

Article

Rapid Biofilm Start-Up and Treatment of Oilfield Produced Water Using a Modified Polyurethane Biosponge Carrier

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Abstract

Oilfield-produced water contains petroleum hydrocarbons, suspended solids, salts and other refractory constituents that challenge conventional biological treatment. Suspended activated sludge systems can suffer from microbial washout, poor retention of slow-growing functional microorganisms and unstable settling, whereas conventional ceramic biofilm packings often provide limited surface area and slow biofilm start-up. Here, a modified polyurethane biosponge was evaluated as a three-dimensional carrier for immobilized microbial growth during the treatment of real oilfield produced water. Three parallel systems were compared under identical start-up and operating conditions: suspended activated sludge without a carrier, Raschig ring-packed biofilm reactors, and the biosponge-based composite system. The biosponge system developed a visible and stable biofilm within 7 days, whereas the Raschig ring system showed only sparse and uneven biofilm coverage. During an 8-day treatment test with real produced water, the biosponge system decreased the COD concentration from 800 to 13 mg L⁻¹ in the aerobic zone and from 1500 to 16 mg L⁻¹ in the anoxic zone, corresponding to removal efficiencies of 98.4% and 98.9%, respectively. In contrast, the activated sludge and Raschig ring systems showed much lower COD removal under the same test period. 16S rRNA gene sequencing indicated that the biosponge selectively enriched petroleum hydrocarbon-associated genera, including *Sphingopyxis*, *Achromobacter* and *Gordonia*, in the aerobic zone, together with fermentative and anaerobic genera such as *Dysgonomonas* and *Clostridium* in the anoxic zone. PICRUSt2-based functional prediction further suggested the coexistence of aerobic hydrocarbon degradation, chemoheterotrophy, fermentation and nitrate respiration potentials. These results indicate that the modified polyurethane biosponge provides a favorable carrier for rapid biofilm establishment and short-term removal of organic pollutants from oilfield produced water. Further long-term operation, adsorption controls and direct functional gene validation are recommended to confirm the engineering robustness and degradation mechanisms of the system. The system also avoided sludge bulking and maintained short-term stability, providing a promising low-cost strategy for treating high-salinity refractory oilfield produced water.

Keywords: oilfield produced water; biological sponge; biofilm carrier; immobilized microorganisms; petroleum hydrocarbon degradation; COD removal



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1. Introduction

Onshore oilfields in China have entered a high-water-cut development stage, resulting in the generation of large volumes of produced water [1,2]. Oilfield produced water is compositionally complex and typically contains petroleum hydrocarbons, suspended solids, sulfides, polymers and inorganic salts [2–4]. Among these components, petroleum hydrocarbons, including alkanes, cycloalkanes and aromatic hydrocarbons, are difficult to remove because of their chemical stability, hydrophobicity and potential toxicity [2,5]. Insufficient treatment of oilfield produced water poses environmental risks. During reinjection, produced water can corrode water injection pipelines, clog formation pore throats, and impair waterflooding efficiency. Direct discharge poses additional environmental risks, as residual contaminants may migrate into soils and surface waters, potentially disrupting aquatic ecosystems and compromising groundwater quality.

Biological treatment remains a compelling option for produced water due to its relatively low operational cost, minimal secondary pollution generation, and scalability for field applications [6]. Among biological processes, suspended activated sludge systems are still widely employed for treating produced water and other industrial wastewaters [7,8]. However, their performance deteriorates significantly when influent contains recalcitrant petroleum hydrocarbons—compounds exhibiting low biodegradability. Under such conditions, functional microorganisms are prone to washout with the effluent; slow-growing hydrocarbon degraders fail to achieve sufficient retention; and fluctuations in hydraulic or organic loading destabilize process performance [7,9]. Sludge bulking further compromises operational reliability [10]. Collectively, these limitations have motivated a strategic shift toward carrier-assisted biofilm systems, where microbial cells adhere to and grow on solid supports, enabling higher biomass retention, enhanced resistance to shock loads, and improved process stability [7,11].

In biofilm reactors, the carrier is an active functional component—not merely a passive substrate. It critically governs both startup kinetics and sustained treatment performance. Ideal carriers must provide a large accessible surface area, balanced hydrophilicity to promote initial microbial adhesion, interconnected pore channels facilitating mass transfer and biofilm stratification, robust mechanical integrity under continuous hydraulic shear, and protected micro-niches conducive to microbial colonization and community succession [11]. Conventional ceramic packings, such as Raschig rings, are cost-effective and mechanically durable, yet their smooth, non-porous surfaces and modest specific surface area hinder rapid biofilm initiation and limit the enrichment of specialized degraders [12]. In contrast, polyurethane sponges feature an open, three-dimensional porous architecture, high void fraction, and elastic deformability—attributes that collectively enhance microbial attachment strength, mitigate biofilm detachment under shear stress, and foster spatially heterogeneous microenvironments supporting functional niche partitioning [11,13,14].

This study evaluated a modified polyurethane biosponge as an immobilized carrier for treating real oilfield produced water. Its performance was benchmarked against two reference systems: a conventional suspended activated sludge process and a biofilm system packed with Raschig rings. Key evaluation metrics included biofilm formation kinetics, COD removal efficiency (particularly during early-stage operation), shifts in microbial community composition, and predicted functional profiles derived from 16S rRNA gene sequencing. The primary objectives were to assess whether the biosponge carrier could accelerate biofilm startup and improve short-term organic removal from authentic produced water—while concurrently elucidating the taxonomic and functional microbial traits correlated with superior treatment outcomes.

2. Materials and Methods

2.1. Reagents, Inoculum, Oilfield Produced Water, and Reactor Setup

The main reagents used in the experiment were as follows: Glucose ($C_6H_{12}O_6$, analytical grade) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ammonium chloride (NH_4Cl , analytical grade) and potassium dihydrogen phosphate (KH_2PO_4 , analytical grade) were obtained from Wengjiang Chemical Reagent Co., Ltd. (Shaoguan, China). Vitamin solution (containing vitamin B_1 , B_6 , riboflavin, etc.) and trace element solution (containing $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, etc.) were prepared in the laboratory according to standard formulations. Guaranteed reagent-grade concentrated sulfuric acid (H_2SO_4) and COD detection reagent kits were purchased from Guangzhou Hangcheng Technology Co., Ltd. (Guangzhou, China). Phosphate buffered saline (PBS) for microscopic observation was obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China).

Two types of microbial carriers were employed: one was a modified polyurethane three-dimensional network elastic biological sponge carrier with an interconnected internal porous structure (porosity $\geq 95\%$, specific surface area $\geq 3000 \text{ m}^2/\text{m}^3$); the other was a ceramic Raschig ring packing ($\Phi 10 \text{ mm} \times 10 \text{ mm}$, specific surface area approx. $320 \text{ m}^2/\text{m}^3$). The inoculum sludge was collected from the aerobic and anaerobic tanks of a municipal wastewater treatment plant, and the inoculation amount was 2% (v/v) of the effective reactor volume. The experimental feed water was actual oilfield produced water collected from a gathering station of an onshore oilfield in China. The water quality characteristics were as follows: aerobic zone influent COD 800 mg/L, anoxic zone influent COD 1500 mg/L, pH 7.2–7.8, and oil content 126 mg/L.

The experimental setup included a sequential batch anoxic-aerobic moving bed biofilm reactor; a YZ-1515X aeration pump (Shenzhen Yuanzheng Electromechanical Co., Ltd., Shenzhen, China); an HD-850 ultra-clean workbench (Jinan Senya Experimental Instrument Co., Ltd., Jinan, China); an AK-480 ultrasonic cleaner (Shenzhen Yujie Cleaning Equipment Co., Ltd., Shenzhen, China); a UV-1800 ultraviolet spectrophotometer (Agilent Technologies (China) Co., Ltd., Beijing, China); a CX23 optical microscope (Olympus Corporation, Tokyo, Japan); and a PHS-3E pH meter (Thermo Fisher Scientific (China) Co., Ltd., Shanghai, China).

2.2. Preparation of Modified Sponge

Polyurethane sponges were sequentially ultrasonically cleaned with anhydrous ethanol and deionized water for 15 min each to eliminate surface impurities and residual processing additives, followed by drying to constant weight in a vacuum oven at 60°C . The pretreated sponges were then immersed in an anhydrous toluene solution containing 5% (v/v) 1,6-hexamethylene diisocyanate (HDI) and 1% (v/v) triethylamine (TEA). The activation reaction was conducted at 50°C for 1 h under a nitrogen atmosphere, during which hydroxyl groups on the sponge skeleton surface reacted with HDI to introduce reactive isocyanate ($-NCO$) groups. Upon completion of activation, the sponges were rinsed thoroughly with anhydrous toluene to remove all unreacted HDI residues. For the grafting procedure, the activated sponges were transferred into a 10 g/L anhydrous toluene solution of methoxy polyethylene glycol (mPEG), with a trace amount of dibutyltin dilaurate (DBTDL) added as the catalyst. The reaction was performed at 40°C for 24 h under magnetic stirring and nitrogen protection, enabling the covalent grafting of mPEG chains onto the sponge surface. After the reaction, the resultant modified sponges were sequentially ultrasonically washed with anhydrous toluene, anhydrous ethanol and deionized water to completely remove physisorbed mPEG and residual solvents. Finally, the

samples were dried to constant weight in a vacuum oven at 50 °C and stored in a desiccator prior to use.

2.3. Reactor Configuration and Experimental Design

Four parallel control systems were established in this study, all operated under identical initial nutrient conditions and environmental operational parameters.

The activated sludge system (AS), with no carrier material supplemented, served as the conventional suspended-growth control. The Raschig ring system (RR) was packed with ceramic Raschig rings at a volume packing ratio of 30% (v/v), with a sludge inoculum dose equivalent to 2% of the effective reactor volume. The modified biosponge system (BS) was packed with surface-modified polyurethane biosponges at the same packing ratio of 30% (v/v), with an identical sludge inoculation ratio of 2% of the effective reactor volume. The non-inoculated biosponge system (NBS) was packed with the same surface-modified polyurethane sponges at a 30% (v/v) packing ratio, with no sludge inoculation introduced. Detailed configurations of all systems are summarized in Table 1.

Table 1. Experimental groups used for comparison.

Group	Carrier	Filling Ratio	Microbial Growth Mode
AS	None	0	Suspended activated sludge
RR	Raschig rings	30% (v/v)	Attached biofilm on ceramic packing
BS	Modified polyurethane biosponge	30% (v/v)	Immobilized biofilm in a porous elastic carrier
NBS	Modified polyurethane biosponge	30% (v/v)	None

The experiment was conducted in three consecutive phases. First, a 7-day biofilm start-up phase was implemented. In the aerobic zone, a sealed batch aeration mode was adopted with the inlet and outlet valves closed and only the aeration device in operation. The aeration intensity was controlled at 0.6 vvm, which ensured sufficient mixing of the system via aeration agitation, and the dissolved oxygen (DO) concentration was maintained at 2.0–3.0 mg/L. In the anaerobic zone, a sealed batch cultivation mode was applied, and low-speed mechanical stirring at 80 r/min was employed to ensure uniform mixing, with the oxidation–reduction potential (ORP) controlled within the range of −150 to −250 mV. Throughout the start-up phase, the reactor temperature was kept constant at 25 ± 1 °C. The mass ratio of carbon to nitrogen to phosphorus (C:N:P) was maintained at approximately 100:5:1 in the aerobic zone and 200:5:1 in the anaerobic zone, and vitamins and trace elements were supplemented in both zones. Water quality parameters and biofilm development were monitored daily during the entire start-up phase. Once initial biofilm formation was confirmed by the observation of a dense microbial layer on the carrier surface, the synthetic medium was replaced with raw oilfield produced water, and the reactor entered the continuous-flow steady-state operation phase. During steady-state operation, the hydraulic retention time (HRT) was set to 12 h for the aerobic zone and 24 h for the anaerobic zone. The temperature, aeration intensity, dissolved oxygen, oxidation–reduction potential and mixing conditions remained consistent with those in the start-up phase, and the steady-state operation lasted for 8 days.

During this evaluation period, mixed water samples were collected daily from both the aerobic and anaerobic zones for chemical oxygen demand (COD) analysis. At the end of the experiment, biofilm samples were carefully scraped from the carrier surface, and suspended sludge samples were collected from the bulk liquid of the reactor. Both types of

samples were immediately cryopreserved at $-80\text{ }^{\circ}\text{C}$ for subsequent high-throughput 16S rRNA gene sequencing and microbial community structure analysis.

2.4. Analytical Methods

Chemical oxygen demand (COD) was determined using the standard potassium dichromate digestion method (APHA 5220C) and quantified spectrophotometrically at 600 nm with a UV-1800 ultraviolet–visible spectrophotometer (Shimadzu, Japan) [15]. Solution pH was measured in triplicate using a calibrated PHS-3E precision pH meter. Biofilm development was tracked qualitatively via time-lapse photographic documentation under standardized lighting and magnification; microbial morphology and spatial distribution within biofilms were further examined using bright-field microscopy on a CX23 optical microscope (Olympus, Japan) at $400\times$ magnification. Sludge settleability was assessed by the standard 30 min sludge volume index (SVI_{30}) test, and mixed-liquor volatile suspended solids (MLVSS) were quantified via gravimetric analysis following standard APHA protocols. COD removal efficiency was calculated as

$$\text{Removal efficiency (\%)} = (C_0 - C_t)/C_0 \times 100$$

where C_0 and C_t represent the initial COD concentration and the COD concentration at each sampling time, respectively.

2.5. Microbial Community and Functional Prediction Analysis

The microbial community was analyzed by sequencing the V3 + V4 region of the bacterial 16S rRNA gene on the Illumina MiSeq platform. The obtained sequences were aligned and taxonomically assigned using the SILVA database [16]. Putative functional profiles were further inferred from the 16S rRNA gene data with PICRUSt2 [17]. Because PICRUSt2 is based on functional prediction rather than direct gene or transcript measurements, the results were used only to indicate potential metabolic capacities and should not be regarded as direct evidence of gene expression or microbial activity [18].

2.6. Statistical Analysis

All experimental results are presented in the form of mean $\pm$ standard deviation. Each experimental group was set with three independent parallel reactors ($n = 3$), and the data shown are biological replicates from independent reactors, rather than analytical replicates from a single sample. One-way ANOVA followed by Tukey's post hoc test was performed using SPSS 26.0 (IBM, Armonk, NY, USA) to compare significant differences in COD removal and biofilm biomass across all systems. A p -value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Material Characterization of Modified Polyurethane Biosponges

3.1.1. Microscopic Morphology Characterization

Scanning electron microscopy (SEM) was employed to characterize the microscopic morphology of PEG-grafted polyurethane biosponges. Observations were conducted at two scales, namely the macroscopic pore structure and microscopic surface features, and the results are presented in Figure 1.

Low-magnification images (Figure 1a) reveal that the sponges fully retain the three-dimensional (3D) interconnected network porous structure of the polyurethane matrix after surface grafting modification. The polymer skeletons are continuous, uniform and well-defined, with open and interpenetrated pores; no structural damage such as pore collapse, blockage or skeleton fracture is detected. With reference to the $200\text{ }\mu\text{m}$ scale

bar, the pore size of the material mainly ranges from 100 to 300 μm , corresponding to a typical macroporous carrier structure [19]. This continuous and open 3D pore system serves two critical functions: it provides ample space for microbial attachment and growth, enabling high biomass retention; meanwhile, it ensures efficient mass transfer of wastewater substrates, dissolved oxygen and metabolic products throughout the pore network, creating favorable structural conditions for the growth and metabolism of microorganisms embedded in the biofilm.

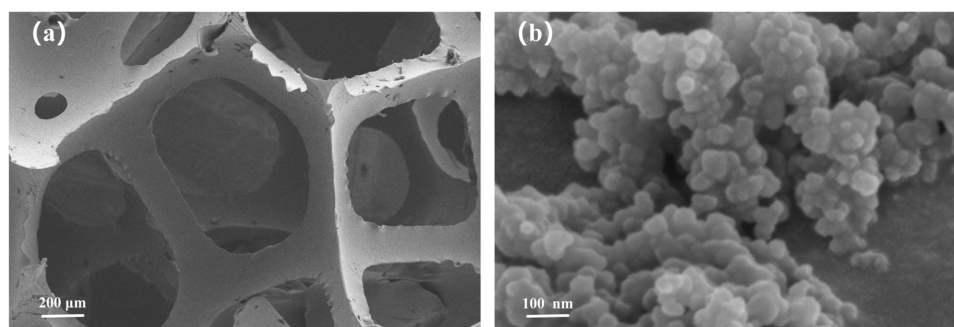


Figure 1. SEM images of PEG-grafted polyurethane sponges: (a) three-dimensional macroporous skeleton structure at low magnification; (b) microscopic surface morphology post-grafting at high magnification.

High-magnification observation of the skeleton surface (Figure 1b) demonstrates that at the nanoscale, the modified sponge skeleton surface exhibits uniformly distributed granular protrusions. Compared with the intrinsically smooth surface of the pristine matrix, the surface roughness is remarkably enhanced, forming a dense molecular modification layer. This morphological feature corresponds to the aggregation state of PEG molecular chains after covalent grafting onto the sponge surface, confirming that the modification effect is confined to the material surface. The nanoscale rough surface effectively increases the actual specific surface area of the carrier, providing more physical anchoring sites for microbial cells. Furthermore, it strengthens the mechanical interlocking between the carrier and microbial extracellular polymeric substances (EPS), thus improving the interfacial bonding strength between the biofilm and the carrier [20–22]. This contributes to shortening the lag phase of initial microbial adhesion and enhancing the stability of the biofilm under hydraulic shear stress.

Overall, the PEG grafting modification process employed in this study well preserves the intrinsic porous framework of the polyurethane matrix without causing structural damage, and simultaneously achieves precise regulation of the surface microscopic morphology. By virtue of the dual advantages of macroscopic structure retention and microscopic interface optimization, the modified sponge provides a solid morphological basis for rapid biofilm formation and stable operation of the subsequent biofilm system.

3.1.2. Characterization of Surface Chemical Structure

Fourier transform infrared spectroscopy (FTIR) was employed to characterize the surface chemical functional groups of the PEG-grafted polyurethane sponges. The measurement was performed in transmission mode with a scanning wavenumber range of 4000–400 cm^{-1} . The obtained spectra were processed with smoothing and baseline correction prior to functional group analysis, and the results are presented in Figure 2.

The spectrum in the high-wavenumber functional group region (4000–2500 cm^{-1}) shows a broad absorption peak at 3284 cm^{-1} . This peak is attributed to the N–H stretching vibration of urethane groups in the polyurethane matrix, and forms an overlapping absorption band with the O–H stretching vibration of terminal hydroxyl groups on PEG

molecular chains. Two distinct characteristic peaks appear at 2969 cm^{-1} and 2867 cm^{-1} , corresponding to the asymmetric and symmetric C–H stretching vibrations of aliphatic methylene and methyl groups, respectively. These signals are jointly contributed by the hydrocarbon backbones of the polyurethane skeleton and the grafted PEG chains [23]. The characteristic peaks in this region confirm that the modification process does not disrupt the aliphatic hydrocarbon segments of the material, and the intrinsic molecular framework of the polyurethane matrix is fully preserved.

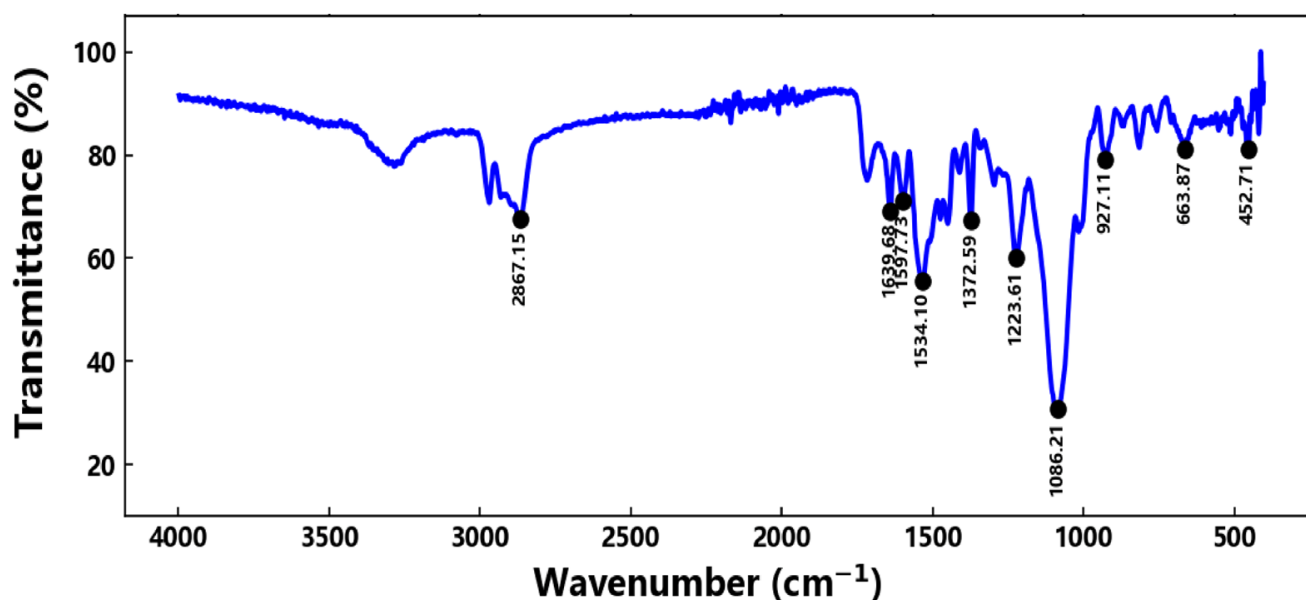


Figure 2. Fourier transform infrared (FTIR) spectrum of PEG-grafted polyurethane sponge.

In the PEG-specific characteristic region ($1200\text{--}1000\text{ cm}^{-1}$), a prominent strong absorption peak is detected at 1086 cm^{-1} , which is assigned to the stretching vibration of aliphatic C–O–C ether bonds. Polyethylene glycol molecules are composed of abundant repeating ethoxy units, endowing their molecular chains with a large number of aliphatic ether bond structures [24]. This intense absorption peak serves as the core spectroscopic evidence for the successful introduction of PEG molecules onto the sponge surface, and also provides the most direct structural confirmation of the surface grafting modification.

This surface chemical modification effectively improves the surface hydrophilicity and interfacial bonding capacity of the carrier, providing a favorable chemical interface foundation for the initial adhesion of microorganisms and the stable growth of biofilms.

3.1.3. Characterization of Surface Wettability

The surface wettability of the PEG-grafted polyurethane sponge was measured via the sessile drop method using a contact angle goniometer. All tests were conducted at room temperature with deionized water as the test liquid. Parallel measurements were performed at multiple randomly selected positions on the sample surface, and a typical test result is presented in Figure 3.

The test results show that the left and right contact angles of the PEG-grafted sponge are 81.83° and 80.89° , respectively, with an average water contact angle of 81.36° . The deviation between the two sides is less than 1° , indicating favorable uniformity of surface wettability on the modified sponge and a relatively homogeneous grafting distribution of PEG molecules across the carrier surface [25,26]. The enhanced surface hydrophilicity exerts a prominent promoting effect on the start-up and stable operation of the biofilm carrier system. On one hand, the hydrophilic surface enables faster and more thorough

infiltration of bacterial suspension into the three-dimensional internal pores of the sponge, which increases the effective contact probability between microbial cells and the carrier surface, shortens the lag phase of initial microbial colonization, and accelerates the biofilm start-up process of the reactor. On the other hand, surface polar groups can establish stronger interfacial adhesion with microbial extracellular polymeric substances (EPSs) through hydrogen bonding, thus improving the adhesion strength between the biofilm and the carrier [27]. This effect reduces biomass detachment under aeration and hydraulic shear stress, and supports stable growth and operation of the biofilm.



Figure 3. Static water contact angle of the PEG-grafted polyurethane sponge.

3.2. Biofilm Formation Process on Different Carriers

Samples were collected on day 3 (start-up phase) and day 8 (stable phase) of biofilm cultivation, respectively, and the dry weight of biofilm attached per unit mass of carrier was determined and calculated.

The comparison of biofilm dry weight across different carrier systems on day 3 and day 8 is presented in Figure 4. On day 3 of biofilm start-up, the biofilm dry weight of the PEG-grafted polyurethane sponge (BS) reached 81.3 mg/g, which was 2.05 times that of the NBS group and 3.68 times that of the RR group, and was also significantly higher than the biomass level of the AS group. This quantitative result directly confirms that PEG hydrophilic modification can remarkably accelerate the initial colonization rate of microorganisms on the carrier surface, enabling the modified sponge to achieve higher biomass enrichment at the early stage of biofilm formation and effectively shortening the biofilm start-up period.

After the system entered the stable operation phase on day 8, the biofilm dry weight of the BS carrier further increased to 186.0 mg/g, remaining significantly higher than that of the other three control groups. This indicates that the modified sponge not only features a faster start-up rate but also exhibits superior biomass loading capacity under steady operation. Its three-dimensional interconnected porous structure provides sufficient attachment space for microorganisms and protection against hydraulic shear stress, thus achieving higher biomass retention. After cultivation, only sparse and unevenly distributed biomass was observed on the surface of Raschig rings. The biofilm attached to the Raschig rings remained thin and discontinuous, with a highly heterogeneous distribution across the carrier surface (Figure 4b). This weak colonization phenomenon is most likely associated with the inherent properties of ceramic fillers, particularly their relatively smooth surface and limited specific surface area. These properties result in fewer initial microbial attachment sites and fail to provide sufficient protective space for biomass within the reactor [28,29]. In contrast, a brown gelatinous biofilm was formed on the outer surface of the sponge, extending along the visible pore walls (Figure 4c). Microscopic observations of

microorganisms on the biofilm are presented in Figure S1. The superior initial performance of the biosponge can be attributed to its interconnected porous structure, high porosity, and hydrophilic modified surface properties [30]. These features not only provide abundant colonization sites but also enhance the contact between microorganisms and the carrier, and help reduce biomass loss under hydraulic shear stress. Furthermore, the elastic porous matrix provides local protection for attached cells, thereby facilitating the stable retention of microorganisms during the initial operation phase.

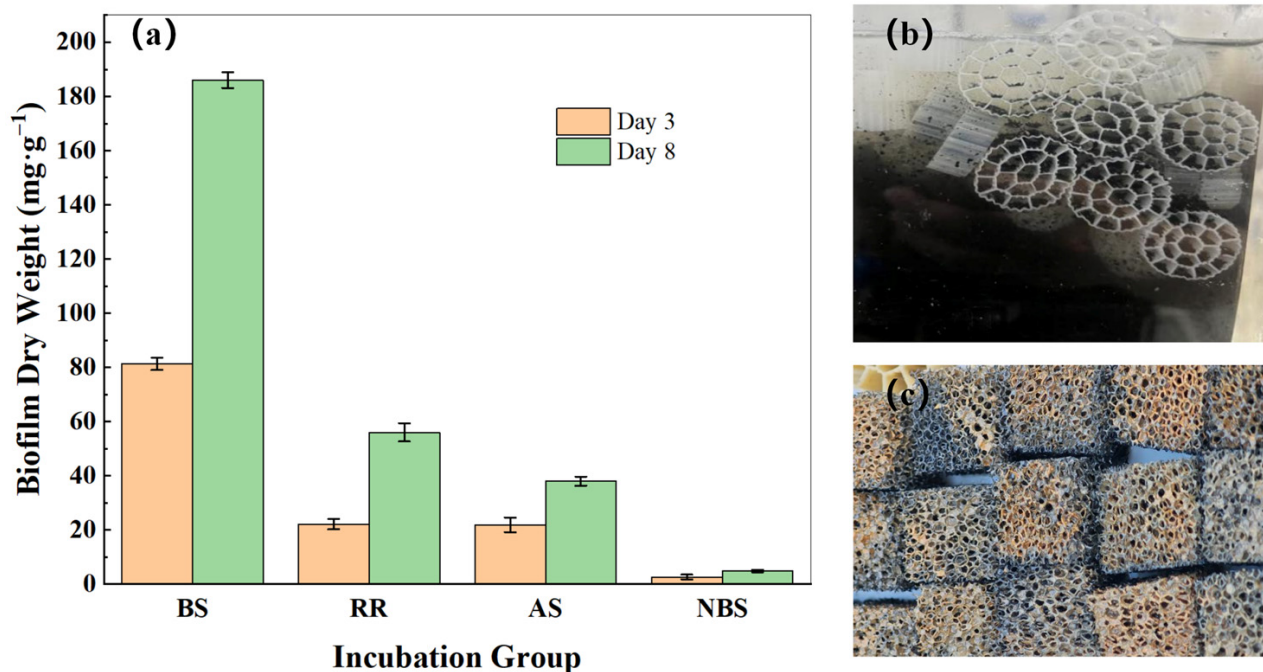


Figure 4. Characterization of biofilm formation performance in different carrier systems: (a) biofilm dry weight on day 3 and day 8; (b) macroscopic morphology of biofilm on RR carrier; (c) macroscopic morphology of biofilm on BS carrier.

The fast and stable biofilm formation on the biological sponge carriers was mainly related to their material characteristics and structural features. The sponge has a three-dimensional interconnected porous network, which provides a much larger available surface area than conventional granular or honeycomb packings [31]. Previous studies have shown that three-dimensional bioelectrode reactors using polyurethane sponge as a biological carrier exhibit excellent treatment performance for pharmaceutical wastewater with complex composition and poor biodegradability [32].

3.3. Treatment Efficiency of Different Systems for Oilfield Produced Water

After biofilm formation was completed, all reactors were switched to treat actual oilfield produced water for continuous operation.

In the aerobic zone, the modified biosponge system (BS) reduced the COD concentration from 800 mg/L to 13 mg/L rapidly within the 8-day test period, achieving a COD removal efficiency of 98.4%. To clarify the contribution of physical adsorption by the sponge carrier itself to COD removal and eliminate its interference with the evaluation of biofilm degradation performance, the non-inoculated modified sponge system (NBS) was set up as the adsorption control group. In the NBS group, where no microbial degradation occurs and organic matter is retained solely via physical adsorption of the carrier, the effluent COD concentration reached 647.1 mg/L after 8 days of operation, indicating that biodegradation accounted for the majority of the total COD removal. In comparison, the

COD concentrations in the Raschig ring system (RR) and the activated sludge system (AS) only decreased to 593.1 mg/L and 723.5 mg/L, respectively (Figure 5a). These results demonstrate that the biosponge carrier significantly enhances the short-term organic matter removal performance under the tested aerobic conditions.

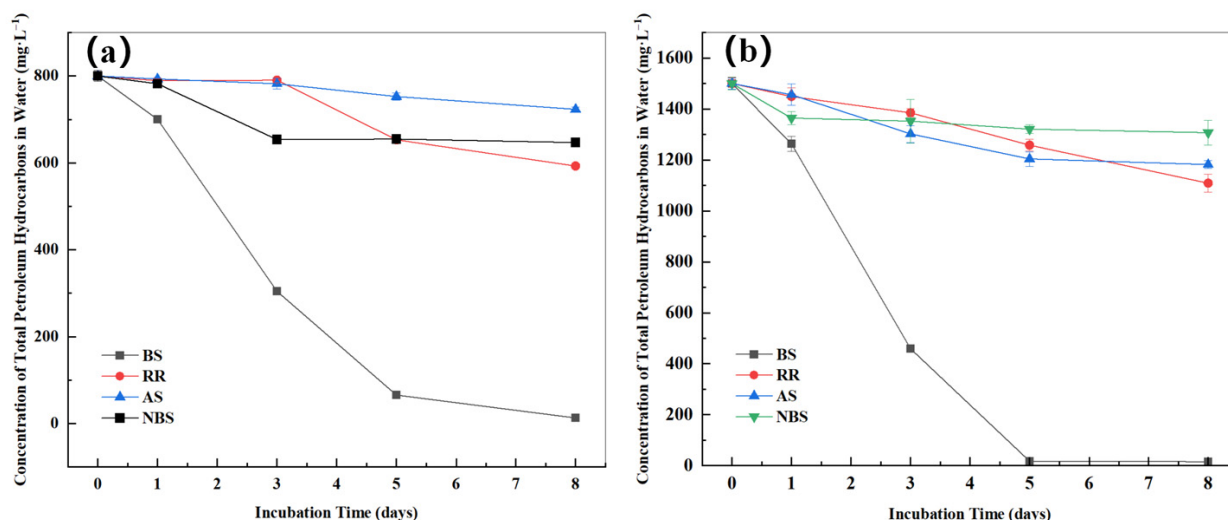


Figure 5. COD concentration in the wastewater of the AS, RR, NBS and BS systems during the 8-day treatment test.

A similar trend was observed in the anoxic zone. The BS system reduced the COD concentration from 1500 mg/L to 16 mg/L, corresponding to a removal efficiency of 98.9%. The COD removal amount via adsorption in the NBS group was 193 mg/L, confirming that biodegradation played a dominant role in the overall removal. In contrast, the final COD concentrations of the RR system and the AS system were 1109 mg/L and 1182 mg/L, respectively (Figure 5b). The COD concentration in the BS system decreased significantly faster, indicating that the immobilized biomass within the porous carrier provides a more favorable microenvironment for organic matter degradation than suspended biomass or ceramic fillers under the tested conditions.

The superior performance of the biosponge system can be attributed to several carrier-associated effects. First, the large accessible surface area and interconnected pores likely increased biomass retention and contact between microorganisms and dissolved or emulsified organics. Second, the porous matrix may have created local oxygen gradients as oxygen was consumed by microorganisms near the outer surface and diffusion into the inner pores became limited [33,34]. Such gradients could allow aerobic and oxygen-limited metabolic processes to coexist in the same carrier [11]. Third, the immobilized growth mode reduced biomass washout, allowing functional degraders to accumulate during start-up and operation [35]. Nevertheless, direct evidence of internal oxygen gradients and adsorption contributions is still needed to further validate this mechanism.

3.4. Microbial Community Differentiation in the Biosponge System

16S rRNA gene sequencing was used to examine the microbial community structure in the biosponge system at the end of operation. At the phylum level (Figure 6), the aerobic zone was dominated by Proteobacteria and Bacteroidota, with relative abundances of 52.2% and 26.4%, respectively. These phyla are commonly associated with organic matter degradation and complex substrate transformation [36,37]. In the anoxic zone, Bacteroidota and Proteobacteria were also dominant, with relative abundances of 37.1% and 33.2%, respectively. The increased abundance of Bacillota in the anoxic zone suggests

that oxygen-limited niches may have promoted the retention of fermentative and anaerobic microorganisms [38].

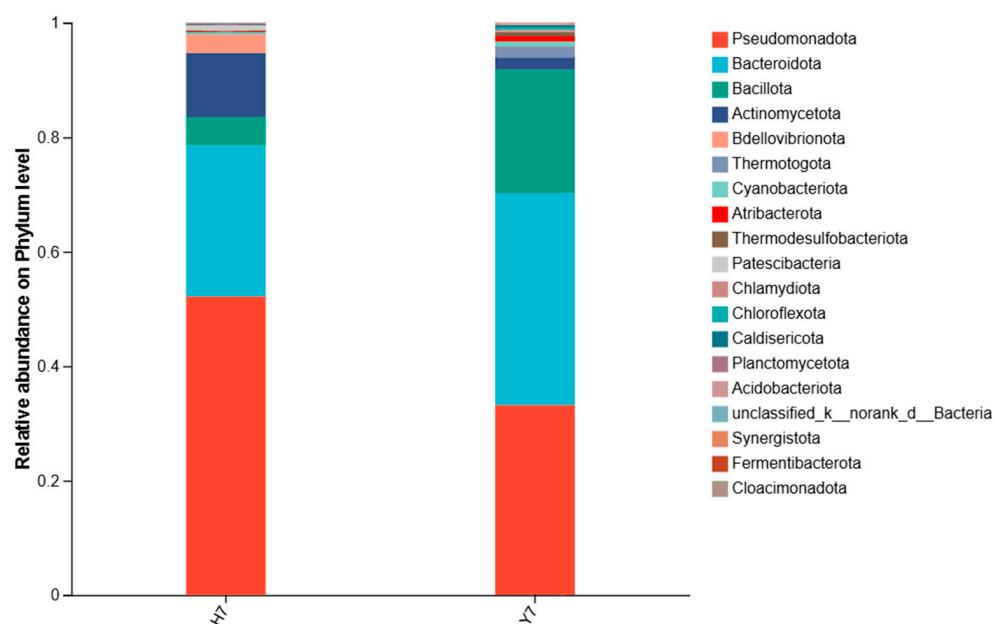


Figure 6. Microbial community composition at the phylum level in the BS system at the end of operation.

At the genus level (Figure 7), the aerobic zone was enriched with *Sphingopyxis*, *Achromobacter* and *Gordonia*, with relative abundances of 11.6%, 10.97% and 9.5%, respectively. These genera have previously been linked to the degradation of petroleum hydrocarbons or aromatic compounds, suggesting that the aerobic region of the biosponge system promoted the enrichment of hydrocarbon-related degraders. *Sphingopyxis* mediates the ortho-cleavage degradation of polycyclic aromatic hydrocarbons via ring-hydroxylating dioxygenases; *Achromobacter* achieves oxidative degradation of alkanes and BTEX through alkane monooxygenase systems and biosurfactant secretion; and *Gordonia* catalyzes the terminal oxidation of long-chain n-alkanes and steranes via the AlkB hydroxylase system [39–41]. In the anoxic zone, *Dysgonomonas* became the predominant genus, accounting for 21.2% of the community, while *Clostridium* was also present, with a relative abundance of 4.1%. *Dysgonomonas* is commonly regarded as a fermentative bacterium and can convert complex organic matter into smaller organic acids, alcohols and hydrogen. These degradation products may subsequently serve as carbon or energy substrates for other anaerobic microorganisms, thereby supporting the continuation of anaerobic degradation processes [42]. The fact that we found these fermentative and anaerobic genera is consistent with the idea that oxygen-limited microenvironments may have formed inside the biosponge carrier. Under continuous aeration, oxygen penetrates from the outer surface of the biosponge carrier into its internal pore channels along with the bulk liquid. Jointly affected by oxygen consumption from the surface-attached biofilm and mass transfer resistance of the porous skeleton, the dissolved oxygen concentration within the carrier declines progressively from the outer layer to the deep interior, naturally forming a continuous redox microgradient spanning aerobic, anoxic and anaerobic microzones. This spatial gradient provides differentiated ecological niches for functional microorganisms with distinct oxygen requirements.

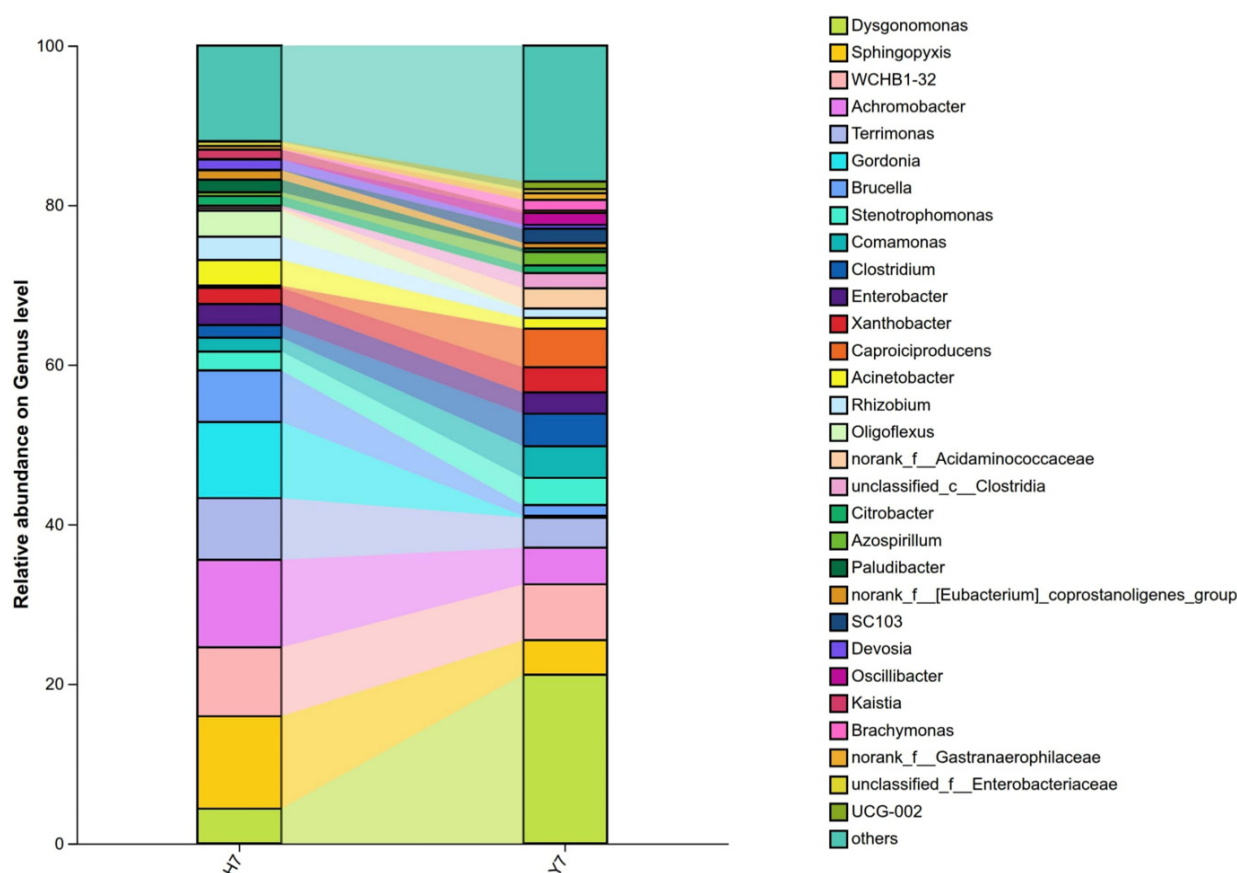


Figure 7. Microbial community composition at the genus level in the BS system at the end of operation. “Others” represents genera with relative abundance lower than 1%.

Overall, the microbial community analysis indicates that the biosponge carrier not only increased biomass retention, but also influenced community differentiation. Hydrocarbon-associated aerobic bacteria were more abundant in oxygen-exposed regions, whereas fermentative and anaerobic taxa were preferentially enriched under oxygen-limited conditions. This spatial differentiation may support cooperative transformation of complex organics in produced water. However, direct functional assays, qPCR of hydrocarbon-degradation genes or metagenomic sequencing would be required to confirm the specific degradation pathways.

3.5. Predicted Functional Profiles

PICRUSt2 was used to infer putative metabolic functions from the 16S rRNA gene data (Figure 7). The predicted functional profile of the aerobic zone showed high relative potentials associated with aromatic compound degradation, hydrocarbon degradation, aerobic chemoheterotrophy and general chemoheterotrophy. These predictions are consistent with the enrichment of *Sphingopyxis*, *Achromobacter* and *Gordonia* and support the hypothesis that the aerobic region contributed to oxidative degradation of petroleum-derived organic matter.

The anoxic zone showed predicted enrichment of functions related to fermentation, nitrate respiration and denitrification. These results suggest that oxygen-limited regions of the biosponge may contribute to the conversion of complex organic substrates into smaller intermediates and may support nitrogen transformation when suitable electron acceptors are present. Because these functions were predicted from 16S rRNA gene profiles rather than directly measured by metagenomics, metatranscriptomics or qPCR, they should be interpreted as metabolic potentials. The predicted functional patterns nevertheless

provide a microbiological explanation for the observed differences between the aerobic and anoxic zones.

3.6. Operational Stability and Practical Implications

During the 8-day treatment test, no obvious sludge bulking was observed in the biosponge system. This behavior can be explained by the immobilization of biomass inside the carrier matrix, which reduces the dependence of system performance on suspended sludge settleability. In contrast, suspended activated sludge systems are more susceptible to biomass washout and settling deterioration under variable hydraulic or loading conditions. The biosponge carrier therefore offers a practical route to improve biomass retention and effluent stability in produced water treatment (see Figure 8).

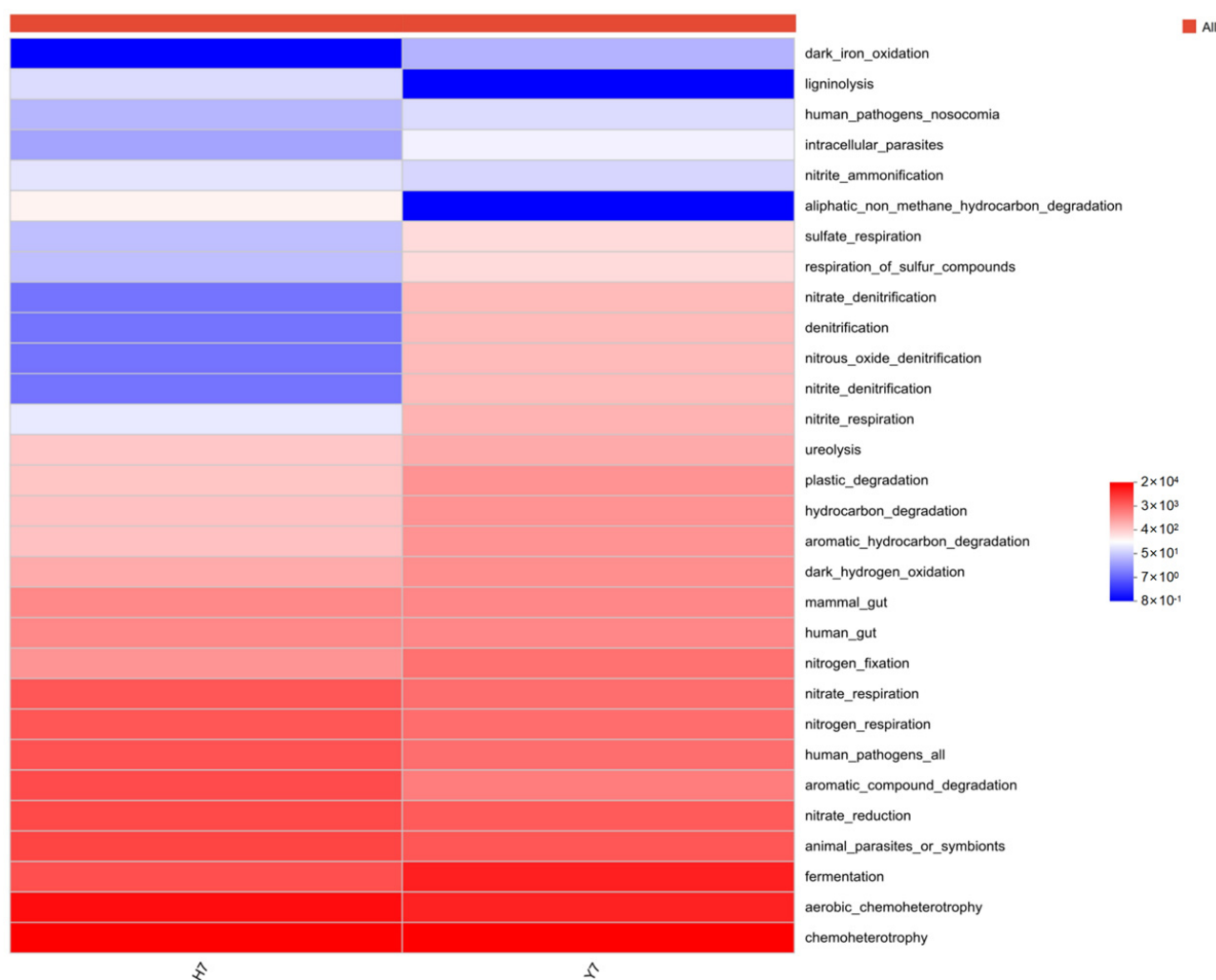


Figure 8. Heatmap of predicted microbial functions in the BS system at the end of operation. The heatmap should be replotted after removing irrelevant categories and retaining functions related to hydrocarbon degradation, aromatic compound degradation, aerobic chemoheterotrophy, fermentation, nitrate respiration and denitrification.

The present results demonstrate the potential of modified polyurethane biosponges as low-cost biofilm carriers for rapid start-up and short-term organic removal from oilfield produced water. However, longer operation under realistic hydraulic conditions is still required before engineering application. In particular, the stability of the system should be evaluated under changes in COD loading, oil concentration, salinity and hydraulic retention time. The contribution of physical adsorption should also be separated from

biodegradation using sterile carrier controls. These additional tests would strengthen the evidence for the durability and treatment mechanism of the biosponge system.

4. Conclusions

A modified polyurethane biosponge carrier was evaluated for immobilized microbial treatment of real oilfield produced water. Compared with suspended activated sludge and Raschig ring-packed biofilm systems, the biosponge system showed faster biofilm start-up and substantially higher COD removal during an 8-day treatment test. The biosponge decreased COD from 800 to 13 mg L⁻¹ in the aerobic zone and from 1500 to 16 mg L⁻¹ in the anoxic zone, corresponding to removal efficiencies of 98.4% and 98.9%, respectively. Microbial community analysis showed enrichment of hydrocarbon-associated genera, including *Sphingopyxis*, *Achromobacter* and *Gordonia*, in the aerobic zone, together with fermentative and anaerobic genera such as *Dysgonomonas* and *Clostridium* in the anoxic zone. PICRUSt2 prediction suggested complementary metabolic potentials related to aerobic hydrocarbon degradation, chemoheterotrophy, fermentation and nitrate respiration. These results suggest that the modified polyurethane biosponge provides a porous immobilization microenvironment suitable for rapid biofilm establishment and short-term produced water treatment. Further verification is still required. In particular, sterile adsorption controls, extended continuous operation, direct measurement of internal oxygen gradients, and functional gene validation would help clarify the degradation mechanism and evaluate the engineering robustness of the system.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr14142290/s1>, Figure S1: Micrographs of the sample: (a) Low-magnification view; (b) High-magnification view.

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