



Implementation of Nature-Based Biofilm Carriers for Sustainable Bioremediation: A Novel MBBR Approach to Treat Fishery Effluents in the Greater Casablanca Region

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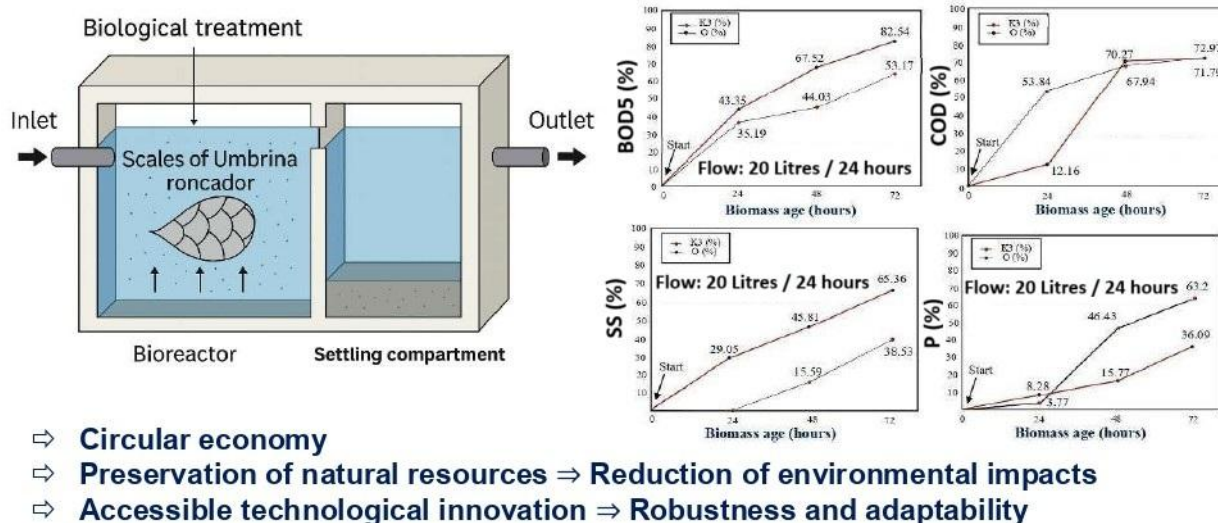
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Graphical abstract

This study presents a compact, nature-based MBBR system for treating fishery effluents in Greater Casablanca. The reactor has two compartments: the first supports biological treatment using *Penicillium chrysogenum* with *Umbrina roncadore* fish scales as biofilm carriers; the second is a settling chamber. Wastewater enters at the base, passes through the biofilm zone, and rises into the settling section. After 72 hours, marked reductions in BOD₅, COD, suspended solids, and phosphorus were observed. Natural carriers performed better than synthetic media across several parameters. The system aligns with circular economy principles and offers a sustainable, low-cost option for wastewater treatment.



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Abstract: Fish processing industries produce large volumes of organic-rich effluents, especially in coastal cities such as Casablanca. This study examines the performance of a nature-based Moving Bed Biofilm Reactor (MBBR) using *Penicillium chrysogenum* and *Umbrina roncadore* fish scales as natural biofilm carriers. The reactor included two compartments: one for biological treatment and one for settling. Real fishery wastewater (20 L/day) was treated for 72 hours under controlled conditions. The system achieved notable removal rates: 82.5% for BOD₅, 72.9% for COD, 65.4% for suspended solids, and 36.1% for total phosphorus. Kaldnes K3 carriers were tested in parallel. These showed greater nitrogen and phosphorus removal. Natural carriers performed better for BOD₅ and suspended solids. The findings confirm the feasibility of using fish scales as low-cost, sustainable supports in biological treatment. This approach supports circular economy principles and contributes to SDGs 6 and 12.

Keywords: Fishery effluents, Natural biofilm carriers, MBBR.

Introduction

The Moroccan fishery sector accounts for 15% of national exports and 50% of agri-food exports. Morocco is the leading fish producer in Africa, mainly through sardine (*Sardina pilchardus*), and ranks 25th globally, contributing 1.2% of world production. With more than 3,500 km of coastline, a fleet of nearly 3,000 vessels, and expanding port infrastructure, the country is a key player in both the Arab world and Africa. Casablanca holds a prominent position among Atlantic ports, alongside Agadir, Dakhla, and Laâyoune, in terms of both landing volume and processing capacity [1].

Processing units generate large volumes of saline wastewater rich in organic matter, fats, and oils. Effluent composition varies depending on the type of product processed [2–5]. When discharged without adequate treatment, these effluents pose a major risk to coastal water quality, marine ecosystems, and public health. This is a growing concern for Morocco, which faces increasing water stress and is approaching the threshold of water scarcity. Improving water reuse and safeguarding available water resources is a national priority [2].

In this context, the treatment of fishery effluents is critical for both the environment and public health. Biological processes, such as activated sludge and biofilm-based systems, have demonstrated both efficiency and cost-effectiveness in wastewater treatment [6]. Conventional suspended biomass processes, though widely applied, show several limitations. These include high sludge production, operational instability, and vulnerability to fluctuations in pollutant loads.

Accordingly, Moving Bed Biofilm Reactors (MBBRs) have emerged as a promising solution. They combine the benefits of suspended and attached biomass technologies. MBBRs offer greater operational stability, shorter retention times, lower sludge output, and enhanced microbial reuse. These features make them well suited to meet current performance and sustainability demands [2,6,7].

Although the MBBR process is recognized for its high performance, ease of implementation, and adaptability to varying loads, its large-scale deployment generally relies on plastic-based carriers such as Kaldnes media. This approach presents several environmental limitations. These carriers are derived from non-renewable fossil resources, and their production contributes to the system's carbon footprint. They have a limited lifespan and must be replaced periodically. Ageing, fragmentation, or loss of carriers within the reactor can lead to the release of microplastics into receiving waters, adding to aquatic pollution.

To avoid shifting the pollution burden, natural, biodegradable, or bio-based alternatives are now being explored as more sustainable options. Natural biofilm carriers such as fish scales, plant-based materials, and revalorized organic waste provide an environmentally sound response, maintaining biological treatment efficiency [8-10]. These materials, low-cost and derived from industrial co-products, support circular economy strategies and organic waste valorization. Their high surface area

and hydrophilic properties promote microbial adhesion and growth, offering an effective base for biofilm development [9,11].

The efficiency of fishery effluent treatment depends closely on the selected biomass and the method of inoculation. One study showed that using natural carriers, such as sardine scales or snail shells, with filamentous fungi like *Penicillium chrysogenum*, led to the formation of robust biofilms. These systems tolerated saline conditions and achieved marked reductions in chemical oxygen demand (COD), suspended solids, and total nitrogen [2,6,9,12] (Fig. 1).

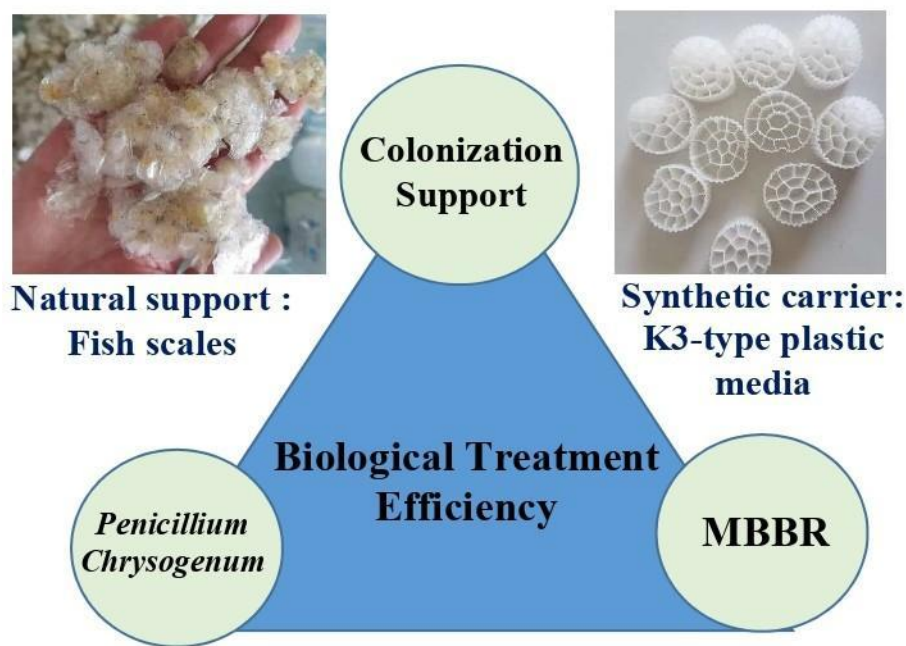


Figure 1. Key Components Influencing Biological Treatment Efficiency in MBBR Systems Using *Penicillium chrysogenum* and Natural Colonization Supports.

Moroccan regulations apply the polluter-pays principle and require strict control of industrial discharges. International standards are also tightening, particularly regarding BODs, total nitrogen, and total phosphorus [13]. In practice, industrial units are required to treat their own effluents or face regulatory penalties, environmental levies, or rising compensation costs. The Moroccan legal framework, notably Laws No. 11-03 and No. 99-12 [14,15], penalizes non-compliant discharges and imposes fees based on pollutant load.

This approach agrees with global trends aiming to tax negative externalities such as water pollution, pesticides, and plastics. In Europe, for example, instruments such as Denmark's pesticide tax and water pollution charges follow the same deterrent logic [16]. Companies are therefore faced with a strategic decision: either invest in treatment technologies and internalize their environmental impacts, or bear the financial and legal consequences of an unsustainable model [16].

Nature-based technologies, which limit sludge production and adapt to variable pollutant loads, offer a strategic solution. This helps to conserve water and to recover resources, in line with the Sustainable Development Goals, particularly SDG 6 and SDG 12. It also promotes more responsible and environmentally sound industrial practices [17].

Materials and Methods

Description of the Experimental Setup

This experiment was carried out using untreated wastewater collected directly downstream of the production line, at the outlet of a fish processing unit in the Greater Casablanca region (small-scale local canneries). The effluents were not subject to dilution or pre-treatment. They originated from key processing steps, including fish washing, gutting, filleting, defrosting, and cleaning of floors, equipment, and containers (Table 1).

Table 1. Effluent-generating activities in the Casablanca fish processing facility.

Process	Nature of Generated Effluents
Reception and washing of fish	Water containing mucus, blood, scales, sand, salt, and suspended organic matter
Gutting and filleting	Effluents rich in organic matter (blood, viscera, fats), total nitrogen, and BOD ₅
Defrosting (frozen fish)	Meltwater with salt, soluble proteins, and lipids
Cleaning of floors, tables, and equipment	Rinse water highly loaded with fats, detergents, disinfectants, and organic matter
Cooking or heat treatment (if applicable)	Water containing melted fats, thermal residues, and high COD
Freezing or final packaging	Cooling water, condensate, or wash water containing organic residues
Cleaning of crates, bins, and iceboxes	Effluents with fish remains, waste, nitrogenous matter, and cleaning agents
Refrigeration circuit maintenance	Wastewater with traces of oil, lubricants, and antifreeze (low pollution if well managed)
Accidental discharges or leaks	Mixed wastewater not contained by drainage systems, often locally concentrated and highly loaded

An MBBR-type bioreactor was used for the experiment (Fig. 2). It was aerated from the base using filtered air supplied by a compressor. The reactor was operated in continuous flow mode at a rate of

20 litres per 24 hours. It comprised two compartments. The first was dedicated to biological treatment using the MBBR process, with *Umbrina roncadore* fish scales as the natural support and *Penicillium chrysogenum* as the microbial biomass. This strain was selected for its ability to degrade organic matter and form stable, resilient biofilms [2,6,9,12].

Samples were collected every 24 hours over a 72-hour period. The monitored treatment parameters were: chemical oxygen demand (COD), total nitrogen (TN), total phosphorus, biochemical oxygen demand over five days (BOD₅), and suspended solids (SS).

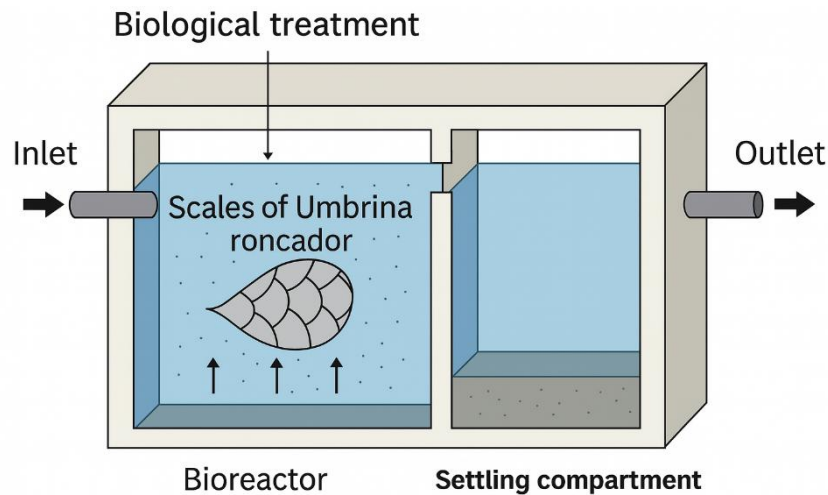


Figure 2. Schematic diagram of a dual-chamber biofilm reactor with *Umbrina roncadore* fish scales and settling compartment for fishery effluent treatment.

The methodology adopted in this study used a Moving Bed Biofilm Reactor (MBBR), specifically designed for treating effluents generated by a fish processing unit in the Greater Casablanca region. In line with principles of circular economy and sustainability, a local natural material was selected as the biofilm carrier : scales of *Umbrina roncadore*, repurposed as a support for microbial colonization (Figure 3).



Figure 3. Types of carriers used in the study : (a) scales of *Umbrina roncadore* used as a natural support, (b) industrial Kaldnes K3 carriers before colonisation, and (c) after colonization by the biofilm.

The experimental approach followed a structured sequence. It began with the physico-chemical and microbiological characterization of fishery effluents to establish their initial pollution profile. Approximately 500 grammes of fish scales were then cleaned and prepared. An MBBR system was

set up, and selected microorganisms were inoculated onto the scales under controlled aeration, temperature (25–28 °C), and agitation to support biofilm formation.

The treatment phase lasted 72 hours. Samples were collected every 24 hours to monitor the removal of key pollutants: COD, BOD₅, total nitrogen, total phosphorus, and suspended solids. All procedures followed the protocols outlined by Fathi *et al.* [2] and Charef *et al.* [9], allowing for performance comparison over time. This approach aims to demonstrate the relevance of a nature-based treatment system using natural packing material in an MBBR reactor to improve the quality of liquid discharges from Moroccan fish processing industries.

Results and Discussion

Characterization of Fishery Effluent

The effluent was collected directly downstream of the production line. Characterization was carried out on several occasions upon reception. Table 2 shows the average values obtained.

Table 2. Quantitative characterization of fishery effluent from a processing unit in the Greater Casablanca region ;

Parameter	Average	Range observed	Permitted limits *	Remarks
Temperature (T)	20.1 °C	18 – 25 °C	≤ 30 °C	Dependent on seasonal ambient temperature
pH	6.53	6.0 – 7.5	6.0 – 8.5	Slightly acidic to neutral water
COD	7,296 mg/L	6,000 – 10,000 mg/L	≤ 500 mg/L	Very high organic load
TN (Total nitrogen)	475.1 mg/L	400 – 800 mg/L	≤ 100 mg/L	Elevated levels linked to proteins and nitrogenous residues
BOD ₅	3,800 mg/L	3,000 – 6,000 mg/L	≤ 120 mg/L	Closely correlated with high COD
Total phosphorus	9.8%	5 – 15%	≤ 10 mg/L	Derived from animal residues and cleaning products
COD/BOD ₅	1.92	1.5 – 2.5	1.5 – 2.5 (indicative)	Indicates a highly biodegradable effluent

* Permitted limits refer to discharges of industrial effluents into non-sensitive surface waters, according to current Moroccan regulations [18].

As indicated by the COD/BOD₅ ratio of 1.92, the effluent shows high biodegradability, which fully supports the use of a biological treatment process such as the one applied in this study. The average COD measured was 7,296 mg/L, indicating a particularly high level of organic pollution. This value is significantly higher than the 4,454 mg/L reported for a fish cannery in the Agadir region by Benyakhlef *et al.* [19], confirming the specific and heavy pollution load associated with urban fish processing effluents discharged intermittently. The measured concentrations of suspended solids, COD, BOD₅, total nitrogen, and total phosphorus all exceed the thresholds set by Moroccan regulations for industrial discharges. These findings show the urgent need to implement suitable and sustainable treatment solutions to reduce the environmental impact of such effluents.

Temporal Evolution of Pollutant Removal in Fishery Effluent Treated Biologically with *Penicillium chrysogenum* on Two Types of Carriers

Figure 1 shows the variation in key pollution parameters (COD, BOD₅, suspended solids, total phosphorus, and total nitrogen) during the biological treatment of real fishery effluent with *Penicillium chrysogenum*. Two types of carriers were used: industrial Kaldnes (K3) media and repurposed *Umbrina roncadore* fish scales.

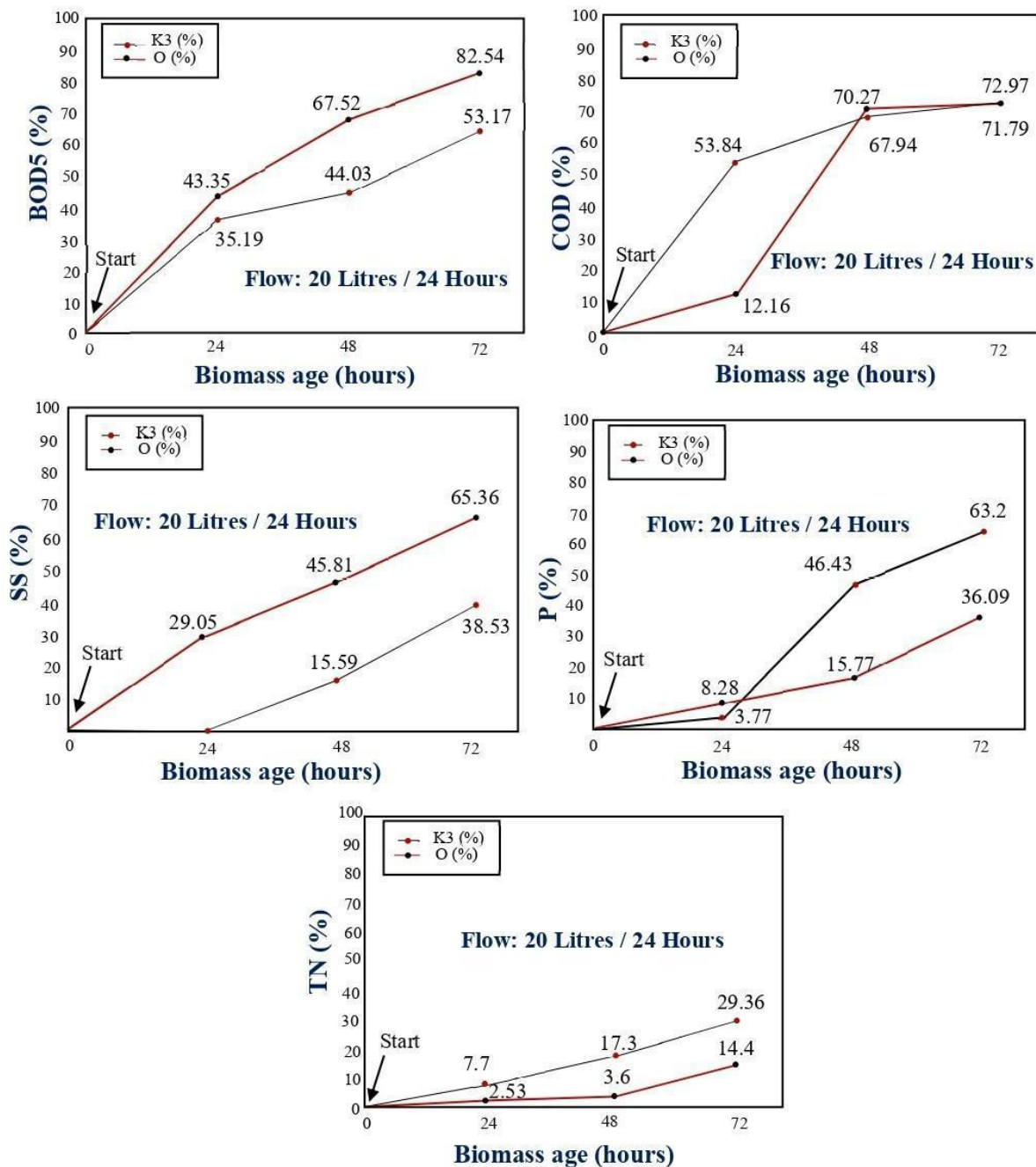


Figure 4. Evolution of pollution parameters during the biological treatment of real fishery effluent using *Penicillium chrysogenum* as a biofilm agent

Overall, the increasing age of the biomass was associated with a continuous improvement in pollutant removal. Significant performance was recorded after only 24 hours, with further enhancement observed at 72 hours. This trend reflects the gradual colonization of the carriers and increased metabolic activity of the attached biomass [20].

For chemical oxygen demand (COD), removal rates after 72 hours reached 71.79% with Kaldnes and 72.9% with *Umbrina roncadore* fish scales. A plateau appeared after 48 hours, suggesting progressive saturation of adsorption sites or limitations linked to substrate availability or biofilm maturity. This observation raises questions about the role of biofilm age and supports further investigation into the effect of longer treatment durations on system stabilization.

The removal of BOD₅ followed a similar trend, with a higher yield for fish scales (82.54%) compared to Kaldnes (63.17%). This result confirms the stronger affinity between the fungal biomass and the natural support, probably related to its porosity and surface properties, which favour the development of dense biofilms [21–23].

For suspended solids (SS), removal was higher with fish scales (65.36%) than with Kaldnes (38.53%). This performance may be linked to better settling, induced by the irregular structure of the natural support, which limits the release of suspended particles [24].

Total phosphorus reduction began after 48 hours, reaching 63.2% with Kaldnes and 36.09% with fish scales. This suggests that the synthetic carrier may offer greater capacity for phosphorus retention or support the growth of polyphosphate-accumulating microorganisms. Initial adsorption on reactor walls may also have contributed to these results [25].

This study also shows that, even at lower temperatures, nitrification remains effective in MBBR systems. This can be attributed to the presence of an adapted biofilm, which may explain the moderate but relevant total nitrogen removal rates—29.36% with Kaldnes and 14.4% with fish scales—despite incomplete nitrification [26].

The results confirm the overall effectiveness of the treatment protocol in reducing organic, nitrogen, and phosphorus loads from untreated fishery effluents. The pilot system, using *Penicillium chrysogenum* immobilized on either *Umbrina roncadore* fish scales or synthetic Kaldnes carriers, achieved satisfactory removal rates for key parameters (COD, BOD₅, suspended solids, total nitrogen, and total phosphorus).

Comparison of the two carrier types showed that natural fish scales performed better in reducing organic loads. This is probably linked to their porous structure, which supports retention and biodegradation. In contrast, Kaldnes media proved more effective in removing nitrogen and phosphorus, possibly because of enhanced colonization by specialised microorganisms, including polyphosphate-accumulating and nitrifying species. These findings highlight promising opportunities

for process optimization. Further research will be needed to refine retention times, support biofilm maturation, and adjust operational conditions. This will facilitate scaling up the system within a sustainable, low-cost framework adapted to the needs of local fish processing industries.

Scanning Electron Microscopy (SEM) analysis provided visual insight into the structure of the biofilm formed on both types of carriers after 72 hours of treatment in the MBBR system. The images revealed dense and organized colonization on the *Umbrina roncadore* fish scales (Figure 5a), characterized by well-developed fungal networks and filamentous structures typical of *Penicillium chrysogenum*. In comparison, the Kaldnes K3 carrier (Figure 5b) also supported microbial colonization, though of lower intensity, with more widely spaced filaments and a less uniform surface coverage. This morphological contrast highlights the strong link between the nature of the carrier and microbial colonization dynamics. The irregular, porous, and naturally adhesive surface of the fish scales appears to provide a more favourable anchor for mycelial development, allowing for sustained interaction between microorganisms and pollutants. This enhanced bioadhesion probably contributes to the improved performance observed in the reduction of organic loads (COD, BOD₅, and suspended solids), supporting more consistent degradation of carbon-based compounds.

In contrast, the more regular and less textured morphology of the Kaldnes media appears less favourable for extensive fungal attachment, but may promote the selection of specific microorganisms such as nitrifying bacteria or polyphosphate-accumulating organisms. This could explain the higher removal rates of total nitrogen and phosphorus. The composition of the biofilm, therefore, reflects not only the density of colonization but also the functional specialization of the system, whether for organic degradation or nutrient removal. These findings emphasize the importance of carrier selection in MBBR design. They point towards targeted engineering of biofilm substrates based on the intended treatment objectives. SEM imaging, by providing detailed insight into the biological architecture, proves to be a valuable tool for assessing performance and optimizing treatment configurations.

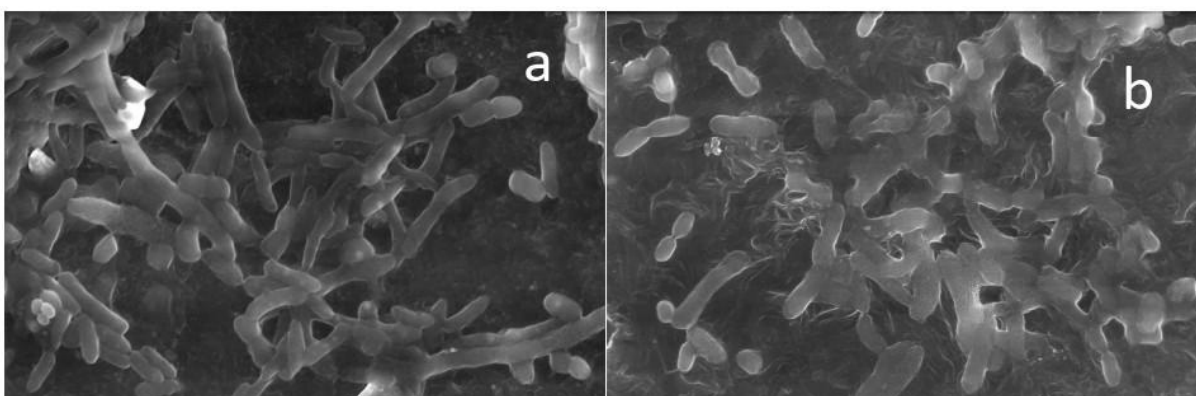


Figure 3. Scanning electron microscopy (SEM) observation of supports colonized by *Penicillium chrysogenum* after 72 hours of treatment: (a) scales of *Umbrina roncadore* and (b) industrial Kaldnes K3 bio-carriers. Magnification $\times 2000$.

These observations are fully consistent with the performance results previously obtained:

- The dense colonization observed on *Umbrina roncadore* fish scales aligns with the high removal rates recorded for BOD₅ (82.54%), COD (72.9%), and suspended solids (65.36%). This efficiency suggests that the porous and organic structure of the scales provides a favourable environment for fungal adhesion, growth, and enzymatic activity.
- The irregular topography and natural composition of these carriers probably supported rapid biofilm establishment, which may explain the performance increase observed within the first 48 hours of treatment.
- On Kaldnes media, colonization was more diffuse and less dense. This may account for the slightly lower BOD₅ (63.17%) and suspended solids (38.53%) removal. These carriers, nonetheless, achieved higher removal of nitrogen and phosphorus. This indicates that the biofilm environment, although less dense, may be more selective for specialized microorganisms active in nitrification or polyphosphate accumulation.

These results show that the nature of the carrier affects not only the amount of colonizing biomass, but also its functional specialization. Natural supports tend to promote intensive degradation of organic matter. Synthetic carriers appear more suitable for targeted processes such as nutrient removal.

It is important to note that some plastic-based biofilm carriers, such as Kaldnes media, may be vulnerable to progressive degradation into microplastics. This is especially the case in systems with dense and diverse microbial consortia, including strains with potential for plastic degradation. Recent studies have shown that large populations of specialized microorganisms can accelerate the breakdown of plastic polymers into smaller particles, increasing the risk of microplastic release into treated effluents [27].

This observation supports the use of natural materials, which are less prone to this type of degradation, particularly in systems with high biofilm-forming potential. A combination of natural and synthetic carriers could therefore be considered in hybrid or sequential systems. Such configurations would help improve overall treatment performance while managing emerging environmental risks linked to carrier materials.

This study provides experimental evidence for the effectiveness of *Umbrina roncadore* fish scales as biofilm supports in MBBR systems. Their use could partially or fully replace synthetic carriers, with comparable performance on key pollution parameters. It also opens the way for multi-compartment configurations designed to optimize metabolic pathways according to specific pollutant targets (organic, nitrogen, phosphorus, particulate). This approach enhances system flexibility without

relying on plastic-based materials. Beyond demonstrating the effectiveness of fish scales in MBBR systems, this work contributes to an integrated strategy aligned with several United Nations Sustainable Development Goals (SDGs) [17] (Table 3).

Table 3. Results in relation to the Sustainable Development Goals (SDGs).

SDG	Goal	Relevance to the Study
SDG 6	Clean Water and Sanitation	Improving the quality of industrial wastewater before discharge helps preserve local water resources and enables safe reuse.
SDG 12	Responsible Consumption and Production	Repurposing fishery by-products (scales) as biological carriers supports rational waste management and reduces the environmental footprint of the industry.
SDG 9	Industry, Innovation and Infrastructure	The development of an optimized MBBR reactor using natural biofilm carriers offers a practical, sustainable, and locally appropriate technical innovation.
SDG 14	Life Below Water	By limiting COD, BOD ₅ , TN, TP, and SS discharges, the process helps prevent eutrophication and protects marine and coastal ecosystems.
SDG 13	Climate Action	The use of low-sludge, additive-free biological treatment indirectly contributes to reducing emissions from conventional treatment methods and pollutant release.

The experimental approach adopted in this study goes beyond the technical challenge of pollution control. It offers an eco-innovative and context-specific solution for Moroccan fish processing industries, and follows global environmental priorities.

Conclusion

The treatment of wastewater from fish processing industries is critical both to conserve water and to limit the environmental impacts of organic, nitrogen, and phosphorus discharges. The results of this study show that integrating a Moving Bed Biofilm Reactor (MBBR) system with *Penicillium chrysogenum* and a locally sourced natural carrier—*Umbrina roncadore* fish scales—achieves significant treatment performance on real effluent.

After 72 hours, the removal rates for COD (72.9%), BOD₅ (82.54%), and suspended solids (65.36%) confirm that the natural carrier is fully functional and represents a credible alternative to synthetic Kaldnes media. Progressive biofilm colonization, strong physico-chemical compatibility between the fungal biomass and the carrier, and the surface properties of the scales (microporosity and specific area) account for much of this performance.

Phosphorus and nitrogen removal, by contrast, remains limited. This is probably linked to the absence of anaerobic zones, the monoculture configuration of the biomass, and the specific characteristics of the effluent. These limitations call for further research into more advanced configurations, including multi-compartment systems, fungal–bacterial co-cultures, and redox-controlled conditions to enhance nutrient removal.

By repurposing a local fishery by-product, this study aligns with circular economy principles and supports sustainable development. It contributes directly to several Sustainable Development Goals (SDGs 6, 12, 13, and 14), by promoting decentralized, low-cost, and environmentally sound treatment solutions. *Umbrina roncadore* fish scales are an effective biological carrier. Several areas for further investigation have emerged from this study:

- Deepen the characterization of natural carriers, focusing on their surface chemical composition (using FTIR spectroscopy), roughness (using profilometry), and microbial adhesion under various environmental conditions.
- Explore other biomass combinations by testing fungal–bacterial associations to enhance metabolic synergies and optimize the simultaneous degradation of multiple pollutants.
- Expand the application of the most promising natural carriers—particularly *Umbrina roncadore* fish scales—to other types of industrial effluents, such as those from the agri-food, dairy, or oil-processing sectors.
- Optimize the system’s hydrodynamic parameters (hydraulic retention time, recirculation rate, and aeration intensity) to improve long-term stability and shorten colonization periods.
- Continue the study of biofilm age by extending treatment beyond 72 hours, to assess performance in stationary or declining biological phases.

This work lays the foundation for an innovative, locally grounded, and adaptable approach, offering promising prospects for the sustainable management of industrial discharges in Morocco and beyond.

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Ethics Approvals: This study was conducted in full accordance with ethical principles. All experimental protocols were carried out in line with relevant guidelines and regulations.

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code availability: Not applicable

Conflict of interest: The authors declare that they have no conflict of interest

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