Extended Abstract

PhD Title: Degradation of natural and anthropogenic pollutant on surface water in aspect of self-cleaning phenomenon

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1. Introduction

Surface water systems are essential to ecological stability, human health, and socioeconomic development, yet they are increasingly burdened by the continuous input of chemically diverse contaminants. Rapid population growth, urbanization, industrial activity, and shifting consumption patterns have intensified the release of organic micropollutants into rivers, lakes, and reservoirs. Many of these compounds, commonly referred to as emerging contaminants, originate from pharmaceuticals, personal care products, lifestyle-related substances, and industrial applications. Although typically present at low concentrations, these compounds' biological activity, persistence, and continuous introduction enable long-term exposure and ecological impact in aquatic environments [1–4].

A defining challenge with emerging contaminants is their incomplete removal through natural attenuation and conventional water treatment technologies. Surface waters possess an intrinsic capacity for self-cleaning through dilution, photodegradation, sorption, plant uptake, and microbial activity; however, this capacity is frequently exceeded under current environmental pressures. Among these processes, microbial biodegradation is the primary biological mechanism for transforming organic contaminants and, under favourable conditions, achieving extensive degradation. In natural aquatic environments, however, microbial self-cleaning remains largely passive and highly variable, constrained by dilution effects, nutrient limitation, ecological competition, and fluctuating physicochemical conditions. As a result, many biologically active contaminants persist in surface waters despite their inherent biodegradability under controlled laboratory conditions [5–8].

Emerging contaminants include both naturally occurring and anthropogenic compounds that differ markedly in physicochemical properties, environmental behaviour, and susceptibility to microbial degradation. Naturally occurring contaminants, such as caffeine and nicotine, are continuously released into aquatic systems through domestic wastewater, urban runoff, and agricultural activities. Although derived from biological sources, their persistent occurrence reflects sustained loading and incomplete attenuation. In contrast, anthropogenic contaminants such as methylparaben and trichlorocarbanilide are synthetic compounds designed for chemical stability and antimicrobial function, characteristics that significantly hinder biodegradation in aquatic environments. These differences highlight the importance of evaluating contaminant removal across a representative spectrum of molecular complexity and biodegradability when assessing microbial self-cleaning processes [9–13]. Pollutant concentrations investigated in this study were intentionally elevated relative to typical levels reported in surface waters. This design choice was made to assess microbial tolerance, adaptation, and degradation capacity under conservative stress conditions relevant to engineered treatment and shock-loading scenarios. Environmental relevance was addressed through the use of wastewater-based nutrient matrices and system-scale integration rather than by matching absolute environmental concentrations [14,15].

Microbial activity plays a central role in regulating the environmental fate of organic contaminants, with bacteria and fungi serving as complementary biological systems for pollutant degradation. Bacterial degradation is typically mediated by intracellular enzymatic pathways that transform low-molecular-weight, water-soluble compounds, whereas fungi, particularly white-rot species, employ extracellular oxidative enzymes that enable the transformation of structurally complex or hydrophobic contaminants. While both systems have demonstrated degradation potential under laboratory conditions, their performance in environmentally relevant water matrices remains limited. Single-microbial systems often exhibit reduced stability and efficiency when exposed to mixed contaminant loads, nutrient-poor conditions, and environmental variability, particularly during scale-up beyond laboratory reactors [16–19].

Constructed wetlands are engineered extensions of natural self-cleaning processes and offer a promising ecological platform for intensifying biologically driven contaminant attenuation. By integrating porous substrates, vegetation, and biofilm-based microbial communities, constructed wetlands create structured environments that enhance microbial activity, prolong contact between contaminants and degradative organisms, and stabilize physicochemical conditions. However, most existing wetland systems rely on naturally colonized, functionally undefined microbial communities, resulting in variable treatment performance and limited control over micropollutant degradation. Consequently, constructed wetlands are often effective for bulk water quality improvement but less reliable for targeted removal of persistent, emerging contaminants [20–22].

A key challenge in advancing biologically driven water treatment is bridging the gap between laboratory-scale microbial degradation studies and ecologically relevant treatment systems. Laboratory experiments typically use nutrient-rich media and highly controlled conditions that do not reflect the complexity of real-world water matrices, while open aquatic systems are unsuitable for controlled evaluation of microbial processes. Furthermore, microbial systems adapted to laboratory conditions often fail to sustain growth and degradation performance in nutrient-limited wastewater environments, limiting their practical applicability [18,23,24].

In this context, the present study advances an engineered microbial self-cleaning approach that intentionally strengthens biologically mediated degradation processes beyond passive natural attenuation. Specialist fungal and bacterial degraders are adapted to wastewater-based growth conditions, enabling contaminant degradation using the intrinsic nutrient content of synthetic wastewater as the sole growth resource. These adapted microbial systems are systematically evaluated across increasing levels of complexity, from laboratory-scale biodegradation experiments to integration within constructed wetland platforms. By combining controlled microbial adaptation with engineered ecological treatment systems, this work shifts the self-cleaning paradigm from uncontrolled natural processes to deliberately designed, biologically driven strategies for enhanced, system-scale removal of emerging contaminants from surface water matrices.

2. Methodology

The methodological framework of this study was designed as a staged experimental system progressing from controlled laboratory-scale biodegradation experiments to a pilot-scale biomimetic constructed wetland. This stepwise design enabled systematic evaluation of microbial adaptation, degradation performance, and system-level stability as ecological complexity increased, while maintaining experimental control.

Four representative emerging contaminants were selected to reflect both natural and anthropogenic pollutant classes commonly detected in surface waters. Caffeine and nicotine were chosen as natural-origin contaminants due to their widespread occurrence, continuous release, and partial biodegradability in aquatic environments. These compounds originate from lifestyle-related consumption and agricultural activities and represent structurally distinct alkaloids with high environmental mobility. In contrast, methylparaben (MeP) and trichlorocarbanilide (TCC) were selected as representative anthropogenic contaminants. These synthetic compounds are widely used as antimicrobial agents, exhibit enhanced chemical stability, and are frequently reported to persist in wastewater-impacted surface waters due to incomplete removal during conventional treatment.

The selected pollutants span a broad range of physicochemical properties, including molecular weight, polarity, solubility, and hydrophobicity. This diversity was intentionally exploited to assess differences in microbial accessibility, degradation pathways, and treatment performance across contaminant classes. Natural-origin compounds were investigated using fungal systems, reflecting their known susceptibility to extracellular oxidative mechanisms, whereas anthropogenic compounds were evaluated using bacterial systems with established capacity for transforming synthetic preservatives and antimicrobials. This classification provided a functional basis for evaluating biologically driven self-cleaning processes under controlled yet environmentally relevant conditions [25–27].

To ensure reproducibility and analytical reliability, caffeine and nicotine were extracted from natural sources to retain matrix relevance, while MeP and TCC were introduced as analytical-grade standards. Pollutant concentrations used in both laboratory and wetland experiments were intentionally elevated relative to typical polluted environmental levels. This design choice enabled robust assessment of microbial tolerance, adaptation, and degradation capacity under stress conditions relevant to shock loading and engineered treatment applications, with environmental relevance addressed through system design rather than by matching absolute concentrations [28,29].

2.1 Lab-scale Microbial Biodegradation Systems

Laboratory-scale experiments were designed to evaluate microbial adaptation, growth behaviour, and biodegradation performance under controlled aqueous conditions. Distinct experimental strategies were applied for fungal and bacterial systems to reflect fundamental differences in microbial physiology, growth requirements, and degradation mechanisms.

2.1.1 Fungal systems for natural pollutants

The white-rot fungus *Trametes versicolor* was selected as the model organism for fungal biodegradation of caffeine and nicotine due to its well-documented extracellular enzymatic system and capacity to transform structurally diverse organic compounds. Prior to biodegradation experiments, fungal adaptation and growth feasibility were evaluated to confirm viability under pollutant-containing and nutrient-limited aqueous conditions. This step was critical, as filamentous fungal growth in liquid media cannot be assumed under environmental stress [25,30–32].

Fungal biodegradation experiments were subsequently conducted in batch systems using different aqueous media representing increasing levels of environmental complexity. Nutrient-rich broth served as a reference condition, while synthetic wastewater was employed as a

nutrient-limited matrix representative of contaminated surface water. In addition, natural extract-based media were used to retain matrix complexity associated with real pollutant sources. Experiments were performed under static aerobic conditions to avoid mechanical disruption of fungal mycelia and to better reflect conditions relevant to passive treatment systems (Figure 1).
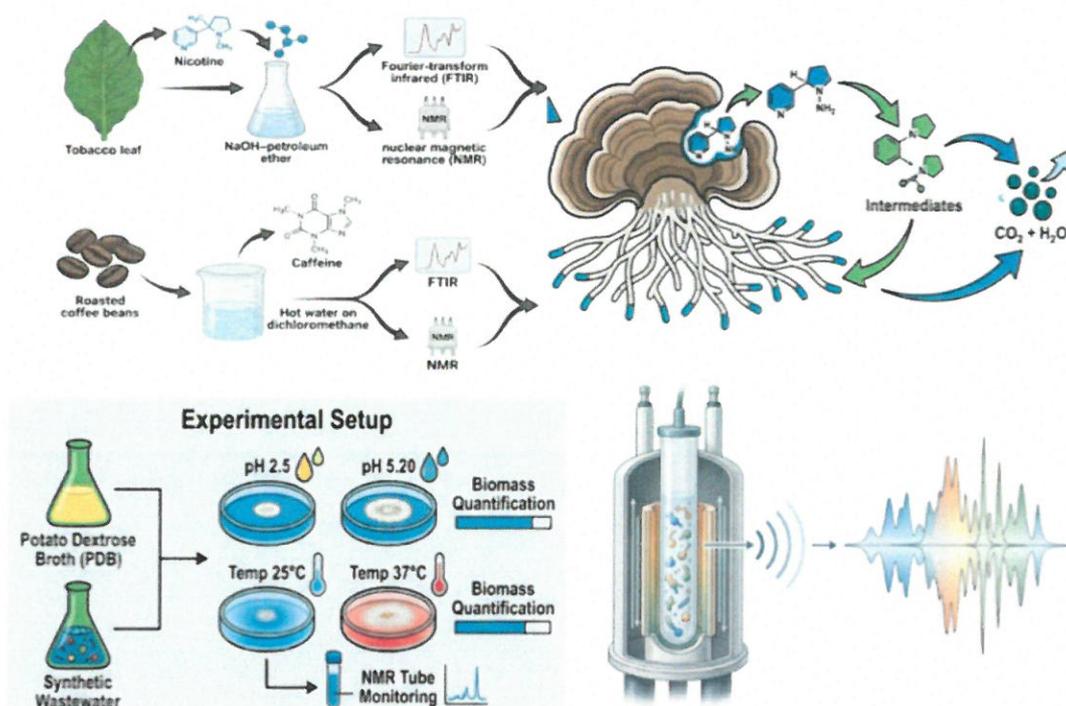

Figure 1. Graphical representation of caffeine and nicotine extraction, fungal degradation, and analysis experiments

Environmental parameters such as temperature and pH were systematically varied to assess their influence on fungal degradation performance. Control systems without fungal inoculation were included to account for non-biological changes in pollutant concentration. Together, these experiments established the functional limits, robustness, and environmental sensitivity of fungal-mediated degradation of natural-origin contaminants.

2.1.2 Bacterial systems for anthropogenic pollutants

Bacterial biodegradation experiments were conducted using a defined consortium consisting of *Rhodococcus* sp. and *Alcaligenes* sp., selected for their demonstrated ability to degrade structurally complex organic pollutants. In contrast to fungal systems, bacteria readily proliferate in liquid media; therefore, preliminary adaptation experiments were not required prior to biodegradation studies.

Batch biodegradation experiments were performed under aerobic conditions using both nutrient-rich broth and synthetic wastewater to evaluate degradation performance under optimal and nutrient-limited conditions, respectively. This comparison enabled direct assessment of bacterial feasibility for application in environmentally relevant water matrices. Experiments were conducted with defined pollutant loading and time-resolved sampling to evaluate degradation kinetics and system stability (Figure 2).

Distinct control systems were maintained to differentiate biologically driven degradation from abiotic losses or background microbial activity. By applying identical experimental logic across both media types, the laboratory-scale bacterial experiments provided a mechanistic foundation for assessing the environmental applicability of bacterial degradation of anthropogenic contaminants [30,33,34].
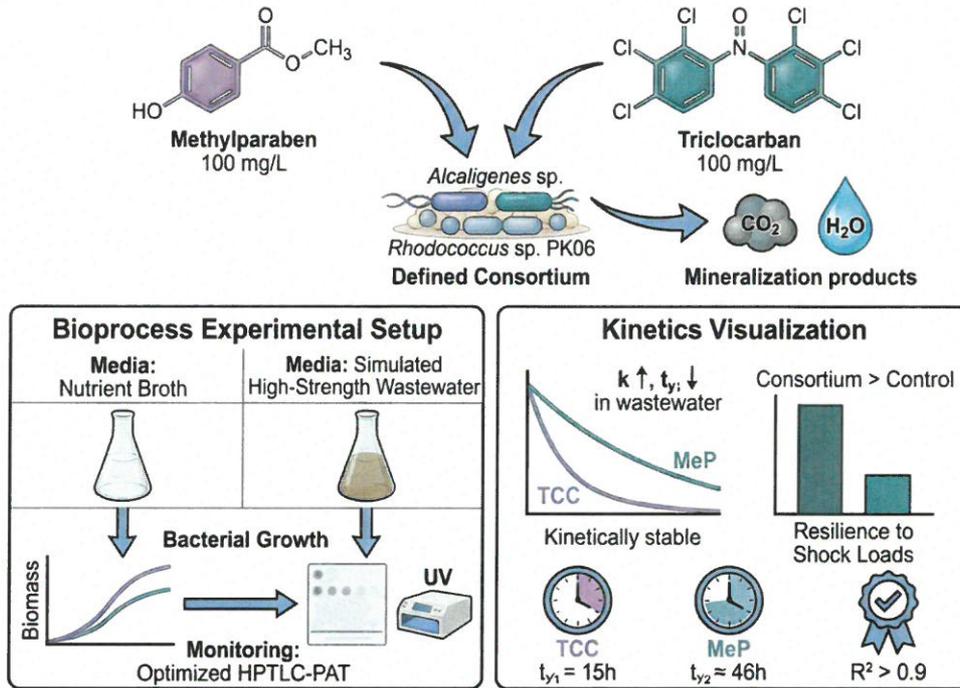

Figure 2. Graphical representation of bacterial degradation of methylparaben and trichlorocarbanilide, and HPTLC analysis

Across all laboratory experiments, microbial inoculation strategies were designed to ensure the rapid establishment of active biomass and reproducible degradation performance under elevated pollutant stress. Fungal systems employed liquid-phase inoculation to promote uniform dispersion of spores and mycelial fragments, while bacterial systems used standardized liquid cultures to ensure consistent biomass input. These tailored approaches were essential for maintaining microbial activity in nutrient-limited aqueous environments and for enabling meaningful interpretation of biodegradation performance.

2.2 Pilot-scale Biomimetic Constructed Wetland System

To bridge the gap between laboratory-scale biodegradation studies and environmentally relevant treatment systems, a pilot-scale biomimetic constructed wetland was developed. While laboratory experiments provided mechanistic insight under controlled conditions, they do not capture spatial heterogeneity, plant–microbe interactions, or long-term operational stability. The constructed wetland system was therefore designed as an intermediate platform that retains ecological complexity while allowing experimental control.

The wetland system was configured as a horizontal subsurface flow reactor using a stratified porous substrate and emergent macrophytes. Synthetic wastewater was employed as the aqueous matrix to ensure reproducibility and to minimize variability associated with real influent waters. The influent was amended with a mixed-contaminant cocktail consisting of

caffeine, methylparaben, and trichlorocarbanilide, representing both natural and anthropogenic pollutant classes under simultaneous exposure conditions [35–39].

Four wetland configurations were established to evaluate system-level biodegradation mechanisms: a bacterial-only system, a fungal-only system, a combined fungal–bacterial consortium system, and an uninoculated control. This design enabled direct comparison of mono-microbial and dual-microbial strategies under identical hydraulic and structural conditions. Aeration and temperature were adjusted to support the metabolic requirements of the selected microorganisms while preserving comparability across systems.

Microbial inoculation strategies were specifically adapted for wetland-scale operation. Bacterial systems employed liquid-phase inoculation to promote rapid dispersion and biofilm establishment within the pore water, while fungal systems used a solid-phase basal inoculation approach to create a stable, hydraulically resistant mycelial network. The combined system integrated both strategies to exploit functional complementarity between microbial groups. Vegetation was included primarily to provide structural stability, support microbial colonization, and facilitate rhizosphere development, rather than to directly uptake pollutants.

Operational parameters, such as pH and plant growth, were monitored throughout the experimental period to assess system stability and, indirectly, evaluate microbial activity. Degradation analysis of wetland samples was performed at stabilized operational stages to distinguish sustained biodegradation from transient adsorption or acclimation effects. Together, the pilot-scale wetland experiments enabled evaluation of microbial adaptation, synergistic degradation mechanisms, and the scalability of engineered self-cleaning strategies under mixed-contaminant conditions.

3. Analytical and Validation Strategy

Reliable evaluation of biologically driven pollutant degradation requires analytical methods that collectively ensure chemical identity, concentration accuracy, and interpretability across different experimental scales and matrix complexities. In this study, an integrated analytical framework was employed to validate microbial self-cleaning performance from laboratory-scale systems to a pilot-scale biomimetic constructed wetland. Rather than relying on a single technique, complementary spectroscopic, chromatographic, and structural analyses were used to provide independent, mutually reinforcing lines of evidence.

Spectroscopic techniques were used as the primary tools for verifying chemical identity and analyzing transformations of naturally derived pollutants. Fourier Transform Infrared Spectroscopy (FT-IR) was applied exclusively at the preparatory stage to confirm the structural integrity of caffeine and nicotine extracted from natural sources. This step ensured that subsequent biodegradation experiments were conducted using chemically intact parent compounds and that observed concentration changes could be attributed to biological activity rather than extraction artifacts. FT-IR was intentionally not applied to biodegradation samples, as its selectivity is limited in biologically complex matrices [40,41].

Nuclear Magnetic Resonance (NMR) spectroscopy constituted the central analytical technique for both quantitative and qualitative validation of biodegradation processes. Quantitative NMR (qNMR) was employed to determine absolute concentrations of caffeine and nicotine prior to biodegradation, providing calibration-independent quantification based on proton signal integration. This approach ensured high accuracy and reproducibility for naturally derived

pollutants without reliance on external calibration curves. At later stages, qualitative NMR analysis was used to monitor concentration changes associated with microbial degradation in both laboratory-scale systems and the constructed wetland. The disappearance of parent-compound resonances provided direct evidence of biodegradation under stabilized operating conditions. Limitations related to analyte solubility were explicitly accounted for, and compounds not amenable to NMR analysis under the selected conditions [42–44].

Chromatographic analysis was applied to robustly quantify anthropogenic contaminants in biologically complex liquid systems. High-Performance Thin-Layer Chromatography (HPTLC) was selected as the primary method for monitoring methylparaben and trichlorocarbanilide during bacterial biodegradation experiments. HPTLC offers high tolerance to complex matrices, allows simultaneous processing of multiple samples, and provides reproducible time-resolved concentration data for hydrophobic and semi-polar compounds. Method development and validation ensured compound-specific detection and reliable quantification throughout degradation experiments conducted under both nutrient-rich and nutrient-limited conditions. By separating chromatographic quantification from spectroscopic transformation analysis, analytical overlap and misinterpretation were avoided [45].

To support the interpretation of system-scale performance, Scanning Electron Microscopy (SEM) was employed as a complementary structural tool. SEM was used solely to visualize substrate morphology, microbial attachment, and biofilm development within the constructed wetland matrix. This analysis provided physical evidence of microbial colonization and substrate suitability, confirming that the engineered wetland environment supported stable microbial retention and spatial organization. Importantly, SEM observations were not used to infer chemical degradation mechanisms but rather to validate the physical and biological integrity of the treatment system.

The combined analytical strategy ensured data reliability through methodological independence and functional complementarity. Chemical identity and transformation were verified spectroscopically, concentration changes were quantified chromatographically using validated methods, and biological system functionality was supported by structural imaging. This multi-layered validation framework minimized analytical bias, addressed matrix-specific limitations, and provided a robust basis for interpreting biodegradation performance across experimental scales. As a result, the analytical evidence supporting microbial self-cleaning in this study is internally consistent, defensible, and suitable for evaluating engineered biological treatment systems under environmentally relevant conditions.

4. Results

4.1 Lab-scale Biodegradation Performance

Laboratory-scale experiments were designed to isolate biological degradation mechanisms from hydraulic, plant-mediated, and physicochemical effects, thereby establishing a mechanistic foundation for engineered self-cleaning. Two distinct pollutant classes were investigated using functionally matched microbial systems: natural pollutants (caffeine and nicotine) using fungal degradation, and anthropogenic pollutants (methylparaben and trichlorocarbanilide) using bacterial consortia. Across all experiments, pollutant concentrations were intentionally higher than those typically encountered in surface waters to evaluate microbial robustness under conservative stress conditions.

4.1.1 Natural pollutants - fungal systems

The white-rot fungus *Trametes versicolor* demonstrated strong tolerance, adaptation, and biodegradation capacity toward both caffeine and nicotine. Growth inhibition assays and biomass accumulation analyses confirmed that neither compound exerted acute toxicity at the selected working concentration (100 mg L^{-1}), provided that environmental conditions, particularly temperature, were within the fungal tolerance range. Temperature emerged as the dominant controlling parameter, with 25 °C supporting sustained mycelial growth and biomass development, while 37 °C imposed measurable metabolic stress.

Under optimized conditions, endpoint-based quantitative NMR analysis revealed cumulative removal efficiencies of approximately 97-98% for caffeine and >98% for nicotine after extended incubation. Removal was consistently associated with substantial formation of fungal biomass, indicating that biodegradation was linked to active metabolism rather than to passive sorption. Synthetic wastewater and complex matrices (coffee and tobacco extracts) supported comparable fungal growth and pollutant attenuation, demonstrating that *T. versicolor* could exploit wastewater-derived nutrients without external supplementation (Figure 3).
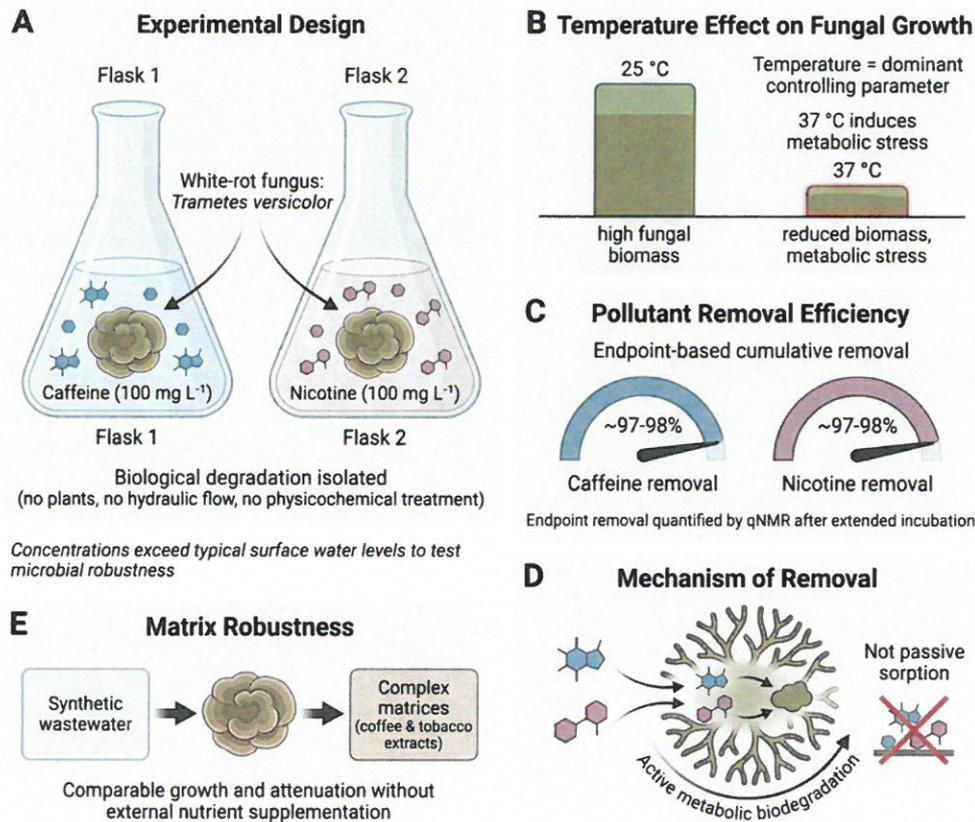

Figure 3. (A) Laboratory-scale biodegradation of caffeine and nicotine (100 mg L^{-1}) by the white-rot fungus *Trametes versicolor*. (B) Temperature effects on fungal growth at 25 °C and 37 °C. (C) Endpoint qNMR-based pollutant removal, showing cumulative degradation efficiencies of ~97–98%. (D) Fungal removal mechanism dominated by active metabolism. (E)

Fungal adaptation and robustness in synthetic wastewater and complex matrices under nutrient-limited conditions

Nicotine imposed a higher physiological burden than caffeine, particularly under acidic and elevated-temperature conditions, but sustained growth and high endpoint removal were still achieved. This confirms that fungal systems can tolerate and transform biologically active alkaloids over long timescales, consistent with their role in natural self-cleaning processes. Importantly, the long adaptation period and slow but persistent removal highlight that fungal degradation contributes primarily to cumulative, long-term attenuation, rather than rapid kinetic removal [26,46,47].

4.1.2 Anthropogenic pollutants - bacterial systems

In contrast to fungal systems, bacterial consortia exhibited rapid and kinetically resolved degradation of anthropogenic contaminants. Time-resolved HPTLC analysis demonstrated effective biodegradation of both methylparaben (MeP) and trichlorocarbanilide (TCC) in nutrient broth and synthetic wastewater.
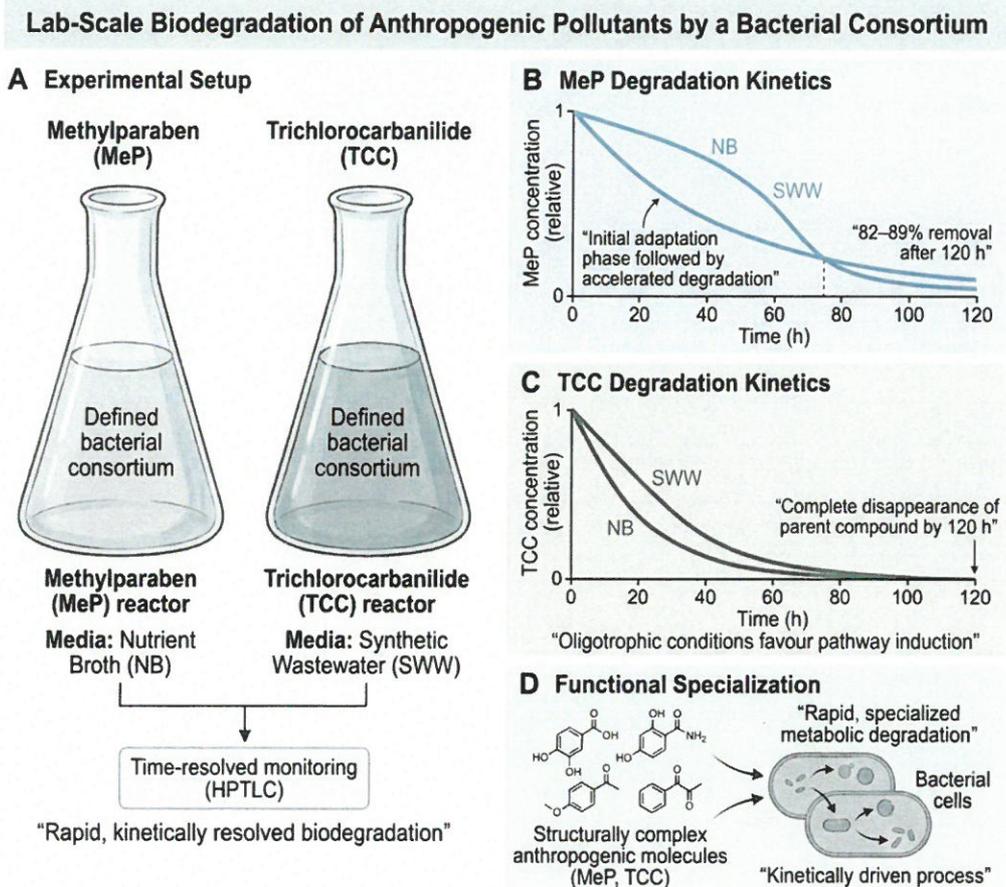

Figure 4. (A) Lab-scale biodegradation of methylparaben (MeP) and trichlorocarbanilide (TCC) by a bacterial consortium in NB and SWW. (B) MeP degradation showing an adaptation phase and 82–89% removal within 120 h. (C) Rapid TCC degradation with complete parent-compound removal within 120 h. (D) Kinetically driven bacterial metabolism of anthropogenic pollutants

For methylparaben, degradation progressed gradually following an initial adaptation phase. Removal reached 82–89% within 120 hours, with nutrient broth supporting slightly faster early-stage activity, while synthetic wastewater promoted stronger mid-stage degradation following acclimation. This crossover behaviour indicates metabolic prioritization of the target compound under nutrient-limited conditions, which is highly relevant for wastewater treatment scenarios (Figure 4).

Trichlorocarbanilide exhibited even more rapid degradation, with complete disappearance of the parent compound within 120 hours in both media. Notably, synthetic wastewater supported faster early-stage removal than nutrient broth, suggesting that nutrient-limited conditions favoured induction of degradation pathways for this recalcitrant chlorinated compound. The bacterial consortium, therefore, demonstrated strong functional specialization toward structurally complex anthropogenic pollutants [6,48–51].

Collectively, the lab-scale results establish a clear functional distinction: fungal systems provide slow but sustained removal of natural pollutants, while bacterial consortia deliver rapid and efficient degradation of anthropogenic contaminants. This differentiation forms the biological basis for engineered self-cleaning strategies tailored to pollutant chemistry.

4.2 Pilot-scale Wetland Performance

To evaluate scalability, adapted microbial systems were integrated into a biomimetic constructed wetland operated under batch conditions with mixed-pollutant loading. Three biologically active configurations were examined: Wetland A (bacterial), Wetland B (fungal), and Wetland C (fungal–bacterial consortium), alongside an abiotic control.

All bio-augmented wetlands achieved stable operation following an initial acclimation phase. Indicators of stability included sustained microbial growth under mixed-pollutant stress, consistent plant development (*Phragmites australis*), and buffered pH profiles within a near-neutral range. Neither fungal nor bacterial populations exhibited collapse under combined exposure to caffeine, methylparaben, and trichlorocarbanilide, confirming that the wetland environment supported long-term microbial viability rather than transient survival [52,53].

SEM analysis provided structural confirmation of extensive microbial colonization of the porous media, demonstrating that the wetland matrix functioned as a stable scaffold for biofilm formation and biomass retention. These observations support the interpretation of pollutant removal as biologically driven self-cleaning rather than adsorption-dominated attenuation.

Clear differences emerged among wetland configurations during the early treatment phase. For caffeine, complete disappearance of NMR signals was observed by Week 4 in the bacterial (A) and consortium (C) wetlands, while the fungal wetland (B) achieved approximately 90% removal, reaching complete disappearance by Week 7. This reflects the suitability of bacterial intracellular pathways for rapid caffeine degradation, enhanced by higher operating temperatures.

Methylparaben exhibited a contrasting pattern. At Week 4, the fungal wetland achieved the highest removal (~89%), followed by the consortium (~63%) and bacterial wetland (~50%). This highlights the effectiveness of fungal extracellular oxidative enzymes for early transformation of aromatic esters. By Week 7, all biological wetlands had completely disappeared methylparaben signals (Figure 5) [54–58].
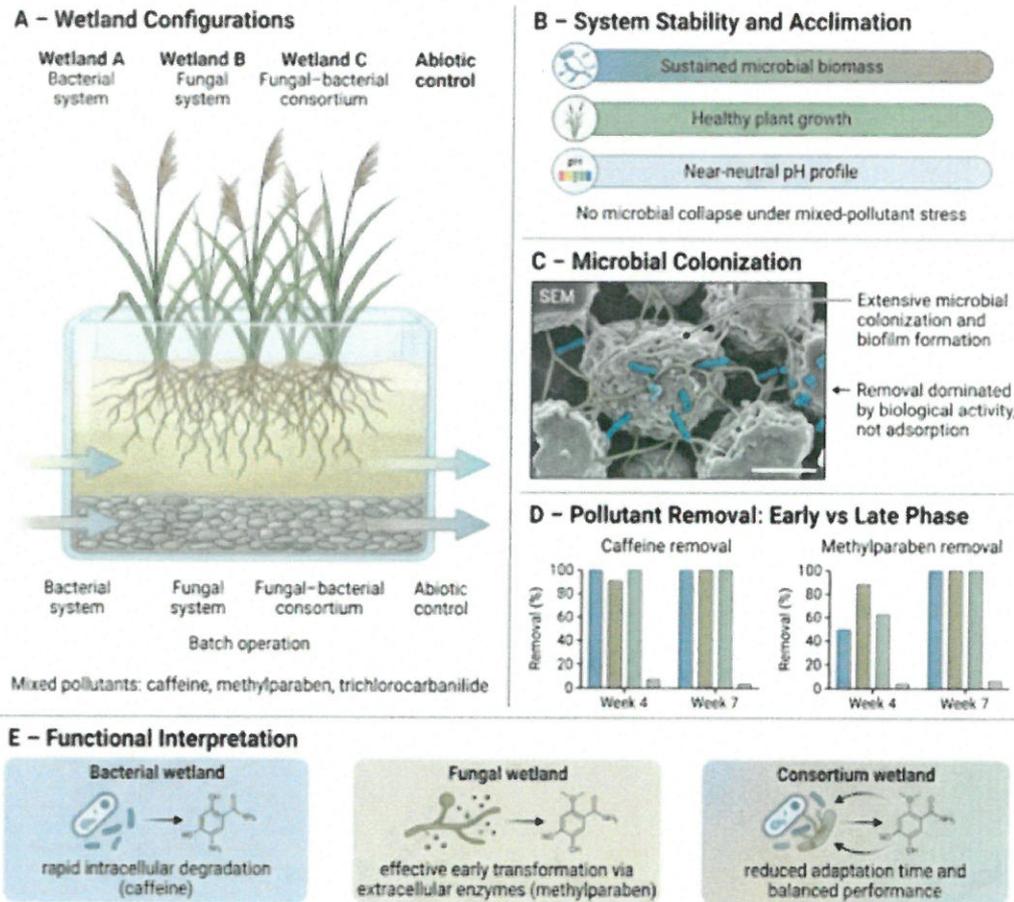
Figure 5. Schematic diagram of the (A) pilot-scale constructed wetland comprising a vegetated porous-media (*Phragmites australis*). (B) System stability and acclimation across all wetland configurations. (C) SEM-based visualization of microbial colonization on wetland media. (D) Pollutant removal performance in biological wetlands. (E) Pollutant degradation driven by microbial metabolic activity

The consortium wetland did not universally outperform monocultures during the initial adaptation phase but consistently reduced adaptation time, achieved faster system stabilization, and exhibited greater resilience under mixed-pollutant conditions, resulting in balanced performance across compounds. This demonstrates functional complementarity rather than simple additive synergy, a critical distinction for system design.

4.3 System-level Interpretation and Scale-up Implications

At the system level, the results confirm that self-cleaning is not a single process but an emergent property arising from microbial adaptation, pollutant chemistry, and environmental structure. Lab-scale experiments established pollutant-specific biodegradation mechanisms, while the constructed wetland validated their persistence under ecological complexity.

The transition from laboratory systems to wetlands demonstrated that microbial self-cleaning mechanisms are scalable when supported by spatial structure, biomass retention, and nutrient-limited conditions. Synthetic wastewater consistently supported microbial activity across scales, confirming that external nutrient supplementation is not required for effective biodegradation.

Microbial synergy emerged as a design logic rather than a universal performance multiplier. Fungi contributed stability, extracellular oxidation, and tolerance to complex matrices, while bacteria provided kinetic efficiency and rapid turnover. Their integration reduced system vulnerability and compressed treatment times, particularly under mixed-pollutant conditions.

Overall, the results demonstrate that engineered microbial self-cleaning can be deliberately intensified and translated from controlled laboratory studies to constructed wetland systems. This validates the central premise of the dissertation: surface-water self-cleaning can be strengthened through pollutant-specific microbial design rather than relying on passive natural attenuation.

5. Conclusions and Significance

This doctoral research demonstrated that microbial self-cleaning of polluted surface water can be intentionally intensified through engineered biological design rather than relying on passive natural attenuation. By progressing systematically from controlled laboratory experiments to a pilot-scale biomimetic constructed wetland, the study validated a scalable framework for degrading chemically diverse emerging contaminants under environmentally relevant conditions.

At the laboratory scale, the work clearly differentiated the functional roles of fungal and bacterial systems. Fungal degradation experiments confirmed that *Trametes versicolor* is highly effective in transforming natural-origin alkaloids such as caffeine and nicotine under nutrient-limited aqueous conditions. Removal efficiencies exceeding 97-98% were consistently achieved across synthetic wastewater and chemically complex natural matrices, demonstrating that fungal systems can adapt from nutrient-rich laboratory media to wastewater environments while sustaining biodegradation using wastewater-derived nutrients alone. These results establish fungi as robust agents for long-term, cumulative degradation of biologically active natural pollutants.

In contrast, bacterial degradation experiments highlighted the kinetic efficiency and metabolic resilience of bacterial consortia toward anthropogenic contaminants. Complete removal of trichlorocarbanilide and high removal of methylparaben were achieved within short timeframes, even under nutrient-limited conditions. Notably, synthetic wastewater often supported degradation performance equal to or superior to nutrient-rich media after microbial adaptation, indicating that nutrient limitation can stimulate pollutant utilization rather than inhibit it. This finding challenges the assumption that clean laboratory media inherently represent optimal conditions for biodegradation and reinforces the environmental relevance of wastewater-based systems.

The pilot-scale constructed wetland experiments demonstrated that these microbial capabilities can be stabilized and sustained within an engineered ecological system. Wetlands planted with *Phragmites australis* successfully supported fungal, bacterial, and combined microbial configurations under mixed-pollutant loading for extended operation. All biological systems

remained operationally stable, maintaining buffered pH conditions and sustained plant growth, confirming effective ecological integration rather than transient microbial survival.

Pollutant-specific behaviour observed at the wetland scale reinforced the importance of microbial differentiation. Bacterial systems achieved rapid caffeine removal, while fungal systems exhibited superior early-stage performance for methylparaben. The combined fungal-bacterial wetland did not merely replicate monoculture behaviour but balanced degradation performance across compounds and reduced adaptation time under mixed-contaminant exposure. This demonstrates that microbial consortia outperform single systems not through simple additive effects, but through functional complementarity, where distinct metabolic and enzymatic mechanisms address different structural challenges posed by complex pollutant mixtures.

Collectively, these findings establish biomimetic constructed wetlands as viable self-cleaning systems for surface water remediation. The engineered wetland configuration provided biomass retention, spatial organization, and environmental buffering, enabling sustained microbial activity under high contaminant loading without external nutrient supplementation or energy-intensive operation. The successful transition from laboratory-scale reactors to a porous-media, plant-associated system confirms the scalability and practical relevance of the proposed approach.

From an environmental perspective, this work demonstrates that polluted surface waters can serve as active biodegradation matrices rather than passive carriers of contaminants. By exploiting inherent nutrient complexity and designing microbial communities with complementary functions, self-cleaning processes can be strengthened in a controlled and predictable manner.

In terms of applicability, the outcomes of this study support the development of low-energy, biologically driven treatment strategies for surface waters impacted by mixed natural and anthropogenic pollutants. The proposed microbial design principles and wetland-based framework provide a defensible foundation for future implementation of bio-augmented constructed wetlands as sustainable tools for surface water protection and remediation.

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