Abstract

The increasing presence of emerging contaminants (ECs) in aquatic environments poses a persistent challenge to natural self-cleaning processes and conventional wastewater treatment systems. In natural aquatic systems, self-cleaning processes operate passively and are strongly constrained by environmental variability, whereas this research addresses self-cleaning as a deliberately engineered and biologically intensified process. Compounds such as caffeine, nicotine, methylparaben (MeP), and trichlorocarbanilide (TCC) exhibit diverse physicochemical properties and are often incompletely attenuated in surface waters due to dilution, nutrient limitation, ecological competition, and environmental variability. Although microbial self-cleaning contributes to contaminant transformation, its efficiency under uncontrolled environmental conditions remains limited. This doctoral research aimed to enhance biologically driven self-cleaning processes through pollutant-specific microbial selection, wastewater-based microbial adaptation, and integration of adapted fungal and bacterial systems within an engineered constructed wetland framework.

The study was conducted at two sequential scales: lab-scale experiments and a pilot-scale constructed wetland system. The originality of this research was the use of synthetic wastewater formulated to resemble polluted surface and wastewater matrices, which served simultaneously as a model contaminated water system and the sole nutrient source for microbial growth. By imposing nutrient limitation, this approach forced direct coupling between microbial growth and contaminant metabolism, thereby improving environmental relevance and scalability beyond laboratory-optimized media. This strategy was intentionally designed to move beyond laboratory-optimized media and to enhance pollutant degradation by forcing microorganisms to utilize wastewater-derived nutrients while metabolizing target contaminants.

At the lab scale, the biodegradation potential of the white-rot fungus *Trametes versicolor* toward caffeine and nicotine was evaluated under controlled, nutrient-limited conditions. Experiments were conducted at an initial contaminant concentration of 100 mg L^{-1} using chemically extracted caffeine and nicotine from natural sources. The effects of temperature (25 and 37 °C), pH (strongly acidic and moderately acidic), and medium composition (potato dextrose broth, synthetic wastewater, and complex natural matrices) were systematically assessed. Due to prolonged fungal adaptation phases and slow biomass development, biodegradation was evaluated using an endpoint-based approach after 45 days of incubation, as fungal-mediated degradation is cumulative rather than kinetically rapid. Pollutant identity and removal were confirmed using spectroscopic techniques such as FT-IR, ^{1}H NMR, and ^{13}C NMR qualitatively and quantitatively.

Caffeine removal reached 97-98% under moderately acidic conditions and in synthetic wastewater at 25 °C, coinciding with maximal fungal biomass formation. High removal efficiencies were maintained in synthetic wastewater even at 37 °C, demonstrating that wastewater-derived nutrients not only sustained fungal growth but also enhanced long-term degradation performance under nutrient-limited conditions. Nicotine removal exceeded 98% under optimal conditions (25 °C, pH ~5.20, synthetic wastewater), with reduced performance observed only under combined thermal and acidic stress. Across all systems, a strong positive relationship was observed between fungal biomass accumulation and cumulative contaminant removal, confirming that adaptation to wastewater directly promoted microbial growth at scale and enhanced biodegradation efficiency.

Continuing further, a lab-scale study investigated the biodegradation of MeP and TCC by a defined bacterial consortium comprising *Alcaligenes* sp. and *Rhodococcus* sp. Experiments were conducted at an initial concentration of 100 mg L^{-1} over a 120-hour incubation period in nutrient broth and synthetic wastewater. High-Performance Thin-Layer Chromatography (HPTLC) was used to quantitatively monitor both pollutants. MeP degradation reached approximately 89% in nutrient broth and 83% in synthetic wastewater, with enhanced mid-stage degradation in synthetic wastewater following bacterial acclimation to the nutrient-limited matrix. In contrast, TCC exhibited rapid degradation, exceeding 97% removal by 96 hours and complete disappearance by 120 hours in both media. These results demonstrate that synthetic wastewater supports efficient bacterial growth and enhances the degradation of both moderately and highly recalcitrant pollutants without external nutrient supplementation.

Upscaling was performed using pilot-scale biomimetic constructed wetlands planted with *Phragmites australis*. Wastewater-adapted fungal (*Trametes versicolor*), bacterial (*Pseudomonas aeruginosa*), and combined microbial systems were integrated into the wetland matrix, operated and analysed for seven weeks under batch conditions using synthetic wastewater containing mixed pollutants (caffeine, MeP, and TCC at 100 mg L^{-1}). Prior tolerance testing confirmed microbial survivability under mixed-pollutant stress. All biologically active wetlands exhibited sustained plant growth, stable near-neutral pH, and persistent microbial activity, confirming successful system accumulation and ecological stability.

Pollutant removal was evaluated at weeks 4 and 7 using ^1H and ^{13}C NMR spectroscopy, where analytically feasible. Caffeine was completely removed by week 4 in bacterial and consortium wetlands and by week 7 in the fungal wetland. MeP exhibited degradation in the fungal wetland 89% at week 4, while complete removal was achieved in all wetlands by week 7. The combined fungal–bacterial system did not universally outperform monocultures during the initial adaptation phase but reduced adaptation time, achieved faster system stabilization, and provided balanced degradation performance across chemically distinct pollutants, demonstrating functional complementarity rather than additive synergy. It enhanced methylparaben removal compared to bacteria alone, while showing similar performance to the bacterial system for caffeine degradation. TCC could not be quantified by NMR due to solubility limitations; however, stable wetland operation and high removal of other pollutants indicate favourable conditions for biodegradation of selected pollutants.

Overall, this research demonstrates that targeted microbial adaptation to synthetic wastewater enhances pollutant degradation by enabling microbial growth and metabolic activity using wastewater-derived nutrients. By linking laboratory-scale optimization with pilot-scale validation in constructed wetlands, the study establishes a scalable, sustainable strategy that actively intensifies microbial self-cleaning beyond passive natural attenuation in contaminated aquatic systems. Collectively, this work reframes surface-water self-cleaning from a passive natural phenomenon into a deliberately engineered, biologically intensified process for contaminant attenuation.