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HABILITATION THESIS

Biodegradation and bioconversion of chemical pollutants in aquatic ecosystems for the protection of environmental factors

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ABSTRACT

The content of the habilitation thesis is structured into four chapters and reflects my scientific achievements obtained after the completion of my doctoral studies in 2011. It also presents the research directions that have guided my activity to date, as well as the strategy I intend to pursue in the future.

The first chapter, entitled **“Scientific and Professional Achievements”**, presents my academic and scientific development up to the present time. In 2012, I was promoted through a competitive examination from the position of Lecturer, and in 2026 I was promoted to Associate Professor. My academic career advancement has been supported by sustained scientific research activities, materialized through the publication of 32 articles in national and international journals (26 of which were published in ISI-indexed journals), as first author, corresponding author, or co-author, 14 papers at national and international conferences and have participated as a team member in five research contracts/projects.

The second chapter, entitled **“Chemical Reactivity and the Mechanisms of Action of Toxic Compounds in Environmental Pollution Processes”**, addresses the behavior and mechanisms of action of chemical substances originating from industrial activities, including wastewater, industrial waste, and accidental contaminations released into the environment through aquatic systems or soil. Particular emphasis is placed on the environmental impact of these contaminants and their interactions within natural ecosystems.

The third chapter, entitled **“Experimental Strategies and Biotechnological Applications for the Removal of Chemical Pollutants from Aquatic Ecosystems”**, presents the research achievements obtained in the field of pollutant removal through the use of microbial suspensions. The experimental investigations focused on monitoring chemical parameters correlated with the biodegradation capacity of microorganisms. The identification of microbial strains specifically adapted to the degradation of individual pollutants contributes to the restoration of biocenoses within wastewater treatment plants affected by accidental pollution events and supports the efficient biodegradation of industrial wastewater. Biological suspensions were cultivated under controlled laboratory conditions using standardized culture media developed within the university laboratory. The experiments aimed to evaluate the ability of microorganisms to remove toxic substances through bioconversion processes that are often difficult to achieve using conventional chemical treatment methods. In addition, the toxicity levels of these compounds were assessed.

Within the subchapter entitled **“Ecological Techniques for the Removal of Toxic Chemical Compounds from Aquifers Contaminated by Industrial Discharges through Microbial Catalytic Conversion”**, I investigated the biocenosis of an artificially established wastewater treatment system using a synthetic polluted medium. The use of microorganisms in bioconversion processes represents a modern and sustainable strategy for reducing environmental pollution. Their capacity to transform contaminants into harmless compounds contributes significantly to water resource protection, remediation of contaminated soils, and mitigation of the adverse impacts of anthropogenic activities on the environment. The study aimed to identify microorganisms with high efficiency in the removal of toxic contaminants, as well as dominant microbial species within the biocenosis of activated sludge adapted to the biodegradation of organic pollutants. Understanding the microbiota involved in pollutant degradation enables the development of optimized strategies for the bioremediation of industrially contaminated waters, including bioaugmentation and biostimulation approaches through the enrichment of contaminated environments with specific microbial populations.

The subchapter entitled **“Control of Chemical, Physical, and Biological Parameters in Pilot-Scale Wastewater Treatment Systems for Enhancing the Removal of Toxic Compounds from Industrial Wastewaters”** presents a series of specific chemical analyses used to determine water pollution levels. The study monitored the evolution of electrical conductivity ($\mu\text{S}/\text{cm}$), dissolved oxygen concentration (mg/L), and pH in the main compartments of the treatment system. Electrical conductivity is an important indicator of the chemical load of water and is frequently used to evaluate pollution levels and control wastewater treatment processes. This parameter reflects the total concentration of dissolved ions such as Na^+ , Cl^- , NO_3^- , SO_4^{2-} , Ca^{2+} , and Mg^{2+} originating from industrial, agricultural, and domestic sources. The main sources of these ions include chemical fertilizers, detergents, domestic wastewater, and industrial effluents containing heavy metals, acids, and other dissolved substances. Conductivity depends on ion concentration and composition, temperature, dissolved oxygen concentration, and medium viscosity; therefore, it is considered a relevant indicator of water mineralization and contamination levels. Monitoring the BOD/COD ratio was performed to evaluate the biodegradability of the chemical medium introduced into the system by the microbial population present in the activated sludge.

The biodegradation capacity of toxic substances through the use of microorganisms is presented in the subchapters **“Biodegradation and Bioconversion of Aromatic Chemical Compounds Using SCLP Biological Cultures (*Saccharomyces* sp., *Chlorella* sp., *Lactobacillus* sp., *Paramecium* sp.)”** and **“Assessment of the Toxicity of o-Chlorobenzylidene Malononitrile through Determination of Oxygen Production by *Chlorella pyrenoidosa* Algal Cells.”** In these studies, the removal performance of o-chlorobenzylidene malononitrile (CBM), the active component of CS tear gas used in military and crowd-control operations, was evaluated through investigations of the mechanisms involved in toxic compound elimination. The performance indicators included specific growth rate (μ), dry cell weight (DCW), biomass productivity (BP), flocculation activity (FA), percentage of growth inhibition (%IR), percentage yield inhibition (%I), Fourier Transform Infrared Spectroscopy (FTIR), and fluorescence analysis of chlorophyll extracts. Additionally, oxygen production (DX) by *Chlorella pyrenoidosa* cultures exposed to CBM was evaluated, and the EC₅₀ value was determined from regression analyses corresponding to 50% inhibition. Metabolic processes occurring within *Chlorella* cells, together with chemical reactions in the culture medium, generated compounds characterized by functional groups identified through FTIR spectroscopy. The experimental results demonstrated that *Chlorella pyrenoidosa* can tolerate elevated CBM concentrations and may contribute significantly to bioremediation processes. The formation of cellular aggregates and maintenance of high viability indicate the existence of adaptive mechanisms against chemical stress. Consequently, this microalga exhibits cellular stability and considerable potential for environmental monitoring and remediation programs targeting contaminated environments.

The subchapter **“Removal of Toxic Compounds Originating from the Petrochemical Industry Using the Microalga *Spirulina platensis*”** presents the results obtained from toxicity studies involving naphthenic acids, a class of chemical compounds originating from the petrochemical industry. The experiments monitored algal growth in media contaminated with different concentrations of naphthenic acids while evaluating pH, electrical conductivity, and dissolved oxygen content (DO). Cell viability was assessed through transmission electron microscopy (TEM) and optical microscopy. The concentration of naphthenic acids in the analyzed samples was determined through acid number measurements and FTIR characterization. The EC₅₀ value was established by extrapolation at a concentration of 110 mg/mL of naphthenic acids. The results demonstrated that the chemical stress generated by these compounds can be

tolerated by *Spirulina platensis*, which biodegrades the toxic substances and utilizes them as carbon sources for cellular synthesis.

In the subchapter “**Anticorrosion Protection Methods for Al, Zn, and Fe Metals in Aquatic Environments through Ecotoxicological Monitoring of Diatom, *Saccharomyces*, and *Spirulina* Microorganisms**”, the corrosion behavior of aluminum, zinc, and iron plates was evaluated under controlled conditions using biological suspensions consisting of diatoms, *Saccharomyces* yeasts, and *Spirulina* algae (DSS). The investigations focused on three major aspects: (1) monitoring the evolution of chemical and biological parameters of the reaction medium (pH, conductivity, total dissolved solids, dissolved oxygen, and optical density) and microbial growth kinetics; (2) analysis of biofilm formation on exposed metallic surfaces; and (3) evaluation of corrosion phenomena using volumetric, gravimetric, and penetration corrosion indices. The results revealed the following corrosion resistance sequence: AlDiat > FeDiat > ZnDiat > AlSpir > ZnSpir > AlSach > ZnSach > FeSpir > FeSach, while biofilm development followed the sequence: AlDiat > FeDiat > ZnDiat > FeSpir > ZnSach > FeSach > AlSach > ZnSpir > AlSpir.

Iron exhibited the most favorable performance, showing very low penetration index values in all tested environments, including saline water. Aluminum demonstrated sensitivity to specific biological environments, with the highest corrosion rates observed in the presence of *Spirulina*, indicating that not all biological systems provide protective effects. Diatoms and *Saccharomyces* suspensions exerted inhibitory effects on corrosion processes. Zinc was found to be the most susceptible metal, experiencing the highest corrosion rates in *Spirulina*-containing environments, followed by saline water, while biological media only partially mitigated corrosion.

The fourth chapter presents future research directions and the strategic plan for the development of my scientific, professional, and academic career.