

University of Naples Federico II



Department of Agricultural Sciences
PhD in Sustainable Agricultural and Forestry Systems and Food
Security

XXXVII Cycle

A multidisciplinary approach to sustainable plastic depolymerization through microalgae enzymatic biocatalysis

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Verifying true biodegradation of plastics demands more than simple weight-loss measurements; it requires a combination of spectroscopic, microscopic, mechanical, and chromatographic analyses to demonstrate chemical, structural, and functional transformations in the polymer. Fourier-transform infrared spectroscopy, especially in its attenuated total reflectance (ATR-FTIR) configuration, is routinely used to monitor the appearance of new functional groups on plastic surfaces. Peaks corresponding to carbonyl (C=O), hydroxyl (–OH), and ether (C–O–C) vibrations serve as unambiguous markers of oxidative weathering induced by microbial enzymes or reactive oxygen species (Campanale et al. 2023; Davidson et al. 2023).

While ATR-FTIR captures chemical modifications at the molecular level, scanning electron microscopy (SEM) reveals their physical consequences. High-resolution SEM micrographs document the formation of pits, cracks, grooves, and generalized surface roughening that accompany microbial colonization (Oberbeckmann et al. 2016). When SEM is coupled with energy-dispersive X-ray spectroscopy (EDX), elemental maps quantify shifts in the carbon-to-oxygen ratio across the polymer surface, offering direct evidence of oxidative incorporation of oxygen atoms into the backbone (Cocca et al. 2020).

Mechanical testing completes the picture by quantifying material weakening. Tensile strength measurements performed before and after microbial exposure detect statistically significant reductions in ultimate tensile strength and elongation at break, confirming that enzyme-mediated chain scission undermines the polymer's structural integrity (Hadiyanto et al. 2021). Although gravimetric assays provide a convenient first estimate of mass loss, they cannot distinguish between true depolymerization and mere surface erosion or sloughing of biofilm, underscoring the need for spectroscopic and mechanical corroboration.

Finally, high-performance liquid chromatography (HPLC) delivers the most direct proof of enzymatic cleavage by detecting and quantifying released monomers and oligomers. For instance, the appearance of terephthalic acid in culture supernatants - measured by reverse-phase HPLC with ultraviolet detection - confirms PET depolymerization and enables calculation of enzymatic yield and kinetics (Ashraf-Khorassani et al. 2025). By integrating ATR-FTIR, SEM-EDX, mechanical testing, and HPLC, researchers can construct a comprehensive, multi-evidence assessment of plastic biodegradation, thereby validating both laboratory findings and field-scale bioremediation strategies.

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Chapter 2

Innovative polypropylene biodegradation by fungal laccase reveals microalgae as emerging biofactories for sustainable bioremediation

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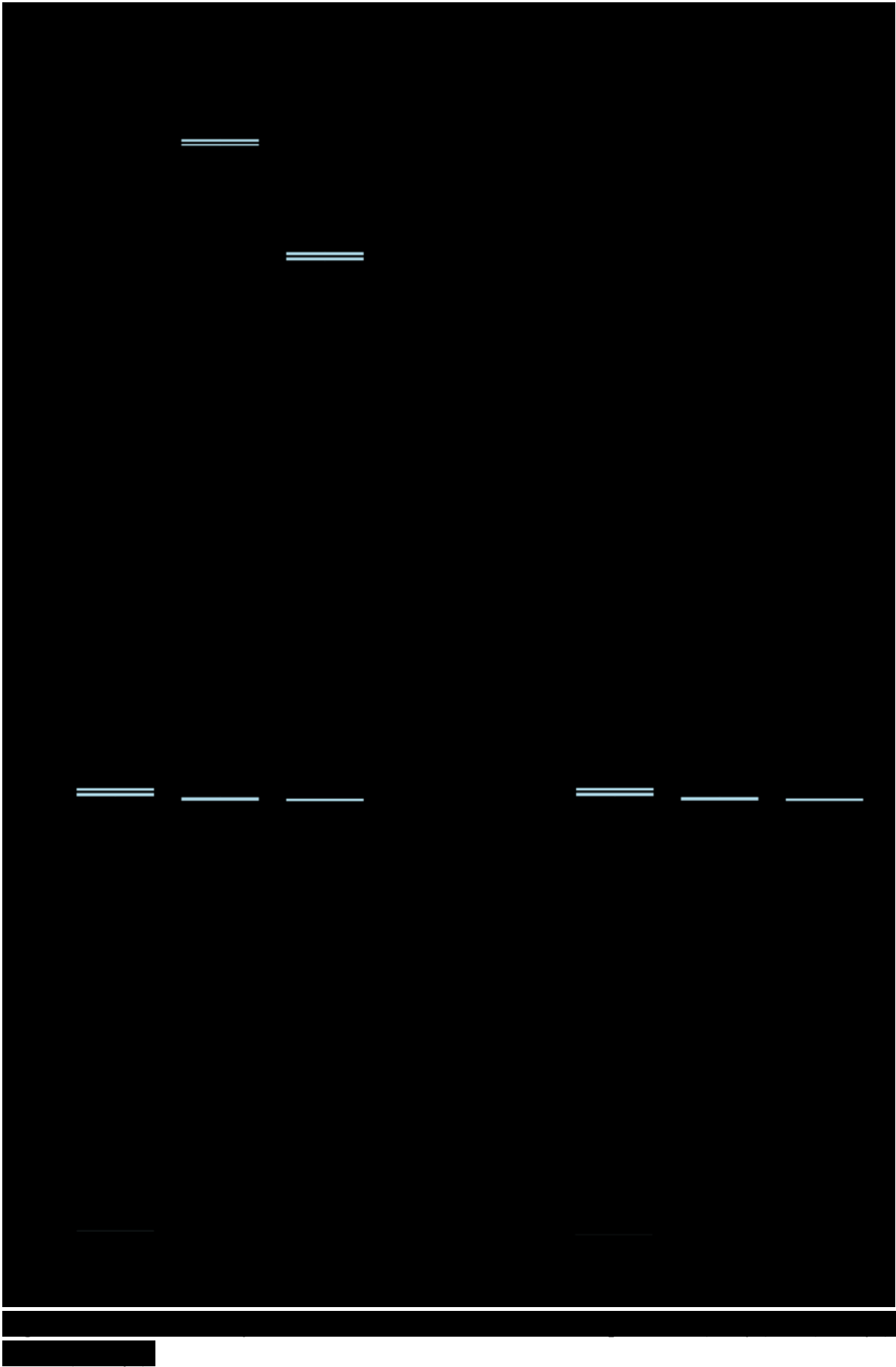


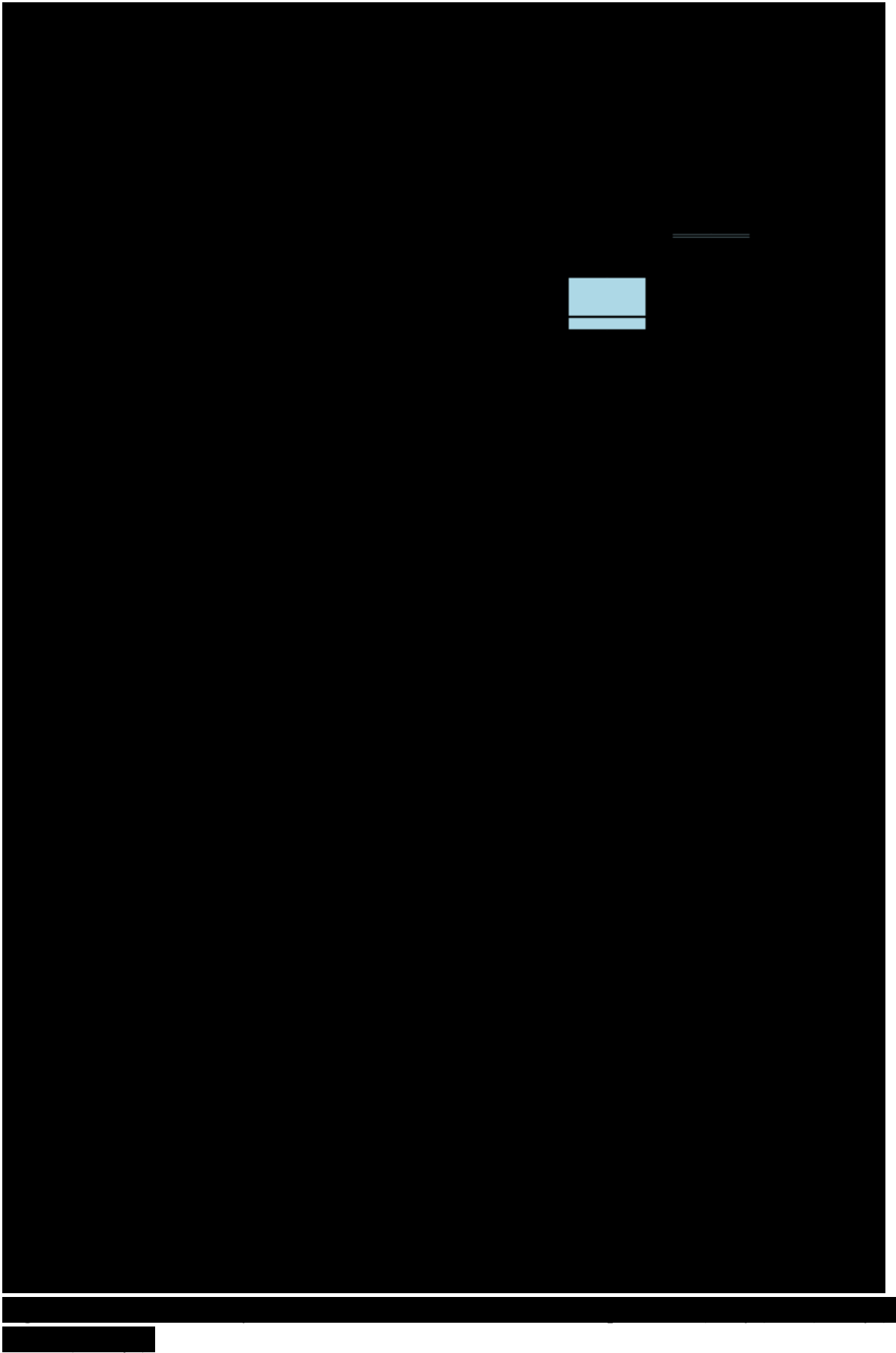
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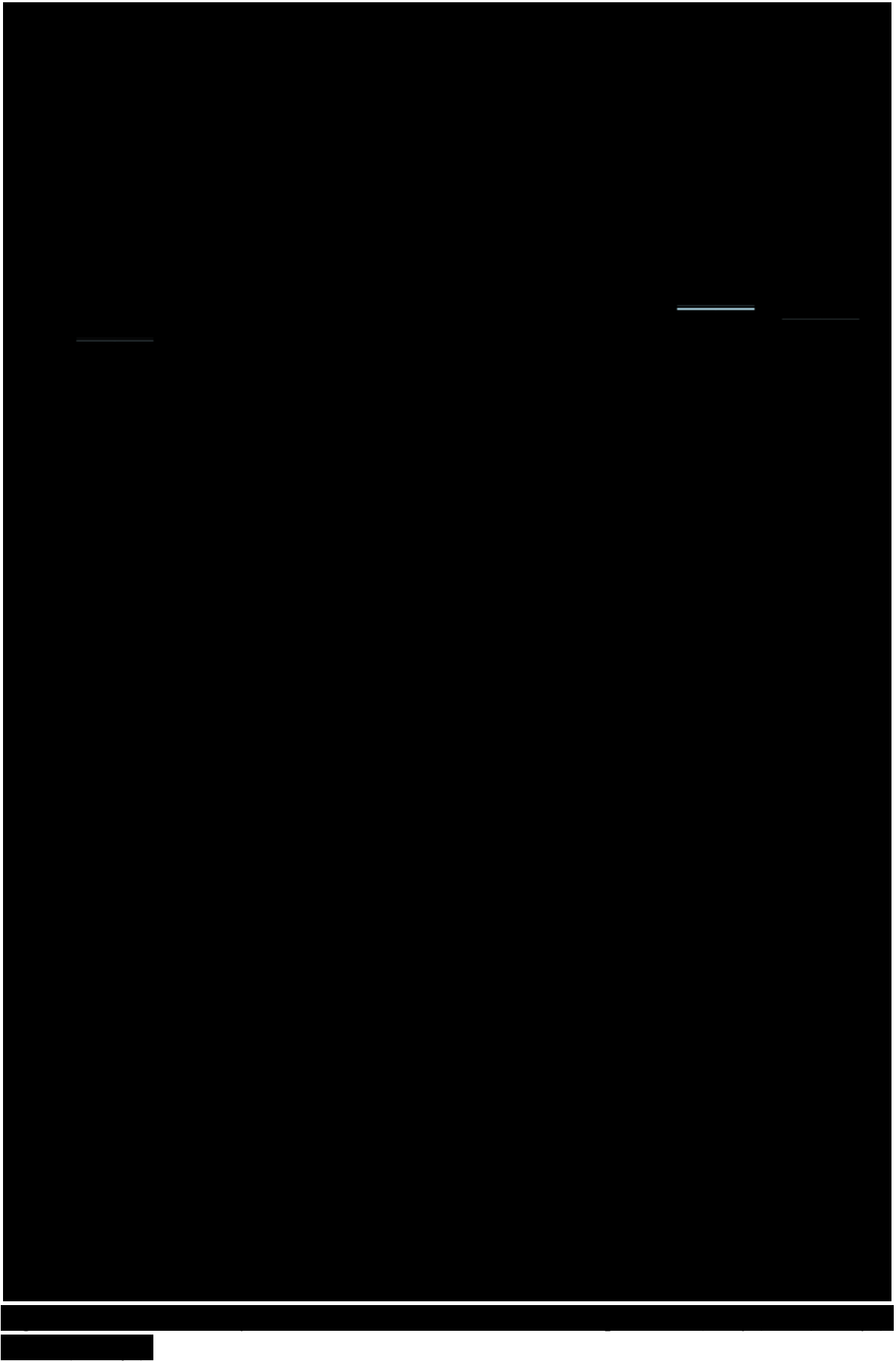
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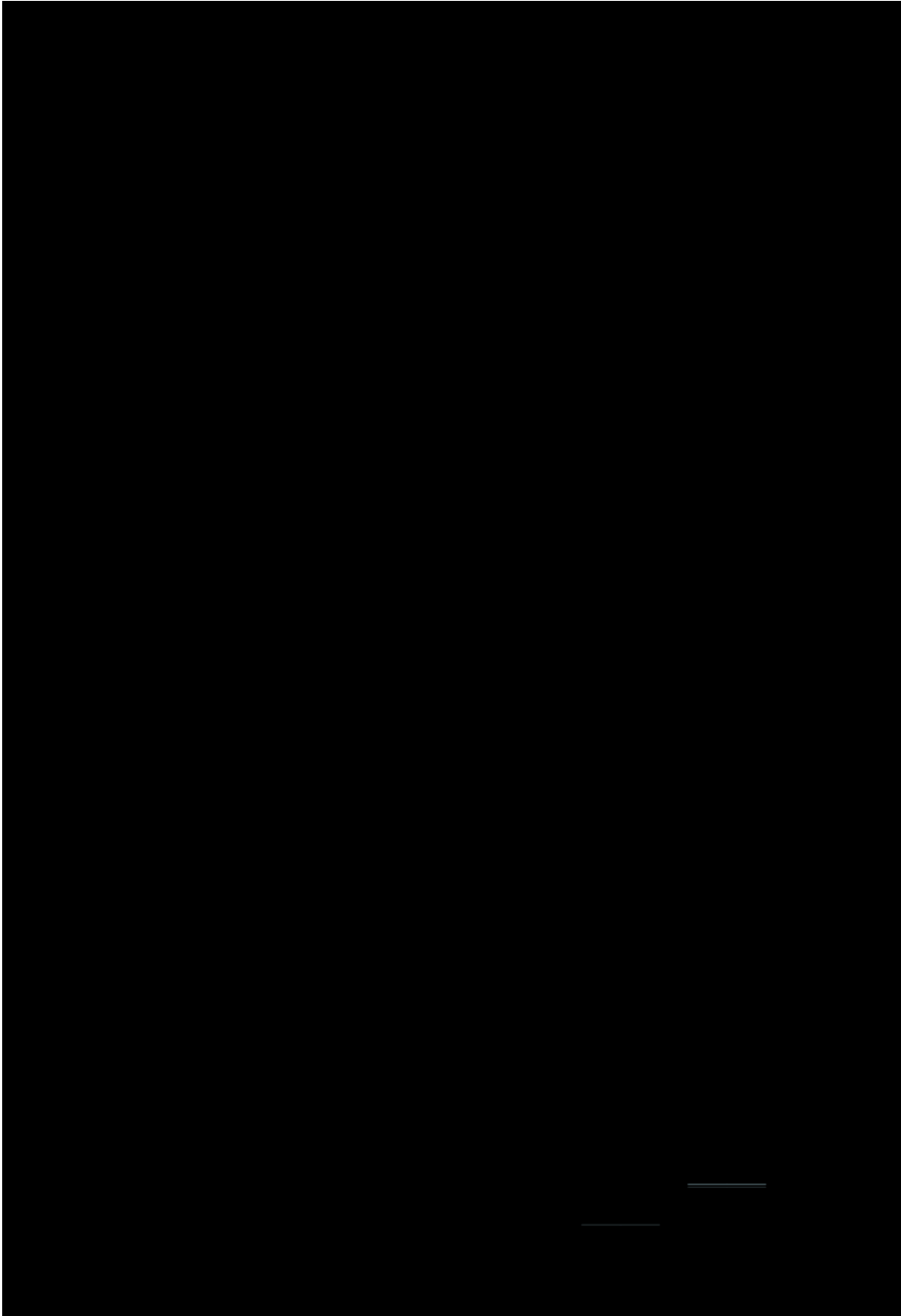
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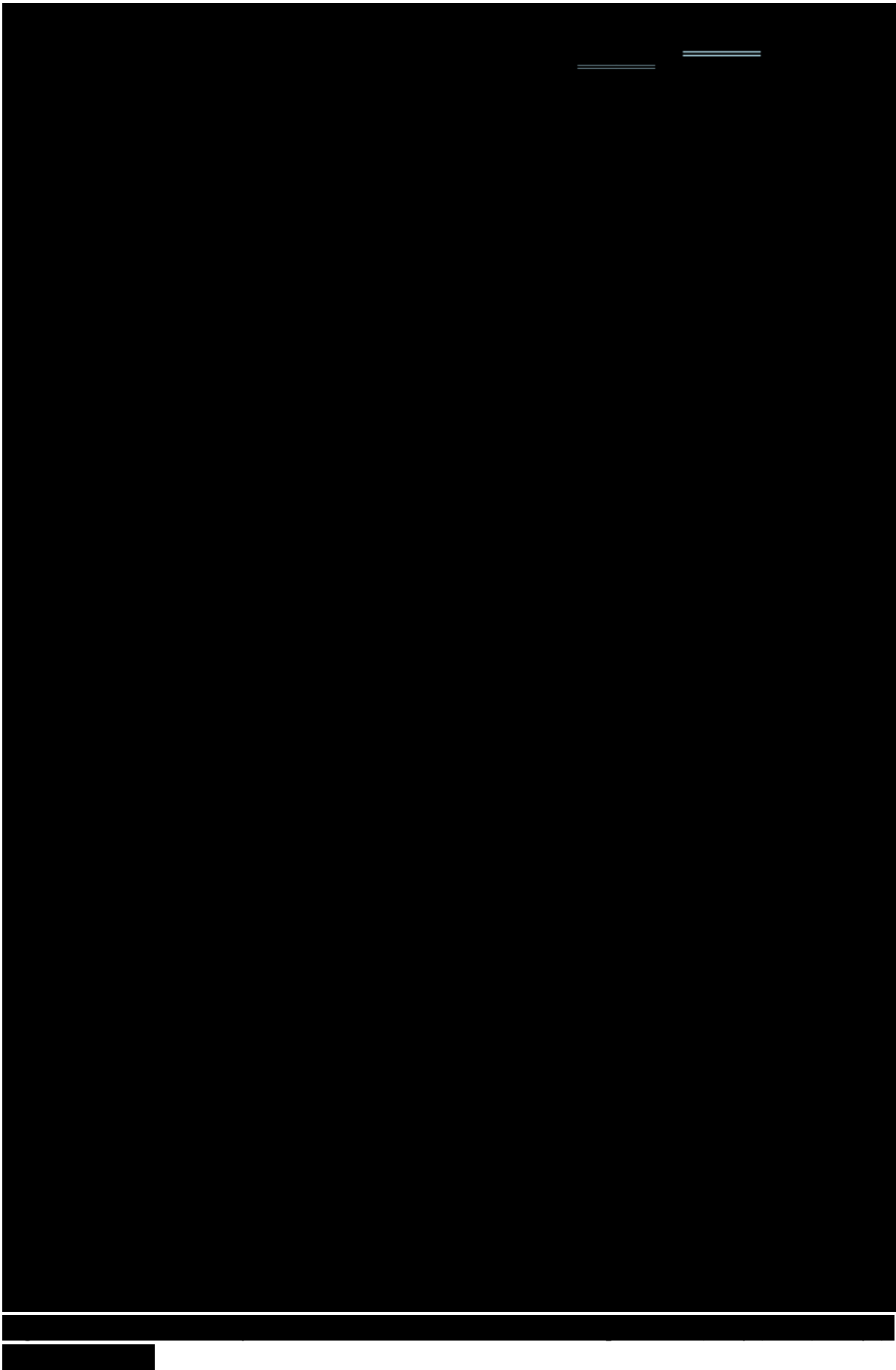
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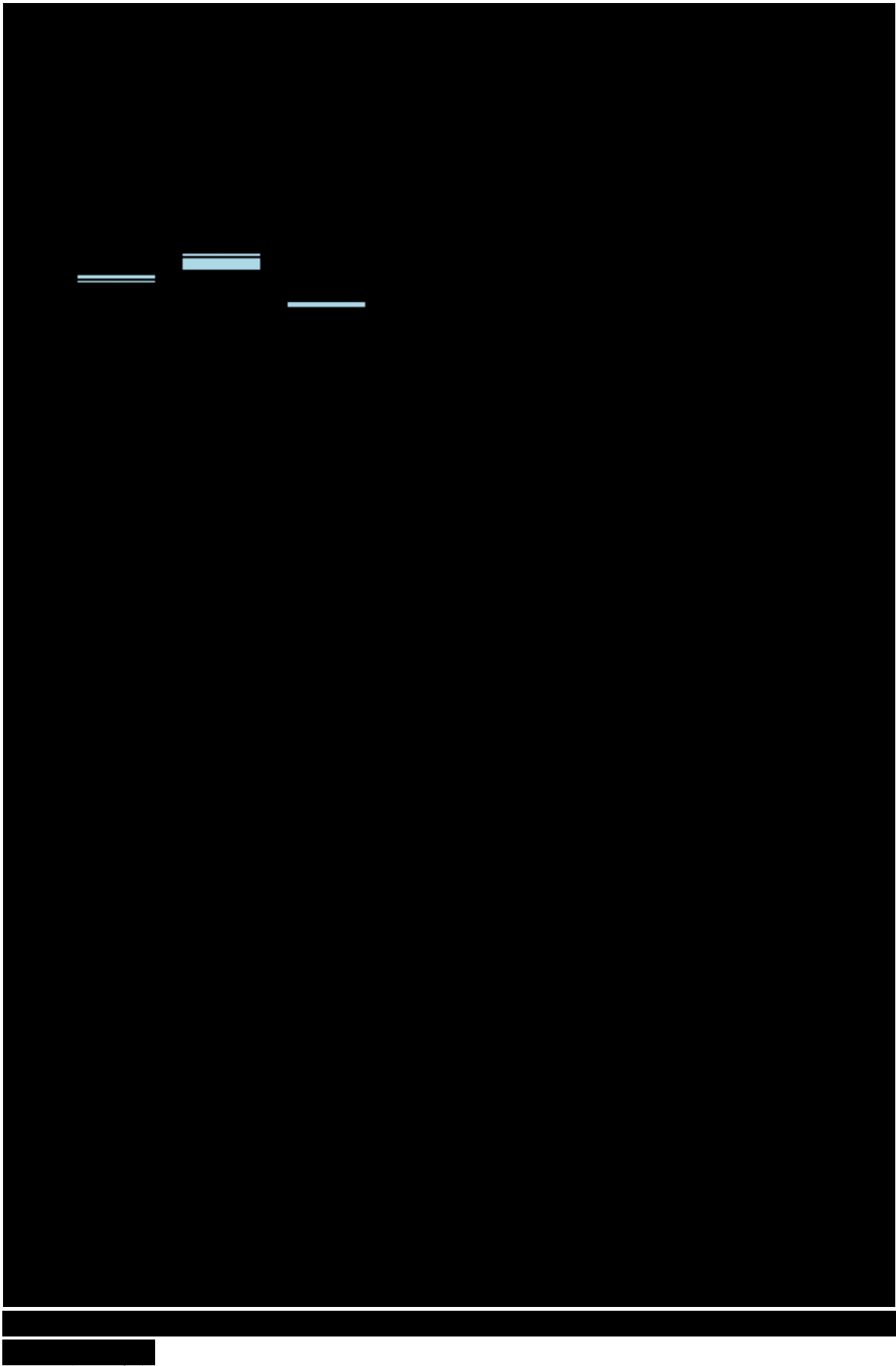










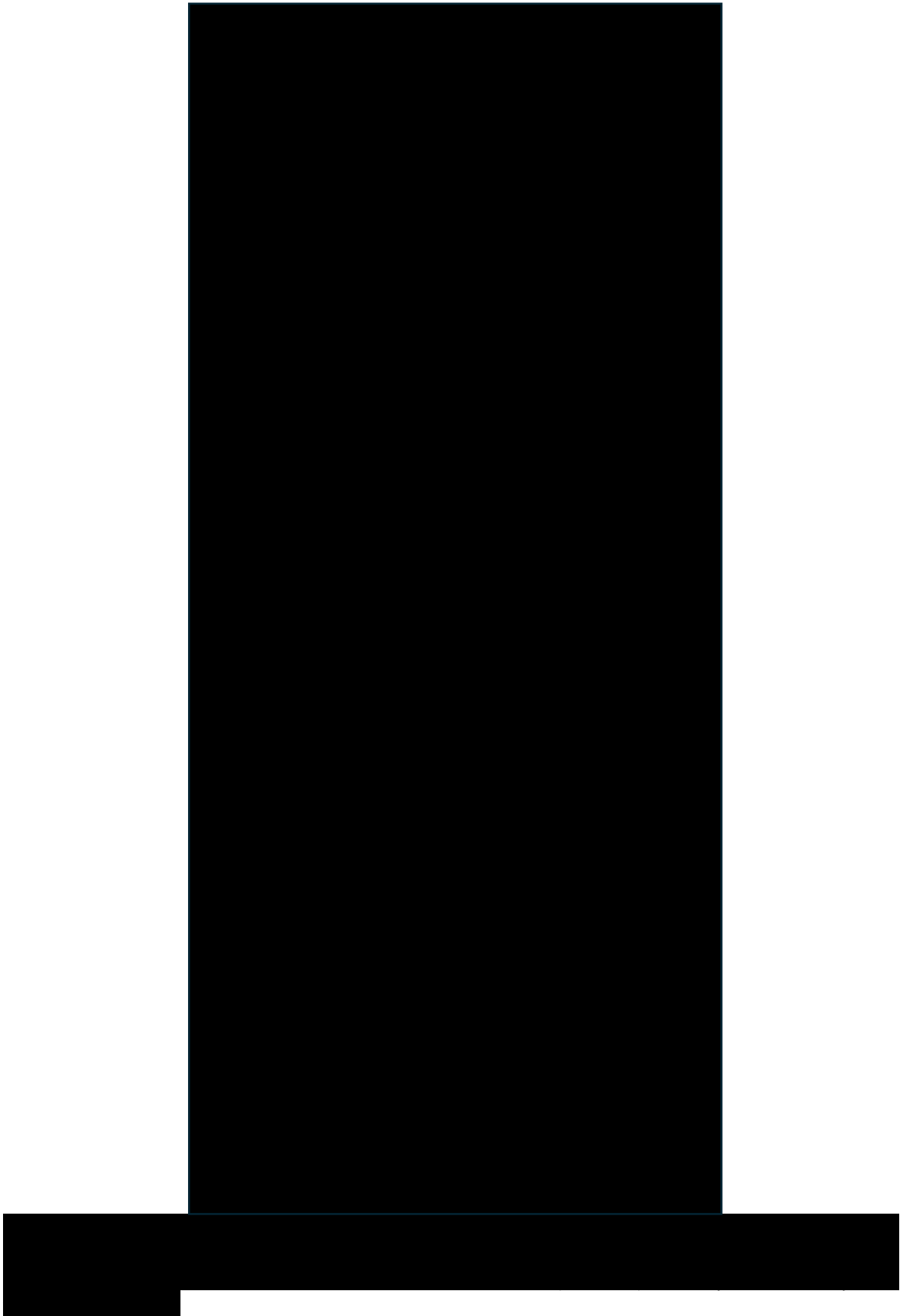


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Chapter 3

Algae versus polypropylene (PP): functional and structural insights into green microalgae-mediated plastic surface oxidation

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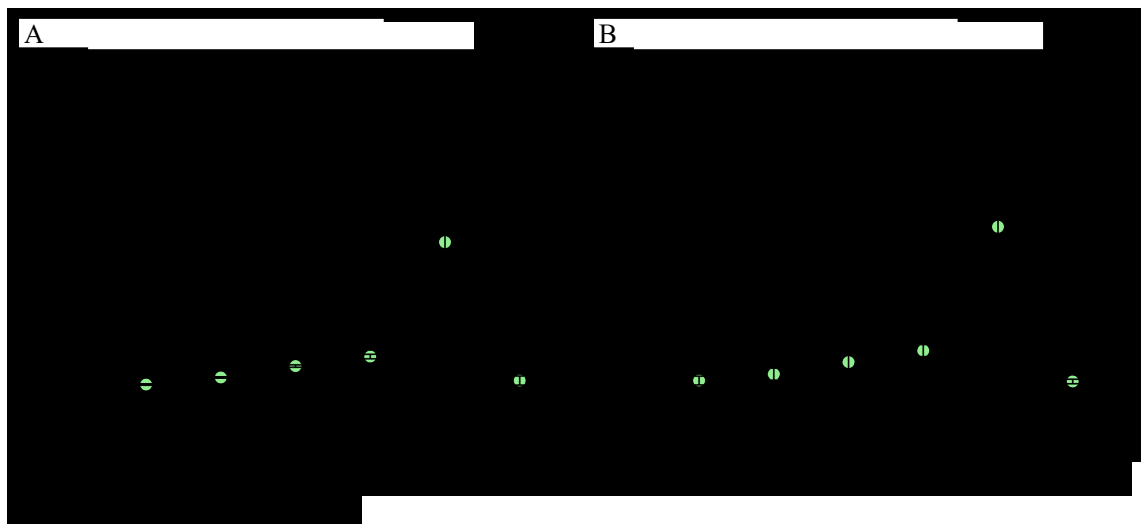
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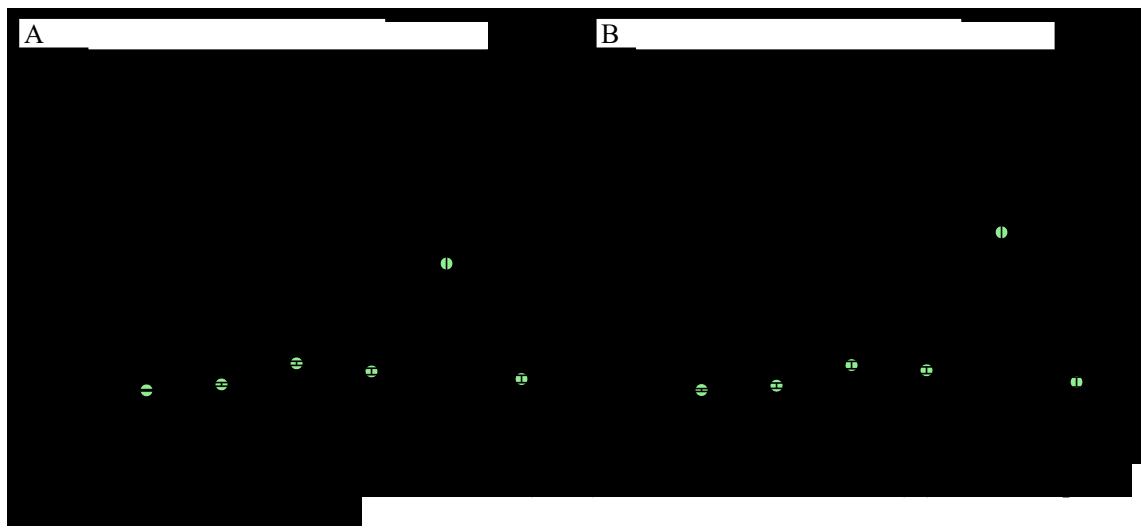
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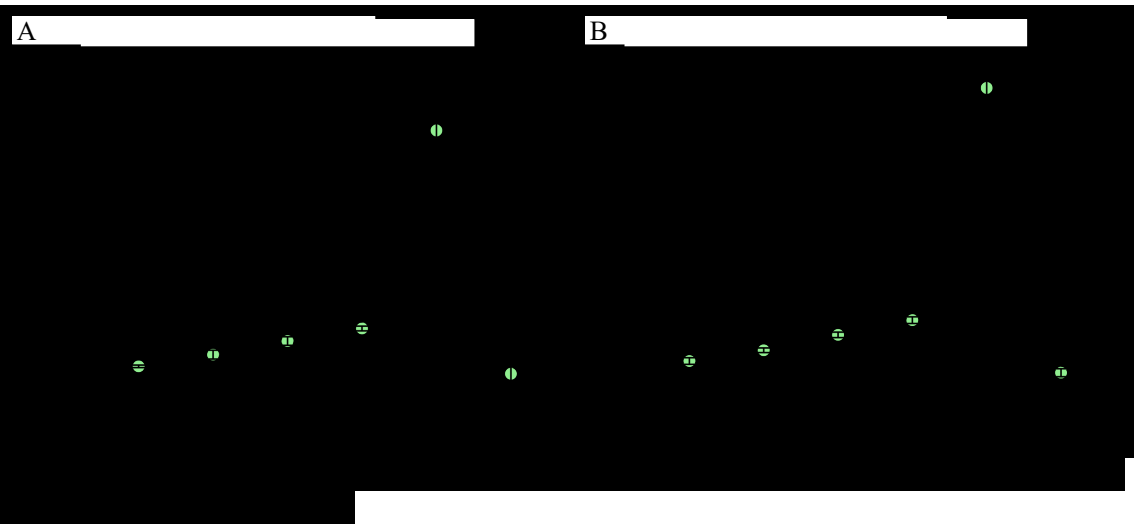
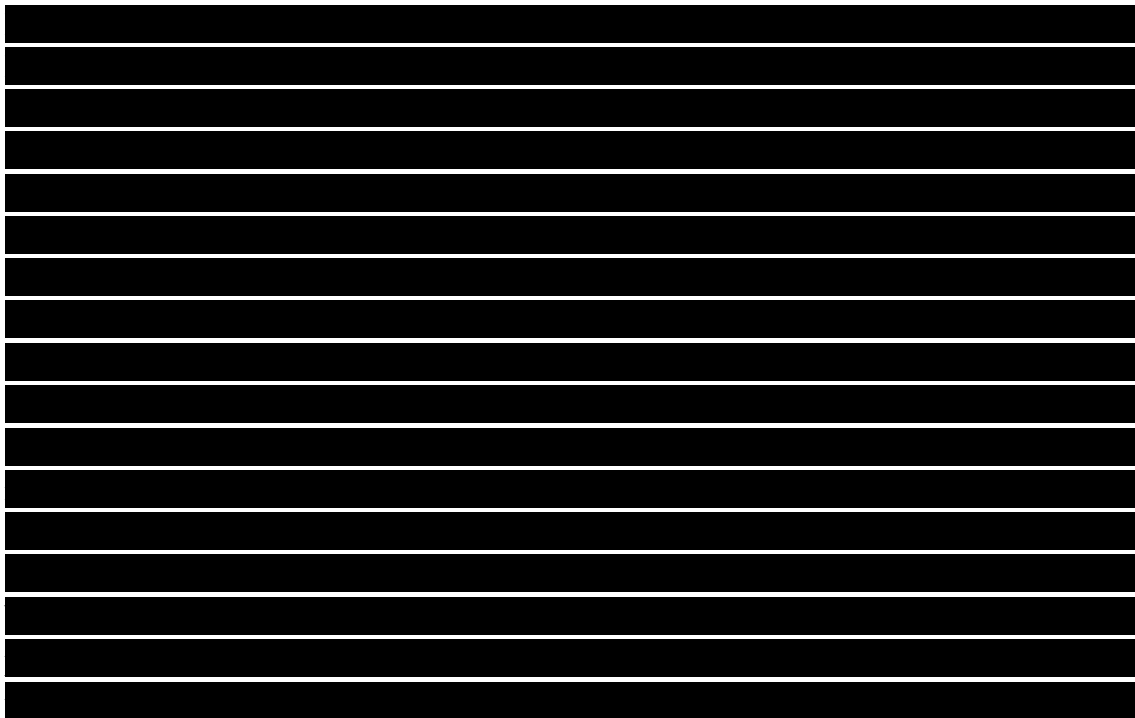
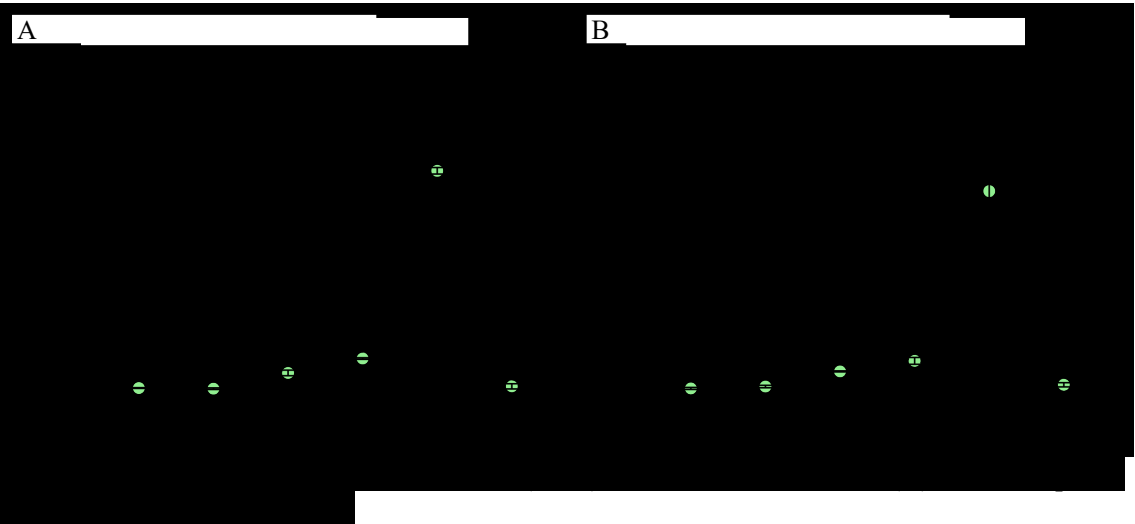


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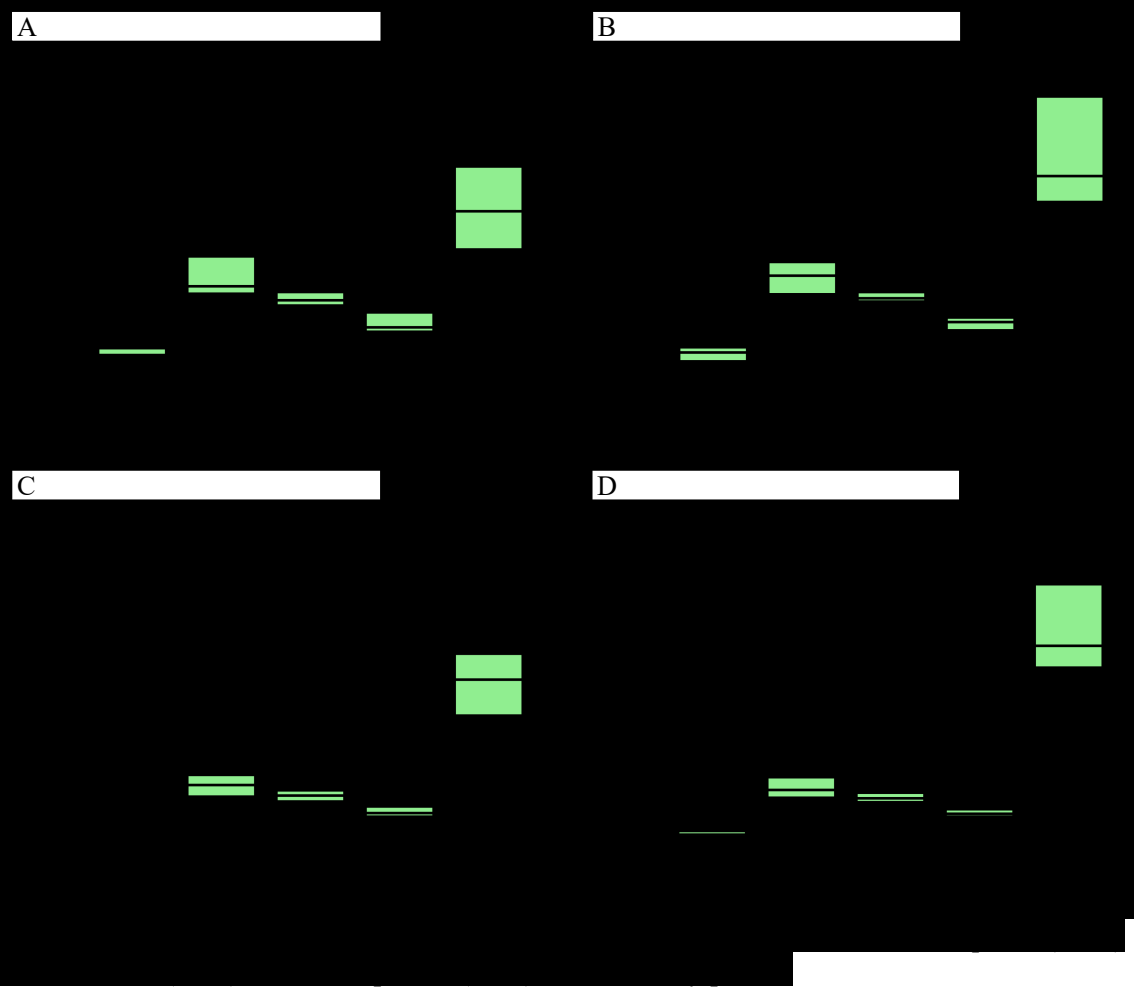
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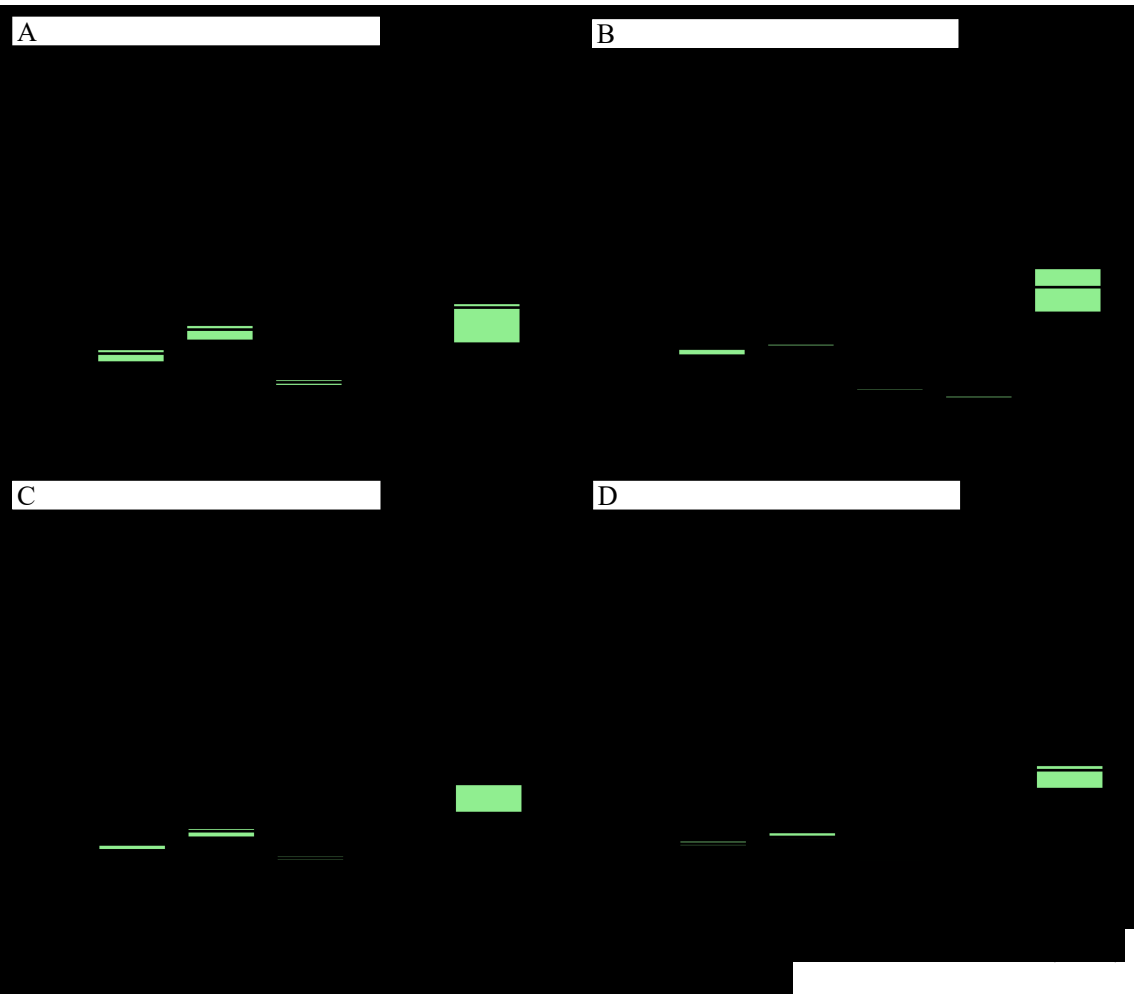
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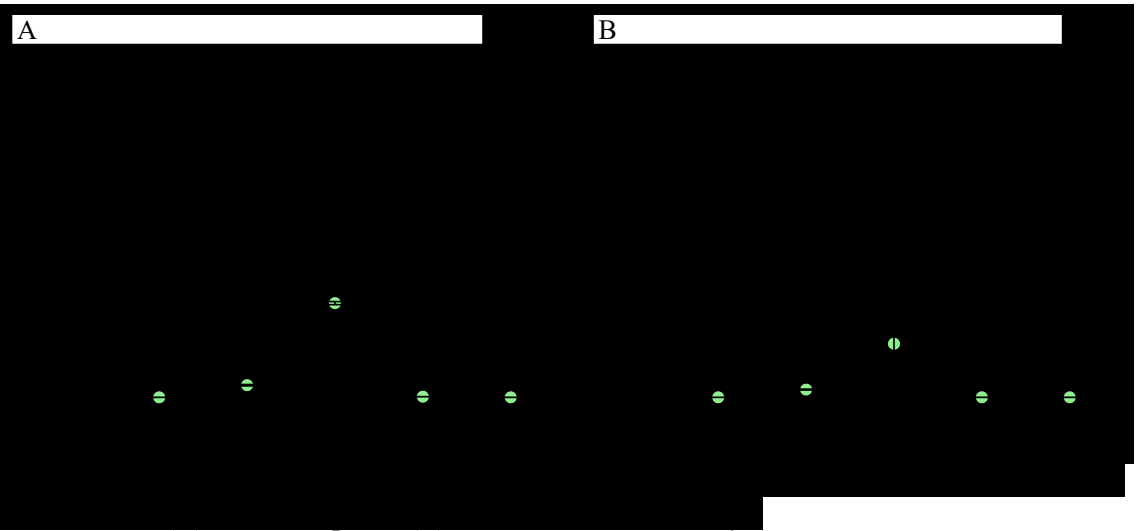


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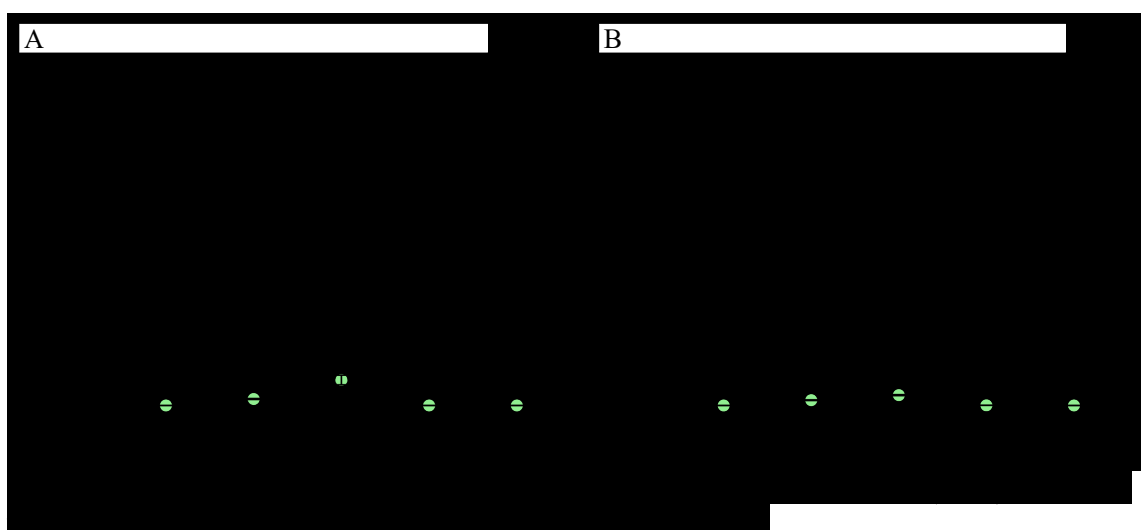
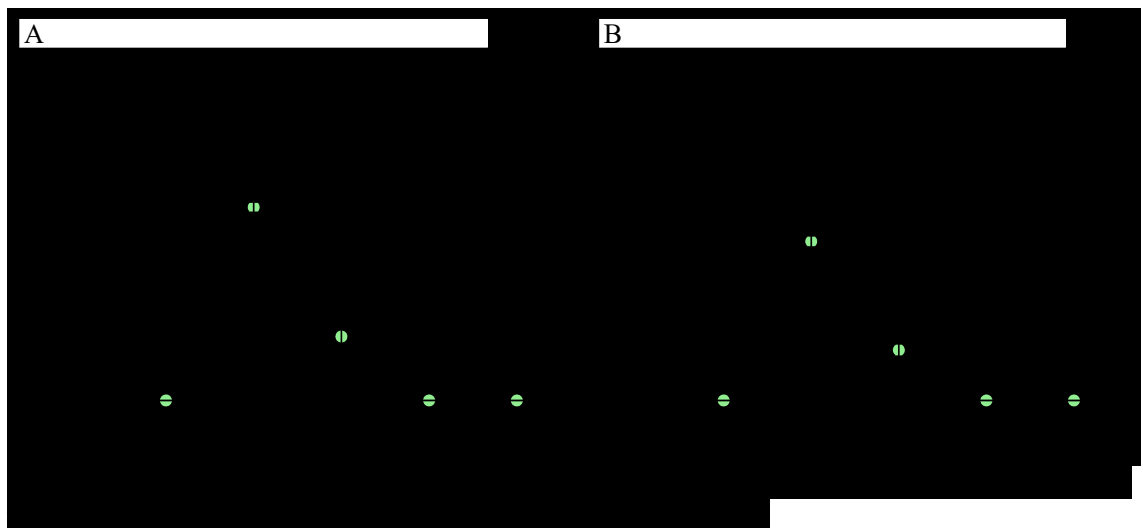
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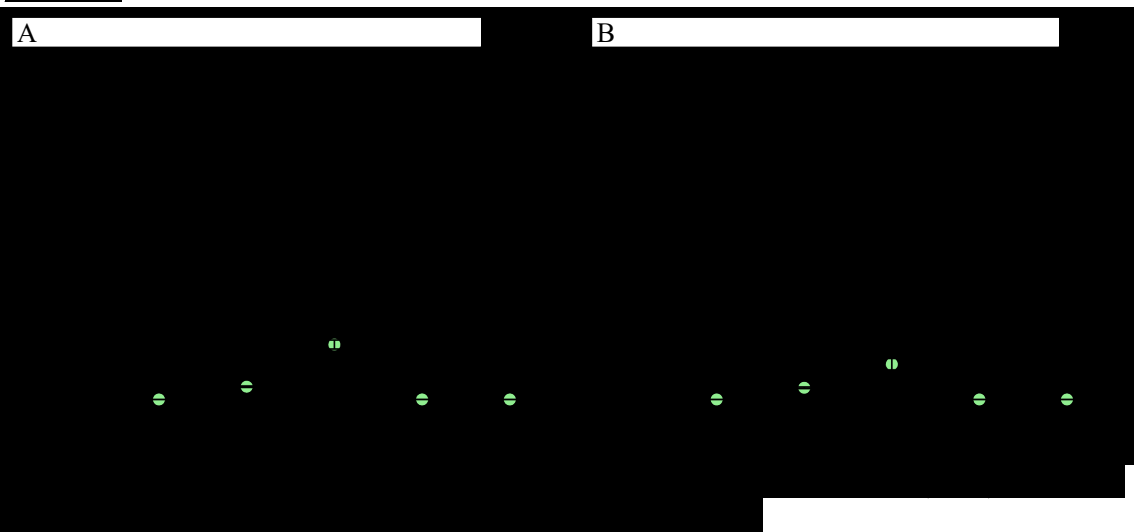


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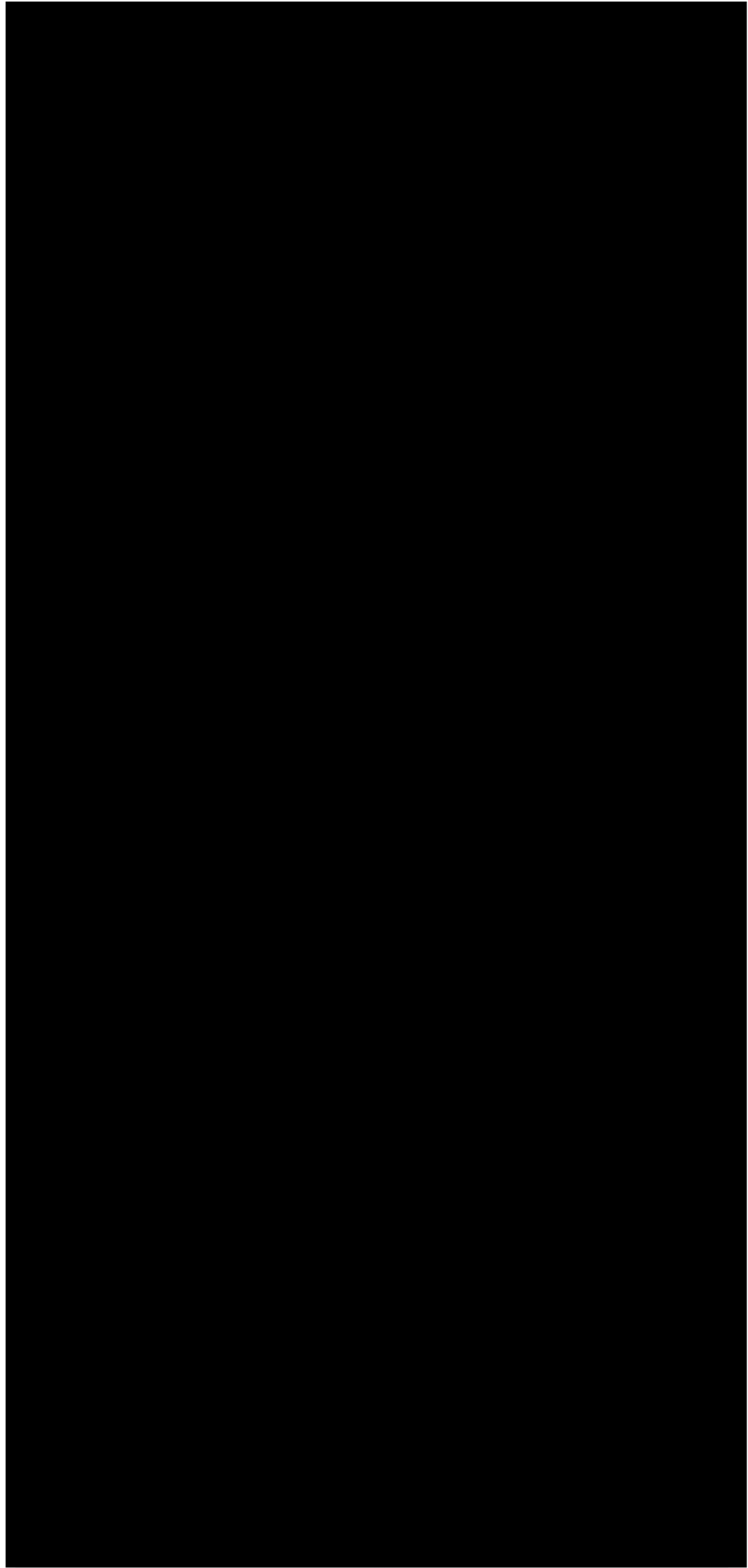
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Chapter 4

Green microalgae mediated Polyethylene terephthalate (PET) oxidation under light and dark conditions revealed bioremediation potential

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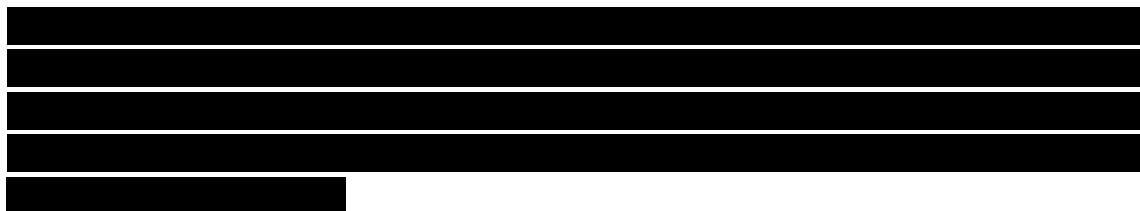
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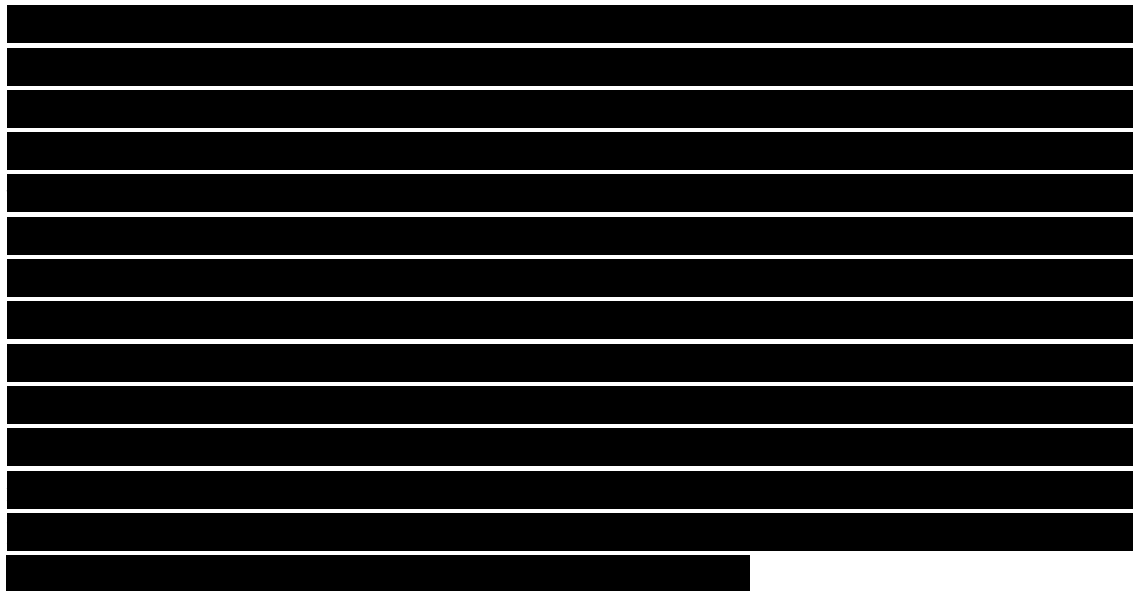
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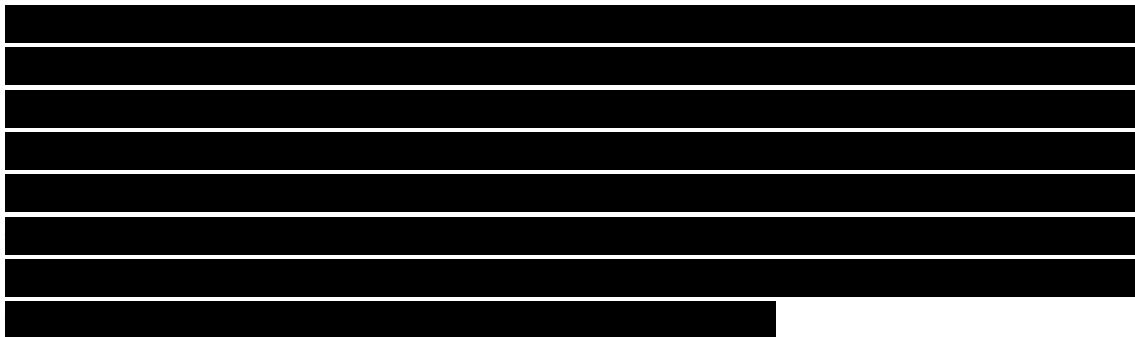
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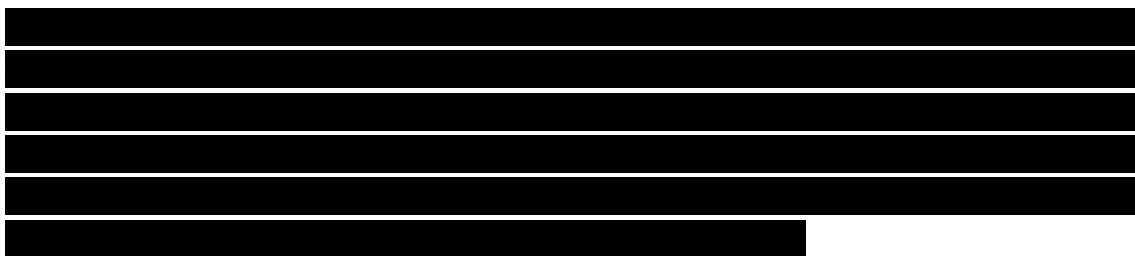
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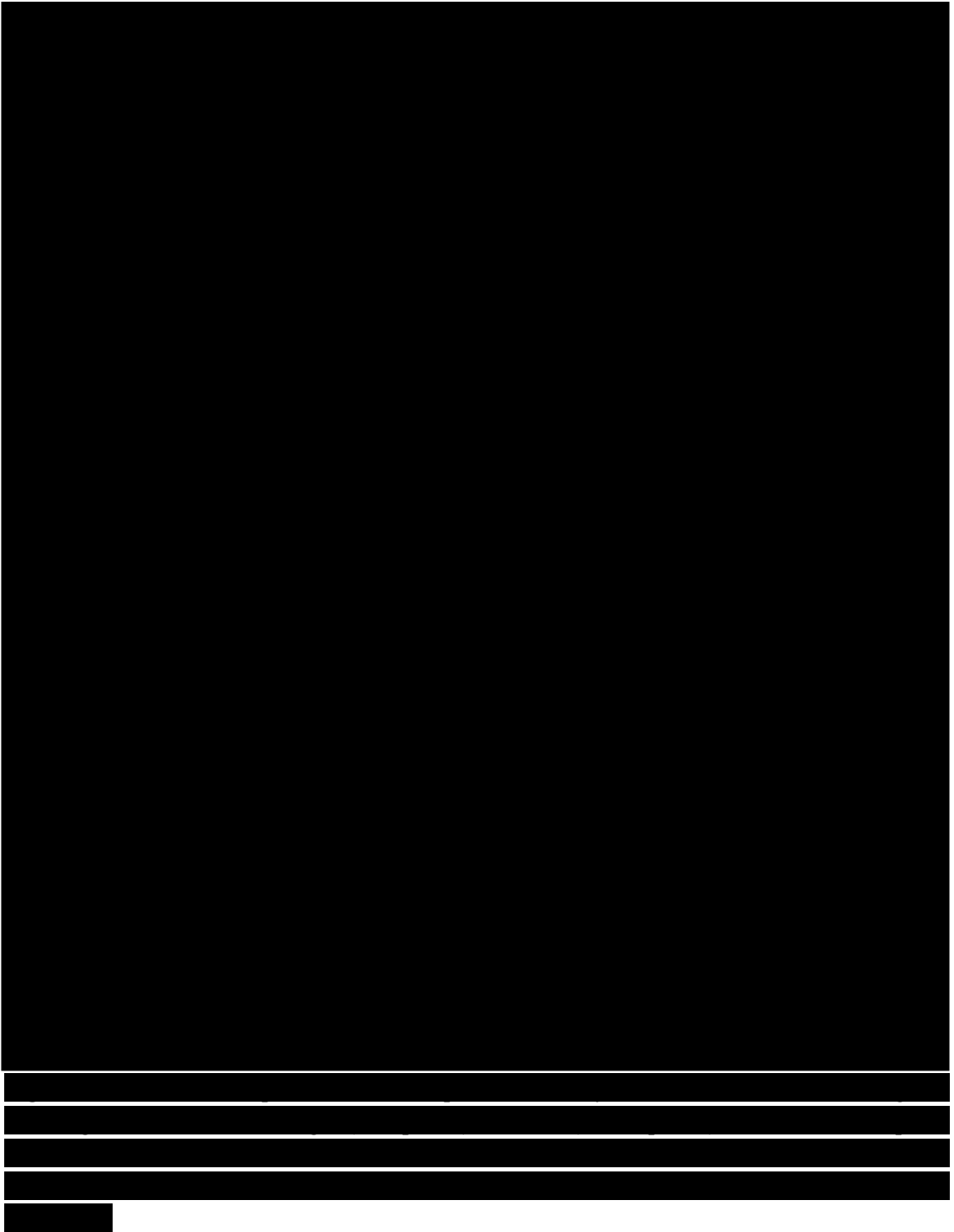
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General conclusions

This research work has extensively explored the potential of green microalgae as innovative agents in the biotransformation of synthetic plastics, with particular focus on polyolefin polymers (PP), polyethylene terephthalate (PET), and the enzymatic activity of laccases. Through a multidisciplinary approach integrating molecular biology, bioinformatics, physiology, biochemistry, and advanced material characterization techniques, various aspects of the complex interactions between microalgae and polymer surfaces were analyzed, contributing substantially to the advancement of knowledge on the biological mechanisms underlying microalgae-based bioremediation.

In the first chapter, the study investigated the capacity of 35 freshwater green microalgae to produce extracellular laccases, enzymes renowned for their versatility in the oxidative degradation of various xenobiotic compounds, including plastic polymers (Geyer et al. 2017; Rodríguez et al. 2006). Bioinformatic analyses conducted on *Chlamydomonas reinhardtii* (#103) and *Botryococcus braunii* (#131) identified several putative genes encoding for laccase-like multicopper oxidases (LMCOs), with conserved copper-binding domains and signal peptides suggesting extracellular secretion. Phylogenetic analyses revealed a noteworthy evolutionary proximity of *C. reinhardtii* LMCOs to fungal laccases from *Pleurotus ostreatus*, indicating functional similarities in substrate specificity and catalytic stability (Janusz et al. 2017; Ramamurthy et al. 2024). Enzymatic activity assays performed at different time points confirmed strain-dependent variability in laccase production, highlighting *Desmodesmus communis* (#126), *Chlamydomonas acidophila* (#100), *Heterochlorella luteoviridis* (#124) and *Botryococcus braunii* (#131) as promising producers of extracellular laccase activity. These findings provide a solid foundation for developing microalgae biotechnological platforms for industrial enzyme production and subsequent application in plastic bioremediation (Shafana et al. 2023). Further studies could focus on heterologous expression of the identified LMCO genes in robust microbial or algal hosts, enzyme purification, and structural-functional characterization to optimize their catalytic properties for industrial-scale applications.

The second chapter experimentally addressed the interaction between five selected microalgae and polypropylene (PP) surfaces, a highly widespread and plastic material. The exposure of microalgae to PP substrates revealed significant physiological and pigment-related responses correlated with polymer presence. Notably, carotenoid accumulation, particularly in early exposure phases, suggested activation of antioxidant mechanisms to counteract oxidative stress induced by contact with the plastic surface (Sharma et al. 2023). ATR-FTIR analysis of polymer surfaces revealed the introduction of oxidative functional groups (hydroxyl, carbonyl, and vinyl), consistent with surface oxidation processes mediated by microalgae metabolites or extracellular enzymatic activities (Hock et al. 2024). In particular, *C. acidophila* (#100), *C. reinhardtii* (#103), and *D. communis* (#126) demonstrated the most pronounced modifications on PP surfaces, indicating differential biotechnological potential among the tested accessions. These results reinforce the hypothesis that certain microalgae, properly selected, can serve as innovative biotechnological tools for the biotransformation of recalcitrant

plastics in contaminated environments (Johnnie et al. 2021). Future work should aim to unravel the exact molecular mechanisms driving the observed oxidative modifications, including potential roles of reactive oxygen species (ROS), biofilm-associated microenvironments, and the contribution of other secreted oxidative enzymes, such as peroxidases or cutinases, in synergistic pathways.

In the third chapter, the investigation extended to polyethylene terephthalate (PET), a widely used polymer of growing environmental concern. Twenty-seven microalgae accessions were initially screened to evaluate their growth tolerance in the presence of PET. Based on growth inhibition rates, six strains were selected for further analysis under light and dark conditions. PET exposure, particularly under phototrophic conditions, induced adaptive pigment responses, with compensatory increases in chlorophyll and carotenoids observed in several strains (Barone et al. 2024; Shoaf et al. 1976). ATR-FTIR analysis confirmed surface modifications of PET, with the formation of hydroxyl and vinyl groups attributable to metabolically active algal biofilms (Moog et al. 2019; Taniguchi et al. 2019). These findings support the role of microalgae not only as colonizers of plastic surfaces but also as facilitators of early oxidative processes, opening promising perspectives for integrated environmental bioremediation strategies (Kim et al. 2020; Vijayan et al. 2024). Given the strong influence of light exposure on pigment dynamics and oxidative capacity, future studies could investigate photobioreactor optimization, modulation of light regimes, and the potential combinatorial effects of microalgae-bacteria consortia on enhancing PET degradation pathways. Additionally, bioinformatic and structural analyses performed in this study provided the first evidence that certain hydrolases from *C. reinhardtii* and *Scenedesmus spp.* exhibit domain architectures and structural homology to known PET-degrading enzymes. Specific sequences clustered phylogenetically with bacterial PETases and displayed conserved α/β -hydrolase domains, suggesting a possible enzymatic basis for PET biotransformation. These *in silico* insights reveal previously unrecognized metabolic potential in green microalgae and reinforce their value as targets for biotechnological engineering and screening.

Taken together, both experimental and *in silico* findings converge to highlight the multifaceted potential of green microalgae in plastic bioremediation. From strain-specific tolerance and oxidative surface modification to the presence of putative PETase-like enzymes, microalgae emerge as both passive modulators and active participants in polymer transformation. Overall, the results of this research demonstrate that green microalgae, thanks to their metabolic plasticity, rapid growth, ease of cultivation, and adaptability to environmental stress, represent promising candidates for the development of sustainable approaches to mitigate plastic pollution. Although the observed processes primarily reflect early stages of polymer surface oxidation and modification, they constitute a crucial step in promoting subsequent degradation and fragmentation processes, potentially integrable with heterotrophic microbial consortia or synergistic enzymatic treatments. Further investigations should focus on long-term degradation kinetics, environmental field trials, scaling up of microalgae-bioreactor systems, and life cycle assessments (LCA) to fully validate the environmental viability and economic

sustainability of microalgae-based plastic bioremediation technologies. Moreover, advances in synthetic biology and metabolic engineering may allow the construction of tailor-made algal strains with enhanced enzyme secretion profiles, increased tolerance to plastic-associated pollutants, and superior colonization capabilities, thereby significantly boosting their efficiency as biotechnological platforms in real-world scenarios.

This study provides compelling evidence that green microalgae can induce oxidative surface modifications on synthetic polymers such as polypropylene (PP) and polyethylene terephthalate (PET). However, several limitations must be acknowledged to appropriately contextualize these findings within the broader framework of plastic biodegradation research.

Although ATR-FTIR analyses revealed the formation of oxidized functional groups (such as hydroxyls, carbonyls, and vinyl groups), these chemical changes were confined to the polymer surface and should not be interpreted as indicators of complete depolymerization or mineralization. As gravimetric weight measurements and CO₂ quantification were not performed under the experimental conditions, these findings can only support that microalgae-mediated transformation represents an initial step in plastic weathering rather than full degradation. This observation aligns with findings from Zampolli et al. (2023), who demonstrated that oxidative modifications by laccase-like multicopper oxidases do not necessarily lead to substantial polymer weight loss in early degradation stages.

The modest laccase-like enzymatic activities detected in microalgal cultures align with previous reports highlighting the limited yet specific action of microbial oxidative enzymes on recalcitrant polyolefins and polyesters (Chow et al. 2022). These enzymes facilitate the introduction of polar functional groups, such as carbonyl, and hydroxyl, which enhance the hydrophilicity of the polymer surface, thereby promoting microbial colonization and enhancing enzymatic accessibility. Although enzymatic activity remains low, even minimal transformation may be ecologically relevant over extended environmental exposure, particularly in synergistic systems involving bacteria or fungi (Restrepo-Flórez et al. 2014).

Abiotic photo-oxidation was rigorously controlled in this study. Control samples containing only plastic substrates exposed to identical light and temperature conditions did not exhibit comparable FTIR spectral changes, suggesting that the observed oxidative modifications are primarily biologically mediated potentially through microalgal exudates or associated microbiota. This finding underscores the importance of distinguishing abiotic from biotic photo-oxidation mechanisms in environmental contexts, as emphasized by Gewert et al. (2015).

Collectively, these results support the hypothesis that green microalgae may serve as early-stage modulators in the biotransformation of plastics, initiating oxidative surface alterations that render polymers more susceptible to subsequent microbial or enzymatic degradation. Integrative approaches combining microalgae with specialized bacteria or fungi as recently explored by Tao et al. (2023) using *Rhodococcus* strains may hold the

key to enhancing depolymerisation rates and revealing synergistic degradation pathways. Integrative approaches that combine microalgae with specialized bacteria or fungi, such as those recently explored by Tao et al. (2023) using *Rhodococcus* strains, may prove instrumental in enhancing depolymerization rates and uncovering synergistic degradation pathways.

To accurately position these findings within the broader degradation continuum, it is essential to differentiate between biofouling, biofragmentation, and biodegradation, as these represent sequential yet mechanistically distinct stages in microbial plastic transformation (Peng et al. 2024). Biofouling involves microbial colonization and biofilm formation on plastic surfaces, often without altering the polymer structure (Dussud et al. 2018). Biofragmentation refers to enzymatic or oxidative cleavage of polymer chains into oligomers, while biodegradation is confirmed only when these fragments are metabolized into inorganic molecules such as CO₂ or CH₄ (Cao et al. 2024). Our findings suggest that microalgae primarily contribute to the first two stages, acting as initiators of surface activation rather than agents of complete biodegradation.

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