt}$$

The mean residence (t_m) time can be obtained from the dataset mathematically as follows:

$$t_m = \frac{\int_0^\infty tE(t)dt}{\int_0^\infty E(t)dt} = \int_0^\infty tE(t)dt$$

Another parameter that aids in the characterization of the curves is the variance or square of the standard deviation (σ^2):

$$\sigma^2 = \int_0^\infty (t - t_m)^2 E(t)dt$$

This descriptive parameter indicates the distribution's breadth (Fogler, 2006).

Hydraulic conductivity measurements:

The measurement of hydraulic conductivity was based on Darcy`s law. *Kv* was determined following a gravity drainage method (Gefell et al., 2019). The method was modified by applying a constant hydrostatic head of 5 cm of water for comparability between reactors. During the test, the water flowing out of the biofilter was collected in a graduated vessel, the volume and time were registered. Then the flow rates were calculated, and the average served for further calculating the K_v.

$$K_v = \frac{Q}{\left[\frac{A\ dh}{dL}\right]}$$

K_v is the vertical hydraulic conductivity, *Q* is the measured flow rate at the outlet, *A* is the outflow area, *dh* is the hydraulic pressure differential (complete water column), and *dL* is the column height occupied by material.

For the measurements of available volume and hydraulic conductivity, the columns were slowly flooded to prevent the trapping of air. Then the water was released, and its volume was measured. The initial values with no biomass were compared to the final values with biomass growth after 72 days.

2.3.2 Biofilter operation

The following experiments focused on studying the operation and validation of lab-scale electroactive biofilters to treat surfactant-polluted water using a specific set-up different from the previous one.

The electroactive biofilter was filled with carbonaceous electroconductive material (3-7 mm), and the control biofilter was filled with gravel (3-8 mm). In both cases, 0.8 L of material was used. The biofilter reactor consisted of a glass cylinder (43cm high and a 6cm inner diameter)—a second internal glass cylinder of 1 cm diameter had 3 mm perforations serving as a passive aerator.

The biofilters were operated indoors at 23±2 ºC under a down-flow mode using a peristaltic pump (Watson Marlow 323). These biofilters were continuously fed, and the flow rate was adjusted from 0.4 L/d to 1.6 L/d, feeding an amount of 5200±200 mg COD/ L.

The inoculum was the same for both systems, using urban wastewater and a heterogeneous electroactive consortium (Metfilter S.L.).

The comparative performance was tested under continuous operation. Two experiments were designed: i) non-steady-state performance at 0.4 L/d during 7-9 days of operation and the sampling for 3 consecutive days; then the reactor was dismantled, bed was flushed with water, and restarted (new flow rate); ii) For the steady-state condition, a biofilter was monitored at 1 L/d, after reaching stable COD values at the effluent.

Another experiment for evaluating the performance and biodegradability of the surfactant mixture was carried out under batch by continuously recirculating a fixed volume of wastewater. The batch experiments were carried out twice, and the results displayed correspond to the last ones. Experiments with both real and synthetic wastewaters were set for several days in a close circuit recirculation at a flow rate of 1 L/d and a tank volume of 1L. HPLC-MS analysis of these waters samples was carried out to provide more precise evidence of molecular degradation and determine the differences in the composition of the synthetic and real waters.

2.3.3 Culture vessel experiments with single surfactants:

Six glass culture vessels of 100 ml (Pobel, Spain) were filled with 35 grams of colonized conductive carbonaceous material. Each surfactant was dissolved in the freshwater medium to achieve a COD value of 3500±200 ppm. Biodegradation tests were performed by adding 20ml of surfactant solution (a solution for each surfactant), and all of them were kept in an orbital incubator at 30 ºC and 120 rpm.

The medium was replaced with new medium every week during the acclimation period. COD and HPLC-MS analysis were made on the 6^{th} and 10^{th} cycle.

Abiotic controls with fresh and autoclaved material were tested to evaluate adsorption. Planktonic controls, without bed material, were maintained for three surfactants to compare the effects of inoculated material for biodegradation.

The Triton X-100 was selected for solvent extraction, given that it had the lowest limit of quantification in the HPLC-MS. For the adsorption evaluation, 2 grams of sample material were taken from the culture vessel subject to four runs. Another sample of 2 grams from a replicate was exposed to the surfactant once. After two-stage organic extraction (methanol and acetonitrile) and resuspension in deionized water, samples were injected into the HPLC-MS.

2.3.4 Microbial community evaluation

Three independent colonized granules from biofilters (*ca.* 4 cm bed depth) were selected and stored at -80 ºC. DNA extraction, PCR amplification, and high-throughput sequencing were performed by an external service for genomic analysis (Institute of Biotechnology and Biomedicine, UAB, Barcelona).

The V3-V4 region of the 16S bacterial gene was amplified by a polymerase chain reaction (PCR) with 338F/806R primers. The protocols of this analysis were set according to Klindworth (Klindworth et al., 2013). High-throughput sequencing analysis was conducted using Illumina-MiSeq from Servei de Genòmica I Bioinformática (Barcelona, Spain).

The data was manipulated and translated into graphics using RStudio. The taxonomy/abundance heat tree was created using the Metacoder package (Foster et al., 2017).

3 Results & Discussion

The integration of electromicrobiology concepts in treatment wetland gave birth to a new generation of electroactive biofilters capable electroremediating pollutants from urban wastewater (Mosquera-Romero et al., 2023). So, providing a strategy for treating industrial wastewater with high organic load of such pollutants. Thus, the following section includes results revealing how downflow-mode electroactive biofilters select for a special microbial community capable of removing a mixture of surfactants from synthetic and real wastewater at low biomass growth yield.

3.1 Effect of flow rate on electrobioremediation performance under continuous operation

The removal efficiency was measured at different flow rates for both reactors, evaluating removal efficiency and rates per bed volume. At seven days of operation, the biomass generated in each reactor was assumed to be similar.

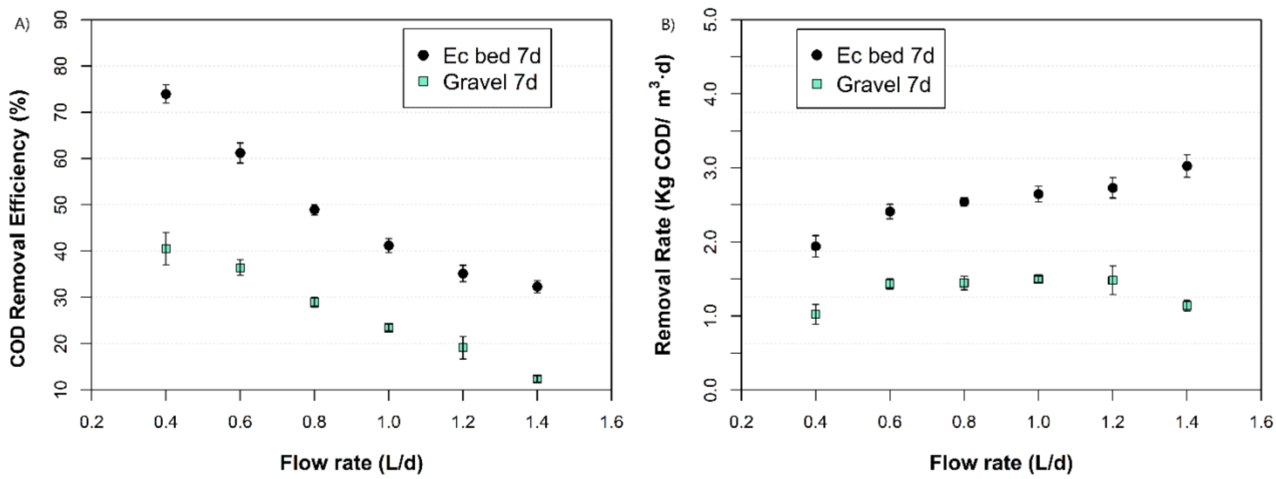


Figure 1. Impact of bed material on electrobioremediation performance at different flow rates. Gravel bed biofilter at 7 days maturity biofilm (green) and electroconductive bed biofilter at 7 days maturity biofilm (black). a) Removal Efficiency (%) at different flow rates. b) Removal Rate per unit of bed volume and time at different flow rates.

The removal efficiency decreased as the flow rate increased for the gravel and electroconductive biofilter under all flow rate conditions. The gravel-based biofilter reached its maximal removal rate at a flow rate of 0.6 L/h, while the electroactive biofilter increased for all flow rates tested. Indeed, the gravel biofilter removed a maximum of *ca*. 1.5 kg COD/m^3·d with no significant variations, while the electroactive biofilter removed *ca*. 3 kg COD /m^3·d at its highest rate.

The gravel biofilter's operation did not reach a steady state due to the rapid and continuous biomass accumulation in the gravel biofilter (Figure 2). Nonetheless, steady state operation of the electroactive biofilter was possible. Therefore, it is possible to compare the impact of a mature biofilm on the degradation performance.

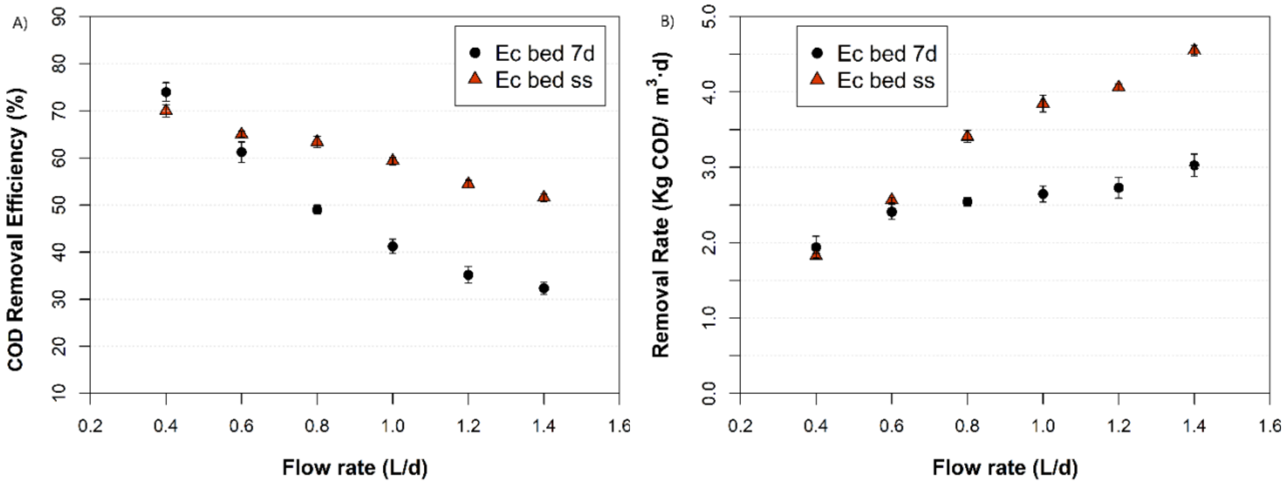


Figure 2. Impact of biomass accumulation on biodegradation performance at different flow rates. Electroactive bed biofilter at steady state (ss) biofilm (red triangles), Electroactive bed biofilter at 7 days maturity biofilm (black dots). a) Removal Efficiency (%) at different flow rates. b) Removal Rate as COD kg per cubic meter of bed and day at different flow rates.

Once the biofilm reaches the state of maturity and stable behavior, the flow rate has a lower impact on the removal efficiency, maintaining a higher percentage of removal for the higher flow rates. The removal rate increased significantly under steady state reaching 4.5 kg COD/m^3·d at 1.4 L/d.

3.2 Impact of OLR on electrobioremediation under continuous operation

The effect of the organic loading rate (OLR) on the electroactive biofilter was observed by altering the concentration of surfactants in the influent while maintaining a steady flow rate of 1 L/d.

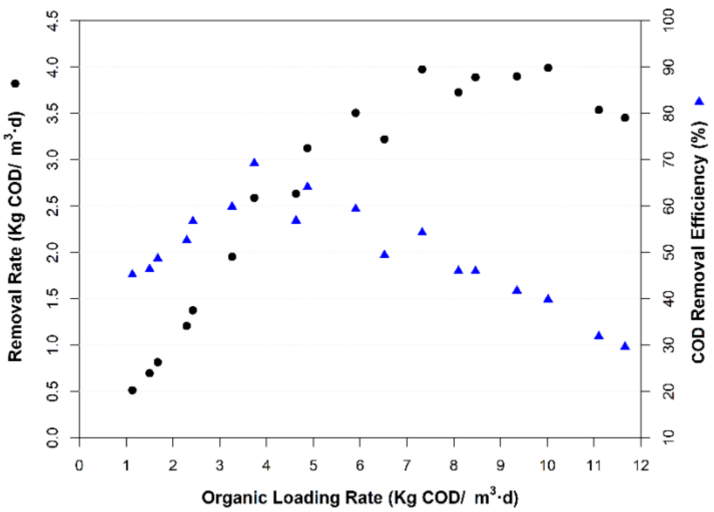


Figure 3. **Organic loading limits the removal efficiency.** Removal rate (left, black circles) and removal efficiency (right, blue triangles) for different values of Organic Loading Rate at a fixed flow rate. The electroactive biofilter was evaluated with a fixed flow rate of 1L/d.

The increase of OLR in the electroactive biofilter induced a steady rise in the removal rate up to *ca.* 4 kg COD/m^3·d. However, an increase in OLR negatively affected removal rates at values higher than 10 kg COD/m^3·d, where surfactants may have a toxic effect.

At OLR levels below 3 kg COD/m^3·d, the main limitation seemed to be related to the availability of organics since both the removal efficiency and rate were relatively low. As the concentration increased, the removal rate (RR) increased until reaching a plateau. The removal efficiency reached its maximum between 4-5 kg COD/m^3·d and then decreased, possibly due to a metabolic respiration bottleneck.

Biofilter systems can degrade pollutants at the highest rate near the maximum RR/OLR ratio. Conventional biofilters are typically used under conditions of low organic loading and relatively high flow rates and granulometries. Biomass accumulation must be reduced to maintain operability at the expense of degradation rates. In electroactive biofilters, significantly higher removal rates can be obtained without detrimental effects on the operation.

3.3 Detergent wastewater biodegradability test

An electroactive biofilter was set up to assess the total biodegradability of detergent wastewater in batch experiments under recirculation mode. Two different detergent mixtures in water were studied, one synthetic and one from a real surfactant production factory.

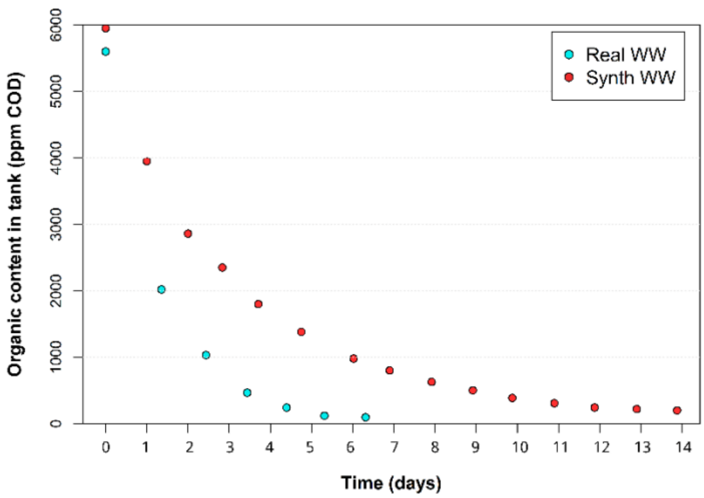


Figure 4. **Biodegradability of surfactant mix in batch**. Evolution of organic content in batch experiments for real detergent manufacture wastewater and synthetic detergent wastewater in an acclimated lab-scale electroactive biofilter.

In such batch experiments, the reduction in available organic matter causes a reduction in the degradation rates, which aligns with previous observations. The real industrial detergent wastewater was degraded in 6 days from 5600 to 97 mg/L COD, reaching an asymptotic tendency in the last few days (Figure 4). The same phenomenon was observed for the synthetic wastewater at the end of the curve, although it took 14 days to achieve COD values as low as 191 mg/L. At the end of each experiment, the degradation efficiency was 98.2% for the real water and 96.8% for the synthetic water.

Analysis of real wastewater composition revealed the presence of SLS, SLES, SDBS, and CAPB (see supplementary section II: table 9). However, after 5 days of recirculation, all pollutants were below the detection limit except SDBS (1 ppm). Regarding synthetic wastewater, the surfactants mentioned above were removed after 10 days of recirculation, except for SDBS (1.27 ppm).

3.4 Degradability of wastewater polluted with single surfactants

Batch flasks containing material with 6 individual surfactants were prepared to more precisely test the preference for metabolizing and biodegradability of certain surfactants. Initially, inoculum from the already active biofilters was supplied to the vessels. Five acclimation runs preceded the measured experiments, in which the COD and optical density were monitored (see supplementary section I: tables 4 and 5).

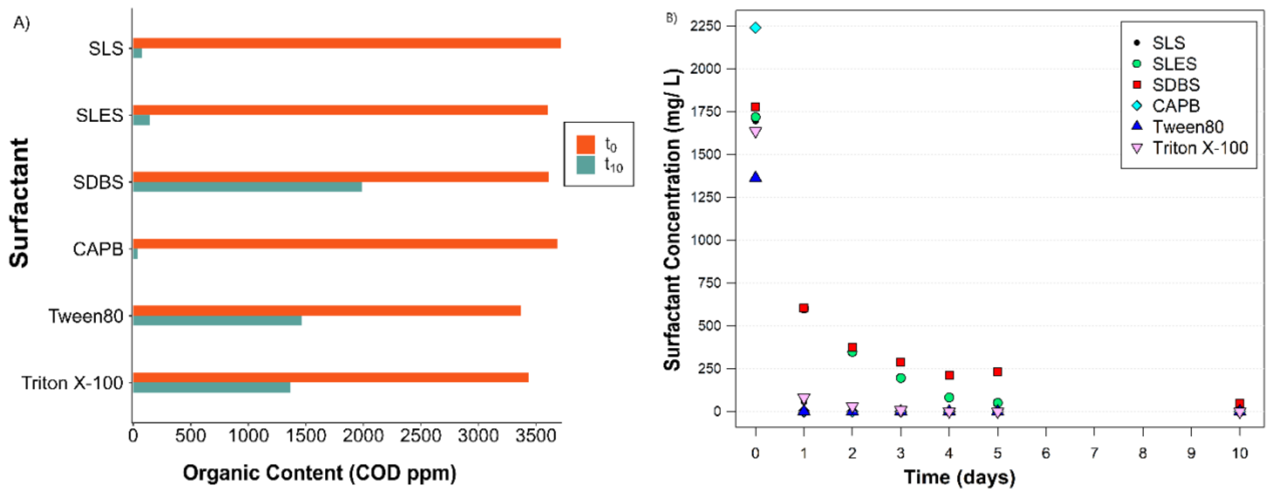


Figure 5. **Single surfactant biodegradation**. Degradation of independent surfactants in flask vessels containing electroconductive material and surfactant solution in a 10-day window, measured by: a) COD content at the beginning and the end; b) mg/L of their characteristics' breakdown masses obtained through HPLC-MS.

All surfactants from our study were analyzed by targeting a characteristic breakdown mass as a proxy for the surfactant concentration, except in the case of Tween 80 due to its particular molecular structure. For the linear structured surfactants (SLS, SLES & CAPB), the biodegradation was nearly complete after 10 days, according to the COD results displayed in Figure 5A (98, 96, and 99 %, respectively). On the other hand, the surfactants with a more complex molecular structure (SDBS, Tween 80, and Triton X-100) were partially degraded after 10 days of incubation as determined by residual COD (45, 57, and 60 %, respectively). However, there was no trace of their characteristic masses at the end of the treatment (Figure 5B).

Indeed, most surfactants quickly degraded despite the high initial concentration. According to the optical density analysis (supplementary section I: table 4), the highest cell density was reached shortly after the first 24h in most cases. The highest cell density was observed for the SLS sample, suggesting the vital role of this surfactant in biological overgrowth. On the other hand, the Tween 80 sample showed very low cell density compared to the others, slightly more than the media blank. Planktonic microbial growth was not observed in absence of electroconductive material, suggesting a strong preference for the microorganisms to grow on the material surface for biodegrading this surfactant.

The COD analysis on the abiotic control samples showed decreased concentration in all cases after including fresh unused granular material (see supplementary section II: table 11). After 4 successive media renewals (abiotic), the COD values no longer decreased, indicating saturation of the bed material. An extract of that material showed a Triton X-100 concentration higher than 200 mg/L for the high-exposure sample and 42 mg/L for the once-exposed sample, confirming that the missing surfactant was adsorbed.

Given the granulometry and specific surface area of materials used in bed biofilters, the adsorption capacity was expected to be low. In previous works, the porosity and specific surface area were studied, among other characteristics (Prado et al., 2019), and it was found that the conductive material was in the same order of magnitude of specific surface area as the gravel (<1 m^2/gr). This similarity suggested a low adsorption capacity, and the rapid saturation of the material confirmed the low relevance of adsorption phenomena on the removal of surfactant for the medium- and long-term operation of electroactive biofilters.

3.5 Effect of the bed material on the biomass accumulation and hydraulic conductivity

An important aspect to consider in biofilm-based systems is the biomass accumulation's impact on the hydraulic conductivity. In this regard, bio-clogging is the blockage of pore space due to surplus microbial biomass accumulation. In biofilters like vertical- flow treatment wetlands, this biomass accumulation is mainly influenced by OLR, grain size, distribution, and wastewater characteristics

(Pucher & Langergraber, 2019). Our experimental conditions were characterized by a relatively high OLR, a highly biodegradable organic fraction, increased bioavailability of surfactants (Nikpay et al., 2017), with nutrient availability that, eventually, enabled a fast and continuous biofilm growth over 72 days.

To determine the vertical hydraulic conductivity, we applied the gravity drainage method based on Darcy´s law, and the results indicate a different biomass growth rate for each biofilter.

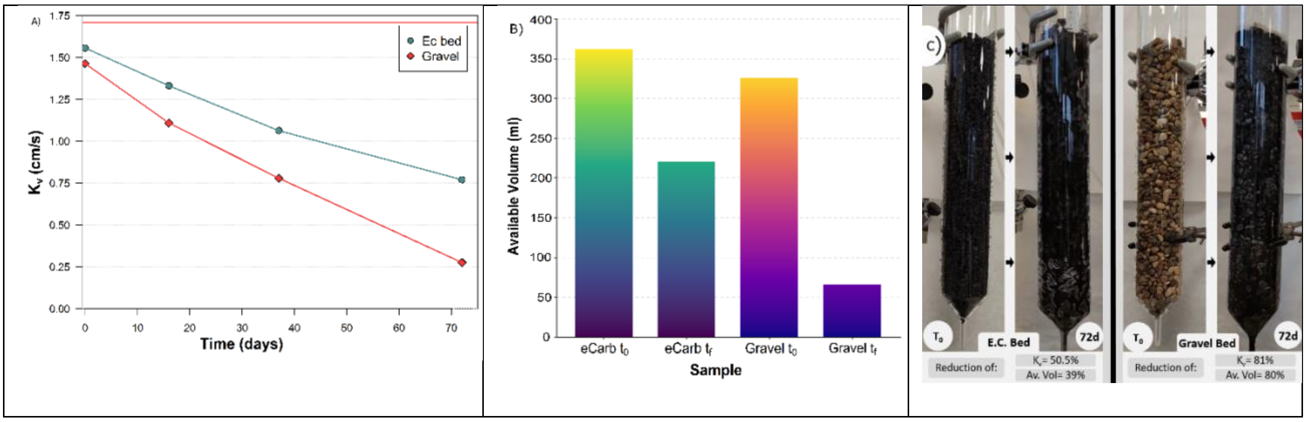


Figure 6. Decrease of hydraulic conductivity and bulk porosity. Effect of the biomass accumulation on: A) the hydraulic conductivity at different incubation time; B) the available volume at the beginning and end of the experiment; C) Image of initial and final state of the electroactive biofilter (left) and gravel biofilter (right).

The maximum hydraulic conductivity (K_v) allowed by the constricted bottom of the glass columns was 1.71 cm/s. The initial values of K_v for the gravel biofilter and the electroactive biofilter were 1.46 and 1.55 cm/s, respectively. This K_v was reduced to 0.27 and 0.77 cm/s, respectively, after 72 days of operation, corresponding to 81.18 % and 50.54 % reductions (Figure 6A). The initially available volume, considering bulk porosity, was 325ml for the gravel bed, and 362 ml for the electroactive bed, and eventually it shifted to 66ml and 220 ml after 72 hours, corresponding to a reduction of 79.70 and 39.22 % (Figure 6B).

The results showed a higher reduction in hydraulic conductivity in the gravel biofilter despite removing less COD than the electroactive biofilter (5.7±2.7 % and 12.7±3.2 %, respectively). An estimation of biomass growth calculated from the difference in available volume at the beginning and end of the operation period indicated a reduction of 259 ml for the gravel biofilter and 142 ml for the electroactive biofilter. This reduction follows the same pattern as the Kv and the dry biomass tests (see supplementary section III: tables 18 & 19).

These results suggest that biomass accumulation can rapidly render the gravel biofilter inoperative, leading to impaired performance due to poorer wastewater distribution and oxygen transfer limitation.

However, the results of this experiment revealed how a high degradation performance under low biomass production/accumulation can coexist in electroactive biofilter. Additional studies have confirmed that electroactive bacteria growing on electrodes result in biofilms of low thickness in comparison with conventional biofilm growing on inert surfaces, probably due to an intrabiofilm accumulation of protons that are not consumed during electron electron transfer to electrodes(Pereira et al., 2023).

3.6 Impact of bed material for selecting microbial communities

All biofilters operated for the microbial community analysis shared the same operating conditions, reactor size, shape, material granulometry, and inoculum. Therefore, the only factors affecting microbial community can be attributed to the bed material's composition and properties.

Duplicate particles were selected from both reactors for microbial community analysis through 16S sequencing, and significant differences were found between the gravel and electroactive biofilter communities. The taxonomic information and normalized relative abundance were graphically displayed as heat trees.

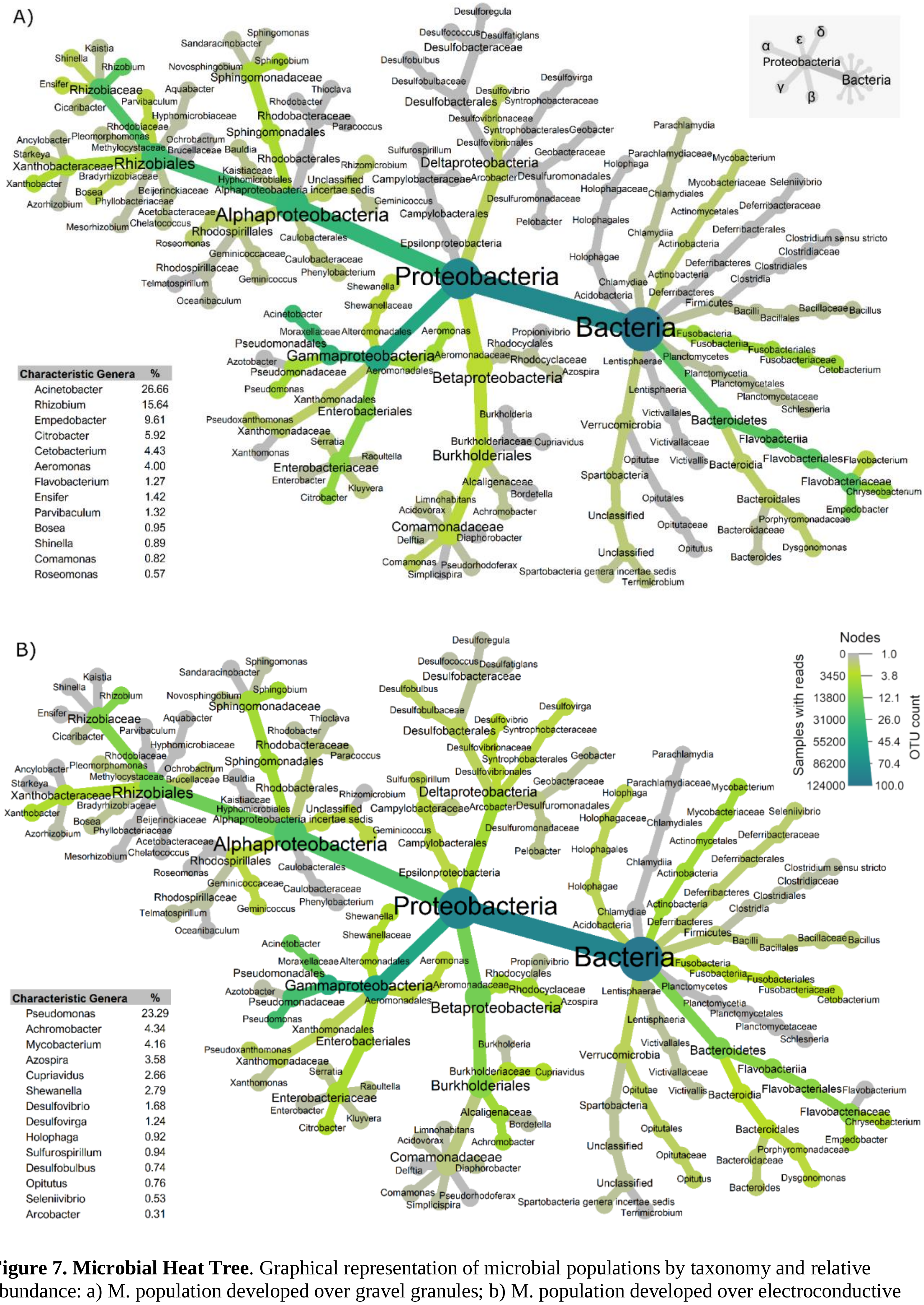


Figure 7. Microbial Heat Tree. Graphical representation of microbial populations by taxonomy and relative abundance: a) M. population developed over gravel granules; b) M. population developed over electroconductive carbon granules.

As an estimation of the alpha diversity, the Shannon index for the electroconductive material samples was 2.99, and for the gravel granules biofilm 2.73. Regarding beta diversity, the Bray-Curtis index between both communities is 0.52±0.04, which indicates a relatively high difference between the communities.

There were common occurrences of many Genera when comparing the microbial populations established in both bed materials. All these common genera were previously classified as aerobes or facultative anaerobes except for *Cetobacterium*, which is micro-aerotolerant. Selecting the most representative differential genera for each population (see supplementary section IV, figure 5), all but one in the gravel population were aerobic. In contrast, the microbial community colonizing the electroconductive bed was all anaerobic or facultatively anaerobic. A similar pattern was observed in the global populations (evaluating 96% of the population). For the gravel population, only 1% were anaerobic, 4.4% were micro-aerotolerant, 26.7% facultative anaerobes, and 63.5% were aerobic microorganisms. For the electroconductive bed population, 10.5% were anaerobic, 2.3% were micro-aerotolerant, 48.6% were facultative anaerobes, and 34.6% were aerobic microorganisms (see supplementary section IV, figure 6).

Some of the major genera such as *Pseudomonas* (Chen et al., 2019; Jovcic et al., 2010; Paulo et al., 2017), *Rhizobium* (Merkova et al., 2018), *Acinetobacter* (Hosseini et al., 2007), *Citrobacter* (Dhouib et al., 2003), *Aeromonas* (Paulo et al., 2017), *Comamonas* (Paulo et al., 2017), present in both systems were previously reported to participate in surfactant biodegradation. These genera suggest a primary degradation role of the aerobic and the facultative anaerobes dominating the outer layers of the biofilm.

Looking deeper into the anaerobes and facultative anaerobes of the electroconductive carbon biofilter's population, electroactive microorganisms represented 10.8% of the global population including *Shewanella Xiamenensis* (Truong et al., 2021), *Cupriavidus Metallidurans* (Alviz-gazitua et al., 2022), *Arcobacter Butzleri* (Fedorovich et al., 2009), *Bacillus Cereus* (Islam et al., 2017), *Citrobacter Freundii, Desulfovibrio Desulfuricans, Geobacter Lovlyi* and *Geothrix Fermentans* (Koch & Harnisch, 2016). There are additional members that are frequently found in electroactive biofilms or belonging to genera with electroactive members, adding up to an additional 31%.

Bacteria from *Pseudomonas* genus is known for their metabolic versatility (aerobic and anaerobic) and their capacity for surfactant degradation, with some producing electron shuttles (Rabaey et al., 2005). At the genus level, *Pseudomonas* represented 4.1% of the gravel population, whereas, in the electroconductive bed population, they represented up to 21.9%. Surprisingly, a single unclassified species dominated the latter, accounting for 19% of the community. This population can be vital for protecting the anaerobic layers of the biofilm since some bacteria from *Pseudomonas* genus have been previously reported to thrive in microaerophilic conditions and to deplete O_2 at the interior portions of biofilms (Alvarez-Ortega & Harwood, 2007).

The presence of surfactants in the studied systems could facilitate electron transfer mechanisms, given that the cellular membrane permeability is modified by surfactants (Sotirova et al., 2008). Previous studies on microbial electrochemistry have shown an increase in the electrical current generation or power generation after adding small doses of surfactants (Oluwaseun, 2015; Ren et al., 2012; Song et al., 2015), and vast reductions in the internal resistance (Pasternak et al., 2020). Furthermore, molecules such as phenazine-1-carboxamide (PCN), known electron shuttles (Rabaey et al., 2005), combined with surfactants were able to boost the current generation of a gram-positive bacteria (Pham et al., 2008).

3.7 Bioelectrochemistry as a booster for conventional downflow (aerobic) biofilters to treat wastewater

The role played by electroactive microorganisms in the performance of flooded METland® systems has already been demonstrated (Peñacoba-Antona et al., 2022b; Prado et al., 2020) to overcome microbial electron acceptor limitation typically present when anoxia meets any inert material bed. Indeed, the use of aeration was excluded from microbial electrochemistry in routinary basis, under the assumption that oxygen inhibits electroactive bacteria by competing with electrode for harvesting electrons from microbial metabolism. However, and according to the microbial community analysis, the electroconductive material supports the formation an anaerobic community capable of respiring the electroconductive bed, together with aerobic and facultative anaerobic microorganisms capable of using O_2, presumably, in the outer layers of the same biofilm. Actually, the presence of bacteria from *Geobacter* genus was previously reported in down-flow electroactive biofilter where oxygen was available (Aguirre et al., 2020). In this context, such complex electroactive metabolic pipeline helps regulate biomass formation and boost microbial metabolism.

This phenomenon could only be attributed to the properties of electroactive microbial communities forming the biofilm. An important contributing factor to this limited growth could be the biofilm's acidification due to proton accumulation as a consequence of the charge unbalances during electrode respiration (Franks et al., 2009). More recently, energetic considerations in the electron transfer mechanisms have been pointed out as growth regulating mechanisms, increasing product/biomass yields in the presence of electroactive microorganisms (Moscoviz et al., 2017). This phenomenon was confirmed and expanded with the demonstration of a hybrid metabolism (fermentation-respiration) that, through the use of extracellular electron transfer, alters the NAD+/NADH ratios, increases product/biomass yields, and increases cellular metabolic fluxes (Tejedor-Sanz et al., 2022).

The high treatment performance of electroactive biofilters, together with the long-term operation with limited biomass accumulation, suggest the feasibility of the technology for the treatment of wastewater polluted with surfactant content. According to the results, achieving sewer discharge limits for urban or industrial wastewater effluents is feasible.

The microbial resilience provided by the sheltering properties of the biofilm and of the biofilter bed material enables the survival of microbial communities despite the presence of pollutants in concentrations that are toxic under planktonic communities. Additionally, these properties allow a more diverse microbial community, including electroactive bacteria, in this manner, increasing the metabolic toolkit.

Finally, the results obtained in this study let us to conclude that passive-aerated electroactive biofilters are sustainable and efficient solution for biodegrading detergents at low energy cost and no sludge production, so we can anticipate their application to the treatment of laundry wastewater, car wash effluents, or similar waste, especially in remote sites with no access to sewer systems.

Credit authorship contribution statement

Eduardo Noriega: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **María Isabel López**: Analytical supervision, Formal analysis. **Abraham Esteve-Núñez:** Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest to the best of their knowledge that could influence the work reported in this article.

Acknowledgments

This work has been made possible by the Spanish Ministry of Science, Innovation and Universities (Grant Ref: DIN2018-009782) and the University of Alcalá (UAH) through the support of the Industrial PhD fellowship of Eduardo Noriega Primo. This research was co-financed by Metfilter S.L.

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