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**Feasibility study on the potential recycling of thermoplastic and thermosetting
polymers**

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Abstract

The increasing accumulation of polymeric waste and the limited recyclability of high-performance materials pose significant environmental and technological challenges. In particular, thermosetting polymers, such as epoxy resins, exhibit excellent mechanical and thermal properties but remain difficult to recycle due to their permanently cross-linked structures. This thesis presents a feasibility study on the recycling potential of thermoplastic and thermosetting polymers using microbiological and combined physico-chemical approaches, with a specific focus on epoxy-based systems.

A comprehensive literature review is first provided, covering the chemistry, curing mechanisms, characterization techniques, and conventional recycling strategies for thermoplastic and thermosetting polymers, as well as current knowledge on epoxy biodegradation and associated analytical methods. The review highlights substantial gaps in understanding the biodegradability of cross-linked thermosets and the limitations of existing assessment techniques, particularly at early stages of degradation.

Experimental investigations were conducted in two main phases. In the first phase, environmental microbial isolates were screened for their ability to interact with and degrade selected thermoplastic and thermosetting polymers. Microbial growth, enzymatic activity, and polymer surface modifications were evaluated using culture-based assays, spectroscopic analysis, and microscopy. The results confirmed a higher susceptibility of thermoplastics to microbial action, while epoxy resins showed strong resistance, with only limited surface-level modifications.

In the second phase, the effect of surface pre-treatments on epoxy biodegradation was explored. Two industrial epoxy resin systems were subjected to controlled plasma etching under oxygen and oxygen/ CF_4 atmospheres to modify surface chemistry and wettability without altering bulk properties. Surface activation was characterized by contact angle measurements, SEM/EDX, FTIR, and XPS analyses. Plasma-treated samples were subsequently exposed to selected microorganisms to assess microbial adhesion, early biofilm formation, and apparent mass changes. The results

demonstrated that plasma-induced surface modification significantly enhanced surface hydrophilicity and microbial colonization, although evidence of bulk polymer degradation remained limited within the experimental timeframe.

Overall, this work demonstrates that while epoxy resins are intrinsically resistant to microbial degradation, targeted surface functionalization can improve polymer–microorganism interactions and facilitate early-stage biodegradative phenomena. The study underscores the importance of combining biological, chemical, and physical approaches and highlights the need for carefully selected analytical methods appropriate to the specific stage of degradation being investigated.

List of Publications

Journal papers

- Nuccetelli P, Maisto F, Kraková L, Grilli A, Takáčová A, Šišková AO, Pangallo D. Evaluation of Microbial Degradation of Thermoplastic and Thermosetting Polymers by Environmental Isolates. *Coatings*. 2024; 14(8):982.
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Conference Proceedings

- Maisto F., Klištincová N., Mendez Villar A., Pavlović J., Nuccetelli P., Galová D., Sanmartin P., Pangallo D. *Whole genome sequencing (WGS) on mural samples from Pazo de Raxoi (Santiago de Compostela, Spain) using MinION approach*. In: Student Scientific Conference held on April 25th, 2024 at Comenius University. Bratislava, Slovakia.
- Maisto F., Klištincová N., Mendez Villar A., Pavlović J., Nuccetelli P., Galová D., Sanmartin P., Pangallo D. *MinION high-throughput amplicon sequencing 16S rRNA gene of mural sample from Pazo de Raxoi and Patio del Carcel (Santiago de Compostela, Spain)*. In: Student Scientific Conference held on April 25th, 2024 at Comenius University. Bratislava, Slovakia
- Maisto F., Klištincová N., Pavlović J., Nuccetelli P., Galová D., Pangallo D., *Comparison of MinION-based amplicon and operon sequencing to identify the microbial community of different granite ashlar samples*. In 19th International Biodeterioration and Biodegradation Symposium held from 9th - 12th Sep 2024 in Berlin, Germany
- Maisto F., Klištincová N., Pavlović J., Galová D., Nuccetelli P., Pin L., Pinzari F., Pangallo D. *Investigation of Microbial Communities using MinION sequencing in "Book of notes and memories of Father Francesco Zazzera"*. In 16th Preveda Interactive Conference of Young Scientists, 2024.

Local presentations

- There is more than just medicine: What a biologist can do. Educational activity organized by the faculty member for scientific subjects, Mara Paola Paolini. Collaborative activity between the Liceo Saru and the Slovak Academy of Sciences, promoted by the Embassy of Italy in Bratislava. November 16th, 2023, Bratislava.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the G. d'Annunzio University of Chieti-Pescara and where applicable, any partner institution responsible for the joint-award of this degree.

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Thesis Context

The primary purpose of this thesis is to conduct a feasibility study on the potential recycling of thermosetting and thermoplastic polymers. Given the significant environmental impact associated with the disposal of these materials, this study aims to explore microbiological techniques for degrading these polymers, contributing to the development of knowledge regarding alternative and innovative solutions within the recycling industry.

A state-of-the-art review of existing recycling technologies for thermoplastic and thermosetting polymers has been conducted. This review is meant to assess their effectiveness, limitations, and potential for improvement, and to evaluate the technical feasibility of microbial methods for degrading these polymers, focusing on the potential for recovering any reinforcing fibers.

The following chapters of this dissertation present the results of the research conducted during my doctoral studies. This research focused on the application of microorganisms and the evaluation of their ability to erode the polymeric matrix of the materials under investigation. Specifically, it explored the potential of microorganisms to break down the complex structures of these polymers. The use of microorganisms was subsequently complemented by chemical and physical methods to determine whether the degradation process could be accelerated and enhanced through combined approaches. These combined approaches aimed to leverage the synergistic effects of biological, chemical, and physical treatments to achieve more efficient and effective polymer degradation. The investigation also sought to understand the underlying mechanisms by which these combined methods interact and influence the overall degradation process, providing insights into potential optimization strategies for future applications in polymer recycling and waste management.

Due to the industrial nature of this doctoral project and the collaboration with the sponsoring company (COMEC Innovative s.r.l.), certain technical details, such as specific curing profiles, proprietary formulations, and processing parameters, cannot

be disclosed in full. Only the information necessary to ensure scientific reproducibility and clarity has been included, while confidential aspects have been omitted or generalized in accordance with industrial confidentiality agreements.

Chapter 1. Literature review

1. Introduction

Materials science plays a vital role in addressing sustainability challenges, especially concerning polymer materials. Thermosetting and thermoplastic polymers, two major categories, each exhibit unique properties, applications, and distinct recycling challenges. As global sustainability efforts intensify, understanding the manufacturing processes and exploring viable end-of-life solutions, especially biodegradation, becomes critical. This literature review aims to consolidate knowledge on the composition and production of the widespread thermosetting and thermoplastic polymers, examine the most advanced recycling techniques and the difficulties they present, and explore innovative biodegradation methods, with a particular emphasis on the role of microorganisms. Additionally, it will assess the feasibility of biodegradation processes and evaluate current methodologies used to measure the biodegradation of both thermosetting and thermoplastic polymers.

2. Overview of Thermosetting and Thermoplastic Polymers

2.1. Thermosetting polymers

Thermosetting polymers are a class of polymers that undergo an irreversible curing process when subjected to heat or chemical agents. The curing process results in a three-dimensional cross-linked network that imparts excellent thermal stability, mechanical strength, and resistance to solvents and chemicals [1, 2]. Common examples of thermosetting materials include epoxy resins, phenolic resins, and polyurethanes. These materials are widely utilized in high-performance applications, such as aerospace components, automotive parts, and electrical insulations, where durability and resistance to extreme conditions are paramount [3]. The curing process of thermosetting materials is critical, as it determines the final structure and properties of the polymer. Once cured, these materials cannot be re-melted or reshaped, which presents significant challenges for recycling. Traditional recycling methods for thermosets are limited, often resulting in incineration or landfilling [4].

Recent advancements in the recycling of thermosetting polymers have shifted towards exploring biological methods, particularly focusing on microbial degradation [5-7].

Research indicates that certain microorganisms can produce enzymes capable of interacting with thermosetting materials, although the degradation rate tends to be slower compared to thermoplastics [7]. For instance, experiments involving bacterial and fungal strains have shown limited but measurable success in attacking epoxy-based thermosets, with a noticeable reduction in material properties observed after prolonged microbial exposure [7]. These findings highlight the potential of leveraging biological approaches for addressing the challenges posed by the inherent stability of thermosetting materials. While these studies are still in their early stages, they are paving the way for alternatives to conventional methods, such as incineration or chemical treatments. By further optimizing the selection of microbial strains, enzyme activity, and environmental conditions, these approaches might have the potential to contribute to strategies for mitigating the environmental impact of thermoset and thermoplastic waste, offering promising pathways toward more sustainable material lifecycle management. [5- 7]

2.1.1. Types of epoxy

2.1.1.1. Petroleum based

A wide array of aromatic, aliphatic, and cycloaliphatic diols, along with select polyols such as novolacs, are utilized as key raw materials for the production of glycidyl ether epoxies. Among these, bisphenol A (BPA) [2,2-bis(4-hydroxyphenyl)propane] remains the most widely used aromatic diol.

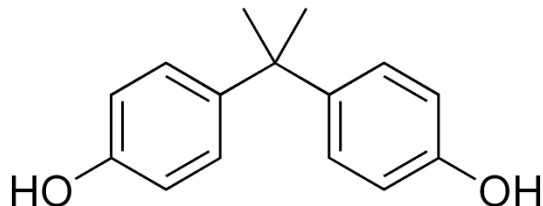


Figure 1: Bisphenol A (BPA)

Source: Wikimedia Commons.

BPA is critical to the epoxy resin industry, accounting for approximately 85% of all epoxies manufactured globally [8]. Despite its widespread use, BPA is a controversial chemical due to the toxicity, especially in its uncured form and is banned in many countries, particularly in products designed for children. Nonetheless, BPA-based epoxy thermosets exhibit outstanding mechanical strength and thermal stability, making them highly desirable for a variety of industrial applications, including construction, transportation, and furniture production [9].

BPA is specifically utilized in the synthesis of diglycidyl ether of bisphenol A (DGEBA), one of the most widely used epoxy resins, which accounts for 80-85% of global epoxy resin consumption [8]. DGEBA resins are highly valued for their versatility and excellent performance characteristics, especially in coatings, adhesives, and composite materials. BPA itself is produced through the reaction of phenol with acetone, using hydrochloric acid (HCl) as the catalyst and thioglycolic acid as a promoter [10]. This process allows for the large-scale production of BPA, which remains a cornerstone of epoxy resin technology.

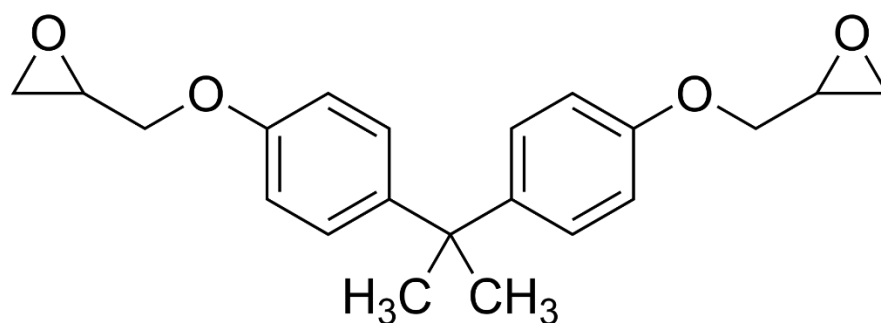


Figure 2: Bisphenol A diglycidyl ether (DGEBA)

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2.1.1.2. Bisphenol A Epoxy Resins

DGEBA alone does not contain oxirane rings; therefore, the addition of other compounds is required to complete the reaction and produce the final product. Key oxirane compounds, such as epichlorohydrin, glycidol, and both aliphatic and aromatic oxiranes, are essential in epoxy production. The process typically involves the polycondensation of oxirane compounds with diols, polyols, or other functional groups, yielding the desired epoxy resins. Among the starting materials, epichlorohydrin stands out as the most significant and widely used aliphatic oxirane precursor. It is a colorless and highly reactive liquid with a melting point of -57°C , a boiling point of 115°C , and a density of 1.18 g/cm^3 . While its solubility in water is low to moderate, epichlorohydrin is highly soluble in many polar organic solvents [11].

Epichlorohydrin is primarily utilized in the production of glycidyl ether epoxies. It can be sourced from both petroleum-based and bio-based feedstocks. In the petroleum-derived process, propylene, a petrochemical feedstock, undergoes a series of four reactions to yield epichlorohydrin (1-chloro-2,3-epoxypropane). First, propylene is chlorinated by exposure to dry chlorine gas, forming allyl chloride. This intermediate is then treated with hypochlorous acid, leading to the formation of 2-hydroxy-1,3-dichloropropane. Finally, the product is subjected to treatment with calcium hydroxide, which converts it into epichlorohydrin (Fig. 3).

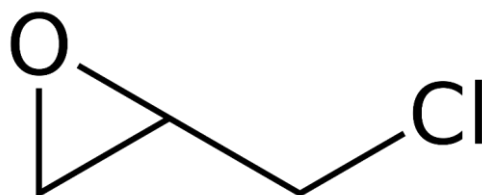


Figure 3: Epichlorohydrin
Source: Wikimedia Commons.

In contrast to epichlorohydrin, glycidol is primarily used for synthesizing epoxy resins by reacting with di- and polyacid chlorides. Glycidol is typically produced via the epoxidation of allyl alcohol. Additionally, compounds containing di- and poly(oxirane) groups are utilized in the synthesis of high-molecular-weight hyperbranched epoxies [12].

Notably, all these di- and poly(oxirane) compounds are typically synthesized via condensation reactions between corresponding diols or polyols and epichlorohydrin in the presence of suitable base catalysts. Alternatively, they can be produced through peracid-based epoxidation processes. As a result, epichlorohydrin remains the primary oxirane precursor for glycidyl-based epoxies, while non-glycidyl epoxies rely more heavily on peracid-mediated epoxidation reactions.

In addition to bisphenol A (BPA), various other bisphenol-type aromatic diols are employed in the synthesis of glycidyl epoxy resins. Although this literature review primarily focuses on the diglycidyl ether of bisphenol A (DGEBA)—the most widely used epoxy resin and the central subject of the research conducted—it is important to acknowledge other types of epoxy resins for the sake of scientific thoroughness.

Notable alternatives to BPA include bisphenol H, bisphenol C, bisphenol S, and tetrabromobisphenol A (TBPA), each imparting distinct characteristics to the epoxy resins they form. For instance, halogenated bisphenols like TBPA are primarily utilized in the production of flame-retardant epoxy resins, which are essential for electronic applications such as circuit boards and housings for devices made from polycarbonate / acrylonitrile butadiene styrene (ABS) materials. The flame-retardant properties

provided by TBPA are critical for enhancing the safety and durability of these electronic components [13, 14].

Another significant bisphenol is bisphenol S [bis(4-hydroxyphenyl) sulfone], commonly used in epoxy resin formulations. Epoxies derived from bisphenol S offer superior thermal stability and resistance to heat deformation, making them suitable for high-temperature environments [15]. Additionally, the sulfone group in bisphenol S contributes to a shorter gel time compared to BPA-based epoxies, enabling faster curing, which can be advantageous in various industrial processes [16].

Bisphenol F [bis(4-hydroxydiphenyl)methane], which exists in ortho-ortho, para-para, and ortho-para isomeric forms, is another bisphenol used in epoxy resin production, particularly for low-viscosity formulations. Epoxy resins synthesized from the para-para isomer exhibit improved solution and melt viscosities, along with enhanced mechanical properties, making them ideal for applications requiring both low viscosity and high performance [17]. The versatility of bisphenol F allows manufacturers to tailor the properties of epoxy resins to meet specific industrial needs.

In addition to bisphenols, novolac resins constitute another class of materials used to produce high-functionality epoxy resins. Novolacs are condensation products of formaldehyde with phenols such as phenol, cresols, resorcinol, catechol, ethylphenols, hydroquinone, and t-butylphenols. Epoxy resins based on novolacs are valued for their high crosslink density, which delivers exceptional chemical resistance and mechanical strength. Leading chemical manufacturers, including Dow Chemical Company and Huntsman Advanced Materials (formerly Ciba Specialty Chemicals), have developed novolac-based epoxy resins that do not rely on BPA, thereby broadening the range of epoxy materials available for industrial applications [17].

2.1.2. Types of curing agents

A wide range of curing agents, also known as hardeners, have been employed to produce epoxy thermosets through the formation of chemical bonds between the epoxy molecules. As mentioned earlier, the epoxide group (oxirane ring) is highly reactive toward compounds containing labile protons, such as amines, carboxylic acids, phenols, thiols, and poly(amidoamine)s, as well as toward Lewis acids and bases. Among these, amines are widely used, although their reactivity and performance characteristics can vary significantly depending on their structure.

For instance, aliphatic polyamines are commonly used for rapid curing of epoxies at room temperature. However, they tend to be volatile and toxic, resulting in thermosets with relatively low heat distortion temperatures. On the other hand, aromatic polyamines require higher curing temperatures but produce thermosets with significantly improved heat resistance, although they tend to cause staining [18]. In general, the nucleophilicity of amine curing agents determines their reactivity, with the order of reactivity increasing from aromatic to cycloaliphatic to aliphatic amines.

In addition to amines, other curing agents are widely utilized. For example, aromatic anhydrides are less toxic than aromatic amines [19] and are known to produce thermosets with high mechanical strength, excellent thermal stability, and low dielectric constants, making them ideal for electronic applications. However, these thermosets may have lower alkali resistance. Other curing agents, such as tertiary amines, imidazoles, and metal alkoxides, including halides and fluoroborates of metals like tin, zinc, and iron, are used in combination with acid anhydrides to cure epoxies [20].

2.1.3. *Types of catalysts*

The synthesis of epoxy resins, including diglycidyl ether of bisphenol A (DGEBA) and other variants, heavily relies on the use of catalysts to facilitate and control the chemical reactions involved, particularly those that form bonds between epoxy compounds and curing agents. The efficiency and selectivity of these catalysts significantly influence the final properties of epoxy resins, such as reactivity, viscosity, thermal stability, curing speed, and chemical resistance.

The process of synthesizing epoxides involves reactions between oxirane groups and functional groups of diols, polyols, or other compounds. The presence of catalysts is essential to promote the reaction, especially in cases where the reactivity of the compounds is relatively low or when faster reaction times are required. Among the most common catalysts used in epoxy synthesis are Lewis acids, Lewis bases, and transition metal-based catalysts.

Transition metal-based catalysts, such as those containing boron, zinc, or titanium, are employed to promote epoxidation reactions and to accelerate the ring-opening reaction of the oxirane with various nucleophiles. For example, titanium (IV) isopropoxide is a well-known catalyst for epoxide synthesis, particularly in the epoxidation of aliphatic compounds [21]. These catalysts are particularly useful when higher selectivity is desired in the synthesis and when the reactions need to be precisely controlled to avoid unwanted by-products [22].

Lewis acids, such as aluminum chloride (AlCl_3) and boron trifluoride (BF_3), are widely used to catalyze the formation of epoxides in reactions involving alkylation of alkenes with peroxides or alkyl chlorides [23]. The efficiency of these catalysts lies in their ability to activate the reactants without destroying the structure of the starting compound. They are also used in oxirane ring-opening reactions, which are useful for obtaining resins with specific functionalities [24].

Lewis bases, such as potassium hydroxide (KOH), are used to catalyze oxirane ring-opening reactions with nucleophiles like amines, phenols, and alcohols. These catalysts are particularly important in the synthesis of epoxy resins, such as DGEBA, where the oxirane ring-opening reaction is a key step in achieving the desired resin

properties [25]. The use of organic bases, such as magnesium complexes, is gaining popularity due to their ability to reduce waste and improve selectivity in reactions.

The most common curing agents for epoxy resins are amines, which can be aliphatic, cycloaliphatic, or aromatic. The reactivity of these amines toward the oxirane group depends on their nucleophilicity, which can be enhanced or regulated using catalysts. Amine-based catalysts, such as tertiary amines, are used to improve the curing speed of epoxy resins at ambient temperature [26]. Aromatic amines, although less reactive than aliphatic amines, provide superior thermal stability and mechanical strength, making them ideal for formulations that require high heat resistance [27].

Alternatively, aromatic anhydrides, such as phthalic anhydride, are used as curing agents in epoxy formulations to ensure superior water and high-temperature resistance. The use of catalysts such as boron or zinc compounds can accelerate the curing process, ensuring that the reaction is completed within a short time without compromising the mechanical properties of the material [28].

2.1.4. Curing process of epoxy

The curing process of epoxy resins involves a chemical reaction between the resin and a hardener, forming a three-dimensional cross-linked network that enhances the material's mechanical and thermal properties. During curing, the reactive epoxide groups of the resin undergo nucleophilic attack by the curing agent, leading to the formation of ether linkages and the development of a rigid, highly stable structure (Fig. 4). The curing process can be affected by factors such as temperature, humidity, and the choice of curing agent. Elevated temperatures generally accelerate the curing reaction, while low temperatures can slow down the process, potentially requiring longer curing times [29]. The stoichiometry of the epoxy and hardener is crucial; an incorrect ratio can result in incomplete curing, leading to suboptimal properties [30]. Additionally, the presence of accelerators or catalysts can further enhance the curing rate, enabling faster processing times [30]. The curing time varies significantly depending on the formulation, with some systems achieving full cure in a few hours, while others may take several days [31]. This variation makes epoxy resins versatile for applications ranging from rapid prototyping to high-performance coatings and adhesives.

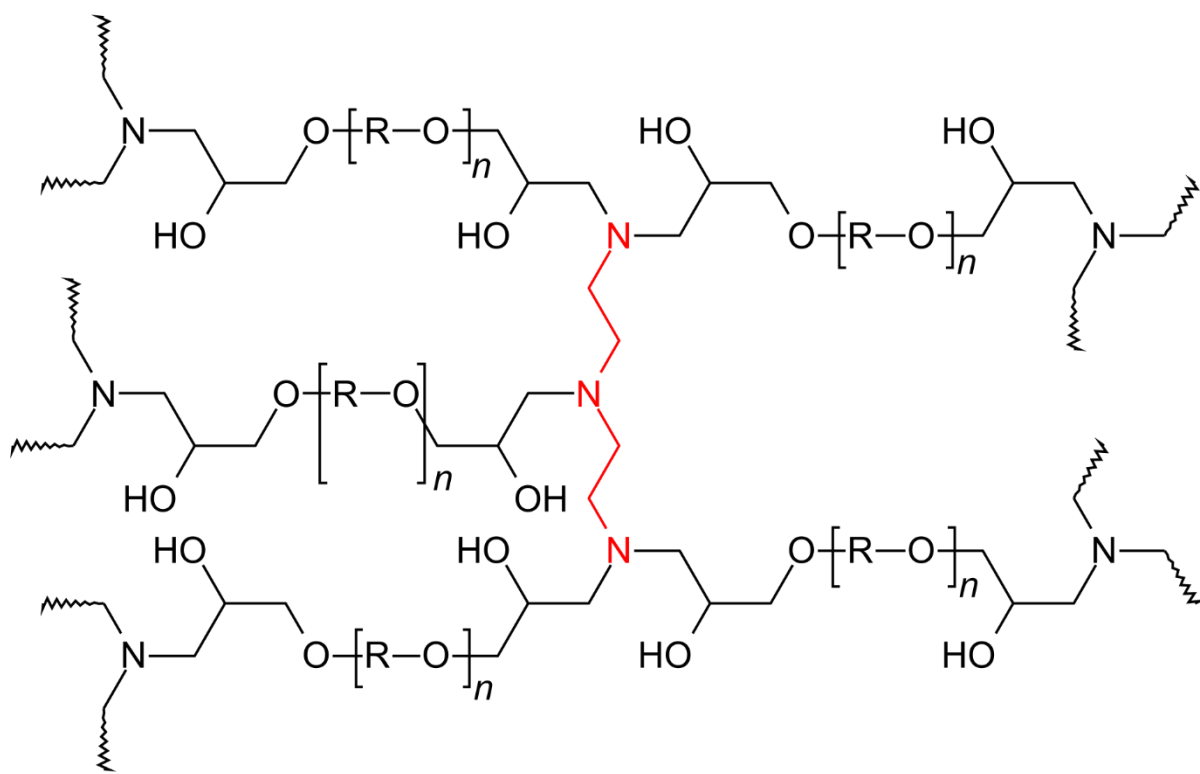


Figure 4: Structure of a cured epoxy resin

Source: Wikimedia Commons.

2.1.5. Characterization

Epoxy resins are typically characterized using a variety of widely employed techniques, including spectroscopic, microscopic, and analytical methods, to analyze their chemical structure, physical properties, and other key attributes. Among the most commonly used methods are Fourier-transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy, which are important for identifying functional groups and chemical bonds within the resin matrix [32]. FTIR provides information on the presence of specific functional groups, while NMR spectroscopy enables more comprehensive analysis, including the calculation of the degree of branching in hyperbranched epoxies [33].

For detecting UV-active groups in the resin structure, UV-Vis absorption spectroscopy is sometimes employed, focusing on the $\pi-\pi^*$ and $n-\pi^*$ transitions of aromatic and other functional groups [34]. Additionally, gel permeation chromatography (GPC) is widely used to determine the molecular weight distribution of the resin, while thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) are essential for evaluating the thermal stability and glass transition temperature (T_g) of epoxy thermosets [35].

Other crucial techniques include dynamic mechanical analysis (DMA), which assesses the viscoelastic properties of the resin, and scanning electron microscopy (SEM), used for examining the morphology and surface structure of the cured epoxy [36]. These methods, in combination, provide a comprehensive understanding of the chemical, thermal, and mechanical properties of epoxy resins.

2.2. Thermoplastic polymers

Thermoplastic polymers are distinguished by their capacity to be repeatedly melted and re-shaped without incurring substantial chemical alteration. This behavior arises from their predominantly linear or mildly branched molecular architectures, which permit the reversible disruption of intermolecular forces upon heating. As these interactions are overcome, the polymer chains gain mobility, allowing the material to flow and be re-formed into new configurations. Polystyrene, for example, is a polymer made from the polymerization of styrene monomers, resulting in a rigid and transparent material that is widely used in packaging, insulation, and disposable cutlery [37]. Its chemical structure consists of a long chain of carbon atoms with phenyl groups attached, contributing to its strength and rigidity (Fig. 5).

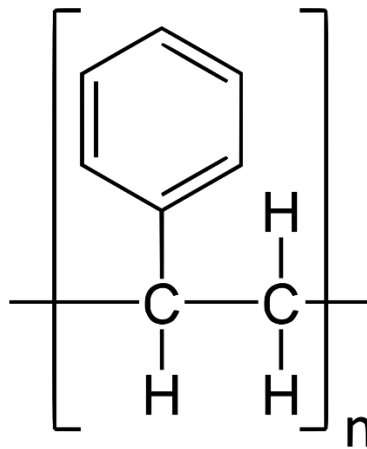


Figure 5: Structure of Polystyrene

Source: Wikimedia Commons.

In contrast, polyethylene terephthalate (PET) is produced through the polycondensation of terephthalic acid and ethylene glycol, forming a polyester with repeating units that provide excellent tensile strength and thermal stability [38]. PET is commonly used in beverage bottles, food containers, and synthetic fibers due to its durability and recyclability. The chemical structure of PET (Fig. 6) includes ester

linkages that can be broken down under certain conditions, allowing for recycling processes that reclaim the material for reuse [39].

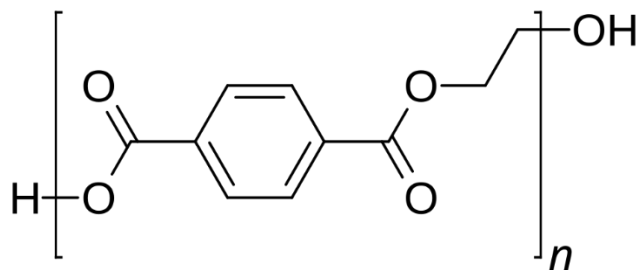


Figure 6: Structure of Polyethylene terephthalate

Source: Wikimedia Commons.

The processing of thermoplastics is generally more straightforward than that of thermosetting materials, as it involves techniques such as extrusion, injection molding, blow molding, and thermoforming. These methods facilitate the efficient production of complex shapes and components, making thermoplastics a popular choice in various applications [40]. For instance, in injection molding, the thermoplastic material is heated until it becomes molten and then injected into a mold where it cools and solidifies, forming the desired shape. This process can be repeated multiple times, allowing for high-volume production with minimal waste [41].

The differences between thermoplastics and thermosetting compounds are significant. While thermoplastics can be remelted and reshaped, thermosetting polymers undergo a curing process that creates a permanent cross-linked structure, rendering them inflexible and non-recyclable through conventional means [42]. This fundamental distinction impacts their applications, with thermoplastics being favored for products requiring flexibility and reusability, while thermosets are often used in applications demanding high strength and thermal resistance, such as in aerospace and automotive components.

2.2.1. Consolidation Process of Thermoplastic Materials

Unlike thermosetting polymers, thermoplastics do not undergo a chemical curing reaction; instead, their processing relies on physical melting and solidification cycles. In fiber-reinforced thermoplastic composites, the consolidation process typically involves heating the polymer matrix above its melting temperature to allow intimate contact and wetting of the fibers, followed by controlled cooling to solidify the structure. Advanced manufacturing methods such as automated tape laying (ATL), automated fiber placement (AFP), and hot-press consolidation are used to achieve uniform heat distribution and minimize void content. This reversible, heat-driven consolidation enables rapid processing and repairability, making thermoplastic composites increasingly attractive for high-performance applications [43].

2.2.2. Characterization

The characterization of thermoplastic polymers involves evaluating their thermal, mechanical, and rheological properties, which dictate their processing behavior and final performance. Techniques such as differential scanning calorimetry (DSC) are employed to measure melting temperature, crystallization temperature, and degree of crystallinity, while dynamic mechanical analysis (DMA) provides insight into viscoelastic behavior across different temperature ranges. Rheological measurements, including melt flow index (MFI) or shear viscosity profiles, are critical for assessing processability in extrusion or injection molding. These characterization methods collectively guide material selection and ensure reliable performance in engineering applications [44].

2.2.3. Carbon fibers reinforced polymers (CFRPs)

Carbon fibers (CFs) are extremely fine, filamentous materials with diameters on the order of micrometers. They are primarily composed of carbon atoms arranged in a highly ordered structure aligned along the fiber axis, which confers exceptional mechanical strength and stiffness compared to most other synthetic fibers [45, 46]. Historically, CFs were distinguished by production temperature: fibers carbonized between 1000 and 1500 °C were termed “carbon fibers,” whereas those treated above 2000 °C were classified as “graphitic fibers.” This distinction has since been abandoned, and both categories are now generally referred to as carbon fibers [47]. Modern classification instead differentiates between high-strength CFs, typically processed at lower heat-treatment temperatures, and high-modulus CFs, which undergo higher-temperature graphitization [48].

Because of their outstanding stiffness, tensile strength, low density, and fatigue resistance, CFs have become essential materials in high-performance structural applications. Since their introduction into industry in the 1960s, CFs have been widely employed in aerospace, automotive, marine, civil engineering, and other advanced technological sectors [49]. The performance of CF composites depends strongly on precursor chemistry, carbon yield, processing conditions, and the degree of alignment of crystalline domains, highlighting the critical relationship between processing, structure, and macroscopic properties [50].

Composite materials consist of two or more constituent phases that remain distinct yet combine to yield enhanced performance. In structural polymer composites, a relatively soft and ductile polymer matrix is reinforced with a strong, stiff material such as carbon fiber, resulting in lightweight systems with superior mechanical properties. These properties depend not only on the intrinsic characteristics of the constituents but also on microstructure and fiber–matrix interfacial interactions [51].

In recent decades, polymers have increasingly replaced traditional materials due to advantages in processing, production efficiency, and cost. Common reinforcement fibers include glass fiber (GF) and carbon fiber (CF), with CF being the second most widely used synthetic reinforcement in structural composites [52]. To further improve

mechanical, thermal, or electrical performance, multifunctional fillers such as carbon nanotubes or graphene are frequently incorporated into polymer matrices. CF-based composites enhanced with such nanomaterials are particularly attractive due to their high aspect ratio, multifunctional potential, and relatively favorable cost-to-performance ratio [53].

3. Recycling

Currently, carbon fiber recycling can be categorized into three broad methods: mechanical, thermal, and chemical, each utilizing specific processes to separate carbon fibers from the thermoset matrix.

3.1. Mechanical recycling

Mechanical recycling is technologically the most mature CFRP recycling method, which involves multiple steps to reduce the size of the waste. In the first part, the composites are disassembled and shredded down to a size of 50-100 mm, then further grinding or milling is applied to obtain recyclates with different sizes ranging from fine powder to heavy recyclates. Due to the resultant short fiber aspect ratio, these recyclates possess a relatively low market value. The use of composite materials recovered with this technique can have two purposes: filling or reinforcement agent. These recovered materials are reported as more difficult to reuse, particularly as reinforcement and, even with low re-incorporation, the resulting mechanical properties are significantly compromised due to poor bonding between the recyclates and the new resin. Their use as a filler is not commercially viable due to the very low cost of virgin fillers [51].

3.2. Thermal recycling

Thermal recycling methods employ high temperatures to decompose the polymer matrix and recover fibers. Processes such as pyrolysis and fluidized bed pyrolysis are commonly used. Pyrolysis involves heating the thermoset composite in an inert atmosphere to temperatures between 400–1000 °C, enabling the recovery of long carbon fibers with high modulus, retaining 50–85% of the tensile strength of virgin fibers. In fluidized bed recycling, shredded composite waste (6–20 mm) is fed into a bed of silica sand fluidized by hot air at 450–550 °C under 10–25 kPa pressure. The recycled fiber length ranges from 5–10 mm, retaining 10–75% of the tensile strength of virgin fibers [54].

3.3. Chemical recycling

Chemical recycling utilizes solvents to break down the polymer matrix and release fibers. This process includes supercritical/subcritical fluid methods and low-temperature solvolysis. Supercritical fluids, such as water, alcohols, and ketones, exhibit properties between liquids and gases, making them effective for decomposing composite waste. However, this method requires specialized equipment capable of withstanding high temperatures and pressures. Low-temperature solvolysis employs solvents and catalysts under normal pressure to degrade the resin matrix, enabling fiber separation and the extraction of degraded resin oligomers. Studies have explored the use of strong acids or bases for this purpose, achieving high resin decomposition rates and retaining over 95% of the original fiber's mechanical properties [55].

3.4. Supercritical / subcritical fluid

The supercritical/subcritical fluid method involves treating waste materials using fluids at temperatures and pressures near their critical points. In this process, the polymer resin is chemically decomposed to release fibers, resulting in recycled products such as fibers, fillers, and depolymerized resin monomers. Supercritical fluids exhibit properties that are intermediate between the liquid and gas phases, including low viscosity, high density, excellent flow, mass transfer, heat transfer, and good solubility. They are also highly sensitive to changes in temperature and pressure, making them well-suited for the recycling and breakdown of composite waste. However, this method requires specialized equipment capable of withstanding the high temperature, pressure, and corrosive nature of certain reaction media. Commonly used supercritical fluids include water, alcohols, and ketones. Current research focuses extensively on optimizing reaction conditions and rates for various media [56 – 61].

3.5. Low temperature solvolysis

Under normal pressure, certain solvents and catalysts can break down the resin matrix of FRP waste into soluble substances, enabling fiber separation and even the extraction of degraded resin oligomers. Studies have widely explored the use of strong acids or bases to treat composite materials. Dang et al. (2021) used HNO₃ to degrade glass fiber-reinforced epoxy resin composites and successfully separated the glass fiber, though the fiber's weight was reduced by 50 wt%. The neutralized extract from the resin degradation was reused in the regenerated resin (less than 30 wt%), improving its tensile strength [62]. Carbon fiber-reinforced epoxy resin was recycled by degrading it in a mixed solution of nitric and sulfuric acids, followed by treatment with polyethylene glycol and KOH to release the carbon fibers. The study found that the mechanical properties of the recycled carbon fibers (rCF) were retained at over 95% of the original carbon fibers (vCF), with a resin decomposition rate of 97% [63]. The low-temperature solvent method can degrade epoxy resin-based composites using acidic or alkaline systems. Acidic systems generally allow for lower temperature processes and faster reaction rates, yielding better quality recycled carbon fiber compared to alkaline methods [64].

4. Biodegradation of Epoxy Resin

The biodegradation of epoxy resins is a complex process that involves the breakdown of their intricate chemical structures, primarily characterized by cross-linked networks formed through the reaction of epoxides with curing agents. The resistance of epoxy resins to biodegradation is largely attributed to the presence of strong covalent bonds, particularly carbon-carbon (C-C) and carbon-oxygen (C-O) bonds, which create a stable and rigid three-dimensional network. This stability poses significant challenges for microbial degradation, as most microorganisms lack the necessary enzymes to effectively cleave these robust bonds [65].

Recent studies have explored the mechanisms by which certain fungi can degrade epoxy resins, focusing on the enzymatic pathways involved. According to Kumar & Chandra [66], ligninolytic enzymes—such as laccases and peroxidases—produced by white-rot fungi enable oxidative depolymerization of recalcitrant aromatic materials. Although primarily adapted for lignin decomposition, these enzymes can also attack synthetic polymers through formation of radical intermediates, thereby facilitating initial epoxy chain scission. Ali et al. [67] further highlights that once the cross-linked structure is partially oxidized, the resulting lower-molecular-weight fragments (oligomers and monomers) become accessible to microbial metabolic pathways.

Klose [68] emphasizes that the biodegradation of epoxy resins can be enhanced through the incorporation of specific additives or modifications to the resin formulation. For instance, the introduction of biodegradable plasticizers or the use of bio-based epoxy resins derived from renewable resources can create a more favorable environment for microbial attack. These modifications may alter the chemical structure of the epoxy resin, making it more susceptible to enzymatic degradation. Additionally, the presence of functional groups, such as hydroxyl (-OH) or carboxyl (-COOH) groups, can facilitate enzymatic action by providing reactive sites for microbial enzymes to target [69].

Lucas et al. [70] further elaborate on the mechanisms of biodegradation, detailing the role of microbial communities in the degradation process. The initial attack on epoxy resins often involves the colonization of the resin surface by microorganisms, which

secrete extracellular enzymes to initiate the breakdown of the polymer matrix. The degradation process typically follows a multi-step pathway: first, the microbial enzymes cleave the strong covalent bonds within the epoxy structure, leading to the formation of oligomers. Subsequently, these oligomers can be further degraded into smaller monomers, which are more easily assimilated by the microorganisms for energy and growth. Moreover, the environmental conditions play a crucial role in the biodegradation of epoxy resins. Factors such as temperature, pH, and moisture content can significantly influence the activity of microbial enzymes and the overall degradation rate. For example, higher temperatures may enhance enzymatic activity, while extreme pH levels can denature enzymes, reducing their effectiveness [71].

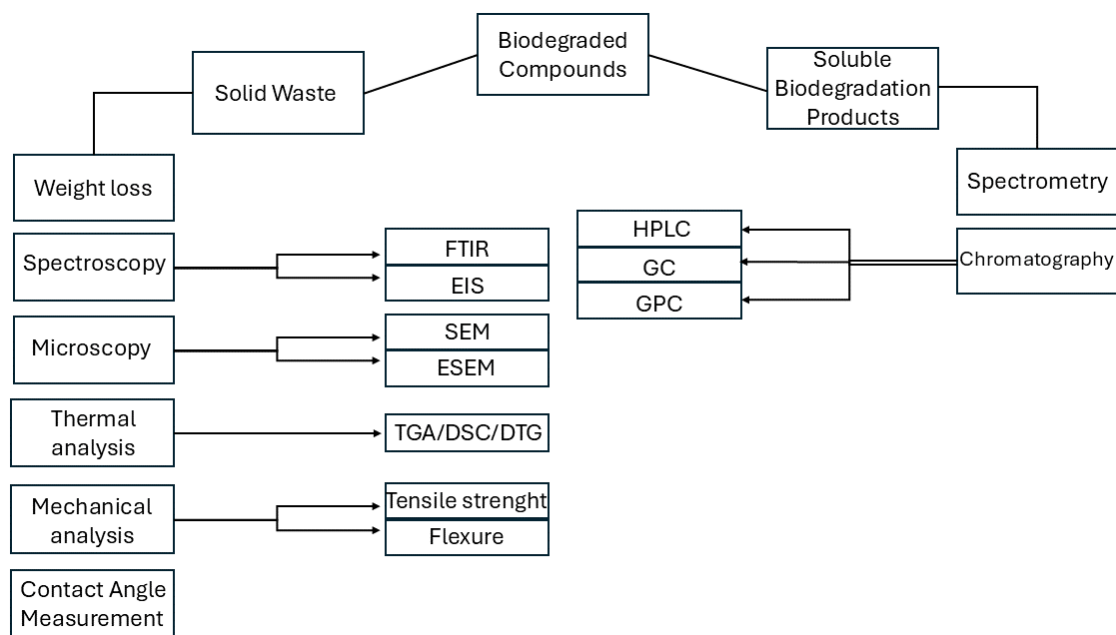


Figure 7: Methods for Assessing Compound Biodegradation

4.1. Analytical Methods of Bio-Degraded Epoxy

4.1.1. *Weight Loss*

Weight loss measurement is a commonly utilized analytical technique to assess the degradation of compounds by enzymatic or microbial activity. This method involves determining the reduction in mass of a sample following exposure to biodegrading processes. It is a straightforward and cost-effective approach, providing insights into the rate and degree of compound degradation under various environmental conditions [72]. However, the simple comparison of the untreated and degraded sample weights has been critiqued for its lack of precision in assessing polymer backbone depolymerization, as the method is significantly affected by systematic errors caused by the degradation of additives such as plasticizers rather than the polymer itself [73]. Moreover, since weight loss measurement requires a noticeable change in mass, it may be challenging to apply finely ground samples that are often used to increase surface area. Thus, it is only suitable to a limited extent and should be employed alongside other analytical techniques, such as TGA, FTIR, and SEM for more accurate and reliable results [74].

4.1.2. *Microscopic Methods*

Microscopic techniques provide a rapid and reliable means of analyzing sample surfaces and are particularly well suited for initial qualitative assessments [75]. Among these, scanning electron microscopy (SEM) offers high spatial resolution, reaching values as low as 1 nm, which enables the detection of subtle morphological changes on polymer surfaces. In contrast to optical microscopy, which is limited by the wavelength of visible light (≥ 200 nm), SEM employs a focused electron beam with a wavelength up to approximately 55,000 times shorter (≥ 0.0037 nm) [76].

When the electron beam interacts with the sample surface, kinetic energy is transferred to the atoms, leading to the emission of secondary electrons that carry detailed topographical information. By raster-scanning the beam across the surface, SEM generates high-resolution images of surface morphology. Conventional SEM operation

requires high-vacuum conditions to prevent electron scattering by atmospheric gases, which restricts its applicability to dry and solid samples. However, environmental scanning electron microscopy (ESEM) allows operation at elevated chamber pressures, enabling the analysis of hydrated or volatile samples [77].

SEM has been extensively applied in epoxy biodegradation studies to visualize surface alterations and microbial colonization. Microscopic observations have revealed fungal growth on epoxy surfaces and the presence of mineral crystal deposits on fungal hyphae, attributed to polymer mineralization processes [78]. These findings demonstrate that microscopic analysis is a valuable tool for qualitative surface inspection and for elucidating microorganism–polymer interactions in thermosetting and thermoplastic materials.

4.1.3. Spectroscopic Methods

Thermogravimetric analysis coupled with Fourier-transform infrared spectroscopy (TGA/FTIR) is a powerful approach for elucidating polymer degradation mechanisms, as it directly correlates mass-loss events with the chemical identity of evolved gaseous products [79]. During TGA, thermal degradation proceeds through mechanisms such as main-chain scission, side-group elimination, and depolymerization. Volatile degradation products generated at each temperature step are transferred to an FTIR gas cell, where their vibrational spectra provide real-time molecular information.

Characteristic FTIR absorption bands allow identification of functional groups present in degradation products. Carbonyl-containing species exhibit C=O stretching vibrations in the 1850–1650 cm^{-1} region, unsaturated compounds display C=C stretching between 1680–1620 cm^{-1} , and ether or epoxy-related structures generate strong C–O–C absorptions in the 1100–1000 cm^{-1} range. The fingerprint region (1400–400 cm^{-1}) is particularly informative, containing skeletal vibrations and deformation modes characteristic of aromatic rings, oxirane groups, and polymer backbones. Monitoring changes in these bands during heating enables direct identification of bond cleavage and structural rearrangements.

Combined FTIR–TGA analysis has been used to investigate microbial degradation of epoxy resins, revealing progressive hydroxylation of the polymer surface evidenced by broadening and intensification of O–H stretching bands ($3600\text{--}3200\text{ cm}^{-1}$) [80]. Concurrent modifications in C–H stretching vibrations ($2950\text{--}2850\text{ cm}^{-1}$) and the appearance or enhancement of carbonyl bands ($\sim 1730\text{--}1650\text{ cm}^{-1}$) indicate oxidative degradation processes involving ether bond cleavage and epoxy network disruption. Corresponding reductions in thermal decomposition onset temperatures further confirm loss of structural integrity in microbially treated samples. Together, these observations demonstrate that FTIR–TGA is a sensitive tool for detecting early-stage oxidative and hydrolytic degradation processes induced by microbial activity.

4.1.4. Thermal Analysis

Thermogravimetric analysis (TGA) is widely used to evaluate the thermal stability and degradation behavior of polymeric materials by monitoring mass loss as a function of temperature under controlled heating conditions, typically in inert atmospheres such as nitrogen or argon. Variations in onset decomposition temperature, multi-step mass-loss behavior, and features observed in derivative thermogravimetric (DTG) curves provide valuable insight into curing degree, cross-link density, and the sequence of degradation reactions occurring within the polymer network.

Previous investigations have demonstrated that microbial pretreatment of polymer-based materials leads to measurable reductions in thermal stability, manifested by lower degradation onset temperatures and increased mass loss during thermal analysis [81]. These results highlight the sensitivity of TGA for identifying early-stage structural deterioration associated with biologically induced modification of polymer matrices.

4.1.5. Chromatographic Methods

Chromatographic techniques enable molecular-level characterization of polymer degradation products by separating fragments based on molecular weight or polarity. For high-molecular-weight degradation products (>2000 Da), gel permeation chromatography (GPC) is particularly effective, as it provides quantitative information on molecular weight distribution, dispersity, and chain-scission behavior. The use of GPC coupled with multi-angle light scattering and viscometric detection has demonstrated that progressive degradation of bisphenol-A-based epoxy resins can be identified through shifts in chromatographic profiles and reductions in molar mass [82].

Low-molecular-weight degradation products, including monomers, dimers, and short oligomers, are more effectively resolved using reversed-phase liquid chromatography (RP-HPLC). Chromatographic methods operating near the critical adsorption regime, particularly when combined with MALDI-TOF mass spectrometry, enable precise identification of epoxy decomposition products and terminal functional groups formed during degradation [83].

Reversed-phase chromatographic approaches have also been shown to differentiate aromatic curing agents, glycidyl monomers, and partially reacted intermediates released during thermal or chemical degradation of epoxy systems [84]. Collectively, these techniques provide essential mechanistic insight into depolymerization, oxidation, and structural rearrangement processes occurring under environmental stress.

4.1.6. Contact Angle Measurement

Contact angle measurements are widely employed to assess changes in surface wettability during polymer degradation, as they are highly sensitive to the formation of polar functional groups and surface roughening caused by microbial colonization. Microbial exposure has been shown to induce significant reductions in water contact angle on epoxy-based materials, consistent with increased surface oxidation and hydrophilization resulting from enzymatic activity and biofilm formation [85].

Similar behavior has been observed for epoxy-coated steel systems subjected to microbial attack, where progressive hydrophilization correlates with biofilm development, reduced barrier performance, and enhanced water uptake [86]. These findings demonstrate that contact angle analysis is a rapid, non-destructive method for detecting early-stage surface degradation.

4.1.7. Mechanical Analysis

Mechanical analysis is routinely applied to evaluate the impact of biodegradation on material performance, as reductions in tensile strength, elastic modulus, impact resistance, or elongation at break directly reflect chain scission, cross-link disruption, and microstructural damage. Mechanical testing of biodegradable polymeric composites following prolonged environmental exposure has revealed substantial losses in performance associated with hydrolytic and microbially driven degradation processes [87].

Such deterioration reflects progressive breakdown of polymer backbones and weakening of interfacial adhesion within composite structures, underscoring the importance of mechanical testing as a complementary method to spectroscopic and thermal analyses for comprehensive degradation assessment.

5. Research Gap and Rationale

While the literature highlights a growing interest in environmentally sustainable strategies for polymer recycling, significant knowledge gaps remain regarding the biodegradability of both thermosetting and thermoplastic materials. Most studies primarily address conventional recycling routes, whereas biodegradation, particularly the microbial degradation of cross-linked thermosets, remains poorly characterized and often confined to specific laboratory conditions. Moreover, the analytical techniques discussed in the literature span different stages of the biodegradation process, and not all are suitable for early-stage or preliminary assessments such as the one adopted in this work. These gaps underscore the need for targeted experimental investigations capable of clarifying the extent to which polymeric materials can undergo biological breakdown and identifying the factors that govern their degradability.

Building on this context, the present research focuses on assessing the biodegradability of selected thermosetting and thermoplastic compounds, with the aim of evaluating the applicability of microbial degradation processes and critically examining the reliability of biodegradation assessment methods when applied at an initial screening level.

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Chapter 2. Evaluation of Microbial Degradation of Thermoplastic and Thermosetting Polymers by Environmental Isolates

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1. Introduction

The importance of recycling thermoplastic and thermosetting polymers has become increasingly pressing in recent years. Growing concerns related to environmental degradation, resource depletion, and the escalating accumulation of plastic waste have intensified the need for innovative and effective strategies to manage polymeric materials. Plastics and synthetic polymers are ubiquitous in modern society, playing a central role in applications ranging from packaging and consumer products to construction, automotive, and aerospace components. In parallel, fiber-reinforced polymers—particularly carbon-fiber-reinforced polymers (CFRPs)—are extensively employed due to their high strength-to-weight ratio and excellent mechanical performance. These materials are widely used in aerospace, automotive, and renewable energy sectors, with global CFRP demand projected to reach approximately 500,000 tons per year by 2030 [1].

Currently, recycling strategies for thermoplastic and thermosetting polymers [2] can be broadly classified into mechanical, thermal, and chemical approaches [3–6]. Mechanical recycling represents the most technologically mature route and involves a sequence of size-reduction and reprocessing steps. Thermal recycling methods rely on elevated temperatures and, in some cases, reactive atmospheres or fluids to decompose the polymer matrix, with pyrolysis and fluidized bed recycling being the most commonly adopted techniques [4,6]. Chemical and electrochemical recycling methods employ a variety of solvents [5], including supercritical and subcritical fluids, often operating under ambient pressure conditions. Despite their effectiveness, these approaches typically require complex and costly equipment and may generate environmentally hazardous by-products, raising concerns related to environmental and human health impacts [5]. Moreover, all conventional recycling technologies tend to compromise, to varying degrees, the physical and mechanical properties of the recycled materials, particularly in the case of thermosetting polymers.

Beyond technical limitations, the disposal and inefficient recycling of plastics and CFRPs constitute a major environmental challenge. These materials persist in natural environments for extended periods, contributing to the contamination of oceans, soils, and the atmosphere, with adverse consequences for ecosystems and human health

[7]. In addition, established recycling routes for CFRPs are often energy-intensive and result in recycled products of reduced quality, limiting their reuse in high-performance applications and increasing dependence on virgin raw materials [8].

In this context, microbial degradation has emerged as a potentially promising alternative or complementary approach for the recycling of plastics and CFRPs [9,10]. Microorganisms possess a remarkable ability to enzymatically decompose complex organic structures into simpler compounds. By exploiting microbial metabolic pathways, polymeric materials may be progressively broken down into oligomers or monomers, thereby contributing to a more sustainable and circular material lifecycle.

Compared to conventional recycling technologies, microbial degradation offers several potential advantages. It typically operates under mild conditions, requires low energy input, and generates minimal harmful by-products, in contrast to solvent-based or thermochemical processes. Moreover, microorganisms are already widely employed in biological treatment systems for the degradation and removal of pollutants from wastewater and contaminated environments, underscoring their relevance for environmentally sustainable remediation strategies [11,12]. In the specific case of CFRPs, traditional mechanical and chemical recycling methods often act aggressively on the embedded carbon fibers, leading to significant deterioration of fiber integrity and limiting their reuse potential in demanding applications [8].

Microbial degradation of polymeric materials generally targets chemical bonds susceptible to enzymatic hydrolysis or oxidation, such as ester, ether, and amide linkages. In CFRPs, the epoxy resin matrix represents a potentially suitable substrate for microbial attack, as microorganisms may preferentially degrade the polymer matrix while leaving the carbon fibers largely unaffected. Carbon fibers exhibit a highly crystalline, graphitic structure, characterized by strong and inert carbon–carbon bonds that confer intrinsic resistance to enzymatic degradation and microbial colonization [13]. Despite this potential selectivity, studies addressing microorganism–polymer interactions, particularly involving thermosetting polymers, remain limited. A deeper understanding of these interactions is therefore essential for the development of innovative and sustainable strategies for polymer waste management and environmental remediation.

Against this background, the scope of this work is to investigate the impact of environmental microorganisms on selected thermoplastic and thermosetting polymers, namely polyethylene terephthalate (PET), polystyrene (PS), and two epoxy resin systems. These materials were chosen as representative examples of widely used polymer classes. The primary objective is to isolate and identify microorganisms capable of growth under nutrient-limited conditions, in which the investigated polymers serve as the sole carbon source. The effectiveness of microbial interaction and degradation is evaluated using spectroscopic techniques, such as Fourier transform infrared spectroscopy (FTIR), to detect chemical modifications, as well as scanning electron microscopy (SEM) to assess surface morphological changes. By elucidating the degradative capabilities of the isolated microorganisms and their effects on the selected materials, this study aims to provide insights into microbial enzymatic mechanisms and to support the development of future biotechnological approaches for polymer recycling and sustainable material management.

2. Materials and Methods

2.1. Target Materials

Epoxy resin samples were prepared using two different industrial resin systems supplied by COMEC Innovative S.r.l. (Chieti, Italy). The first system (RS1) was based on Araldite®, while the second system (RS2) was based on Epikote™. For each system, the individual components were mixed according to the manufacturer's specified mixing ratios and cured under the conditions reported in the corresponding technical data sheets. The cured epoxy specimens were produced using silicone molds, yielding samples with dimensions of 5 mm × 5 mm × 5 mm.

Polyethylene terephthalate (PET; Polymer Institute, SAS, Bratislava, Slovakia) samples were prepared by dissolving granular PET in dimethyl terephthalate (DMT). The resulting solution was cast onto a flat surface using circular molds to obtain specimens with uniform thickness. Following solvent evaporation, thin, paper-like PET samples with a diameter of 3 cm were obtained and used for subsequent analyses.

Similarly, polystyrene (PS; Polymer Institute, SAS) samples were produced by dissolving granular PS in tetrahydrofuran (THF). The polymer solution was cast onto flat supports using circular molds with a diameter of 3 cm. After complete evaporation of THF, thin, paper-like PS specimens suitable for the biodegradation experiments and characterization analyses were obtained.

2.2. Soil and Oil Refinery Activated Sludge (ORAS) Suspensions

Microorganisms were isolated from two environmental sources, namely soil and oil refinery activated sludge (ORAS), and two corresponding microbial suspensions were prepared.

The soil suspension (SS) was obtained by collecting fresh undergrowth soil and suspending it in 360 mL of sterile 0.9% NaCl solution in a flat-bottomed flask. The mixture was incubated at room temperature in an ES-20 orbital shaker incubator

(Biosan, Riga, Latvia) at 100 rpm. After incubation, the suspension was filtered to remove large particulate matter.

The second suspension was prepared from oil refinery activated sludge (ORAS) residue collected from industrial waste oil. The residue was suspended in 250 mL of sterile 0.9% NaCl solution in a flat-bottomed flask and incubated under the same shaking conditions. After incubation, the supernatant was collected and designated as the ORAS suspension (ORAS-S).

2.3. Agar Media and Isolation of Microorganisms

In order to isolate microorganisms present in the SS and ORAS-S suspensions, several selective and non-selective agar media were employed: Luria Bertani agar (LBA), Pseudomonas Isolation agar (PIA), Czapek Dox agar (CZDA) supplemented with chloramphenicol (25 µg/mL), and Azotobacter agar (all from Sigma-Aldrich, Burlington, VT, USA). All media were sterilized by autoclaving at 121 °C for 15 min.

Serial dilutions of both SS and ORAS-S were prepared, starting from a 1:10 dilution and proceeding up to 1:10,000 using sterile 0.9% NaCl solution. Aliquots of 100 µL from each dilution were spread onto the agar plates, which were then incubated at room temperature until microbial growth was observed. Individual microbial isolates were selected based on distinct colony morphology and subsequently purified for further analysis.

2.4. DNA Extraction, PCR, and Sequencing

DNA from selected bacterial isolates was extracted using the DNeasy UltraClean kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Fungal DNA was extracted using the DNeasy Plant Mini kit (Qiagen).

Bacterial identification was performed by amplifying the 16S rRNA gene using primers 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 685r (5'-TCT ACG CAT TTC ACC GCT AC-3') [14]. Fungal identification was carried out by amplifying and sequencing

the internal transcribed spacer (ITS) region using primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') [15].

PCR reactions (25 μ L) contained 50 pmol of each primer, 200 μ mol L⁻¹ dNTPs (Life Technologies, Gaithersburg, MD, USA), 1.5 U HotStarTaq Plus DNA polymerase (Qiagen), 1 $\times$ PCR buffer, and 3 μ L of extracted DNA. The PCR protocol consisted of an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were purified using ExoSAP-IT (Affymetrix, Cleveland, OH, USA) and sequenced at Eurofins Genomics (Ebersberg, Germany). Sequences were compared with those in GenBank using the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on July 4, 2022) and were deposited under accession numbers PP486466–PP486480 (bacterial isolates) and PP488307–PP488309 (fungal isolates).

2.5. Colorimetric Agar Assays

Lipolytic activity was assessed using commercial Spirit Blue agar (SBA; HiMedia Laboratories, Mumbai, India). SBA was prepared by suspending 32.15 g of the medium in 1000 mL of distilled water, autoclaving it, cooling it to 50 °C, and supplementing it with 30 mL of a lipase substrate mixture (1 mL Tween 80, 400 mL warm distilled water, 100 mL linseed oil; sterilized by autoclaving). The mixture was gently stirred and poured into Petri dishes [16]. One hundred microliters of broth medium (Luria Bertani broth, Sigma-Aldrich) containing the specific isolate were plated onto SBA and incubated at room temperature for one week, after which plates were examined for bleaching rings around colonies.

Haloalkane dehalogenase activity was evaluated using a specific pH indicator agar. The medium was prepared by suspending 5.00 g of LB broth and 15 g of bacteriological agar (HiMedia, Mumbai, India) in 1000 mL of distilled water. The pH was adjusted to 8.2 with 1 M NaOH before autoclaving. To monitor pH changes, phenol red (PhR) and bromothymol blue (BT) were added, each at a final concentration of 13 μ g/mL. After autoclaving and cooling to 50 °C, the medium was poured into Petri dishes.

Freshly grown isolates were streaked onto sterile 0.2 μm nitrocellulose filters and grown overnight on LB agar. The filters were then transferred to the indicator plates. Pure 1,2-dichloromethane was applied to the lids, and the plates were sealed with parafilm. After incubation at room temperature for one week, color changes were recorded [17].

Cutinase activity was assessed using polycaprolactone (PCL; Polymer Institute, SAS), dissolved in acetone and added to Minimal Medium (MM: KNO_3 0.5 g/L, KH_2PO_4 0.1 g/L, K_2HPO_4 1 g/L, MgSO_4 0.5 g/L, KCl 0.5 g/L, agar 15 g/L). One hundred microliters of Luria Bertani broth containing the isolate were inoculated in the center of each PCL plate and incubated at room temperature for two to three days.

Safranin staining was performed by preparing an aqueous solution consisting of 2.5 g safranin dissolved in 10 mL 95% ethanol and diluted with 100 mL distilled water. After incubation, plates were flooded with stain for 20–30 min, gently shaken, and washed three times with 1.0 M NaCl for 15–20 min before examining for clear zones [18].

2.6. Biodegradation Experiment

Samples of thermosetting and thermoplastic materials (RS1, RS2, PET, and PS) were prepared for testing. Minimal Medium (MM) was used as the culture medium, and each material was inoculated separately with five different microorganisms. Each compound–microorganism combination was placed in an individual Erlenmeyer flask.

The five microorganisms were selected based on positive results in colorimetric agar assays. The flasks containing MM, plastic materials, and microorganisms were incubated in an orbital shaker at 100 rpm and room temperature for 30 days. After incubation, samples were collected, placed in Eppendorf tubes, and sterilized by heat prior to analysis. The experiment duration was intentionally limited to 30 days to evaluate the feasibility of implementing a biodegradation process on an industrial scale.

2.7. Fourier Transform Infrared Spectrometry Analysis

Fourier transform infrared (FTIR) spectra were collected in the mid-infrared range (4000–400 cm^{-1}) using a NICOLET 8700™ spectrophotometer (Thermo Fisher

Scientific, Madison, WI, USA). For each sample, spectra were acquired at three different surface locations after 30 days of microbial exposure, and the arithmetic mean of the three spectra was calculated to minimize the effects of heterogeneous surface degradation.

Spectral resolution was set to 4 cm^{-1} , and each spectrum was averaged over 100–200 scans. Baseline correction [19] and spectral smoothing were performed using OMNIC software (Ver. 8.1.0.10). Peak integration for PET samples was carried out using OriginPro 2023 software, applying the most appropriate integration method based on peak characteristics. Integrated peak areas were compared with those of pristine samples to quantify changes in functional groups resulting from microbial degradation.

2.8. Scanning Electron Microscopy (SEM)

The surfaces of samples subjected to microbial attack were examined using a JSM JEOL 6610 SEM (JEOL, Tokyo, Japan) at an accelerating voltage of 15 kV. To prepare non-conductive samples, a thin gold layer was applied using a Balzers SCD 040 sputter coater (Balzers Union Limited, Balzers, Liechtenstein) to dissipate surface charge. SEM images were captured using AzTec software Ver. 2.1 (Springfield, NJ, USA) and processed using ImageJ software Ver. 1.8.0 (LOCI, University of Wisconsin, Madison, WI, USA).

3. Results and Discussion

3.1. Target Materials

The two three-component epoxy resin systems investigated in this study, designated as RS1 and RS2, are both protected by industrial patents, which limits the availability of detailed information regarding their exact chemical structures and formulations. As a result, only partial compositional data can be obtained from publicly accessible technical documentation and complementary laboratory analyses.

RS1 is composed of Araldite LY 3508, a bisphenol-A-based epoxy resin characterized by a relatively high molecular weight and enhanced thermal stability, making it suitable for applications requiring elevated thermal performance. The curing agent used in this system is Aradur 1571, which contains dicyandiamide (DICY) as a latent hardener. DICY remains largely inactive at room temperature, providing extended processing time, and undergoes thermal activation upon heating, leading to the formation of a highly cross-linked epoxy network. The curing process is further promoted by the presence of Accelerator 1573, also DICY-based, which accelerates reaction kinetics and contributes to achieving uniform and complete polymerization.

RS2 consists of Epikote 05910, another bisphenol-A-derived epoxy resin known for its excellent chemical resistance and long-term durability. The curing agent, Epikure Curing Agent 05900, similarly employs DICY as a latent hardener and exhibits curing behavior comparable to that of Aradur 1571. In addition, Epikure Catalyst 05900 is incorporated as an accelerator; this component is supplied as a homogeneous, agglomerate-free dispersion of DICY in a liquid epoxy resin matrix, ensuring efficient heat-activated curing and the formation of a consistent cross-linked structure.

In contrast to the epoxy systems described above, the chemical structures of polyethylene terephthalate (PET) and polystyrene (PS) are well established in the literature and are not subject to industrial patent protection. Their known and well-defined molecular architectures provide a useful reference framework for comparison with the more complex and proprietary thermosetting epoxy formulations investigated in this work.

3.2. Isolate Identification and Enzymatic Characterization

A total of 18 microorganisms isolated from soil and ORAS samples were identified by DNA sequencing (Table 1). The soil sample was characterized by the presence of members of the genus *Flavobacterium*, whereas the ORAS sample was dominated by members of the genus *Pseudomonas*. Both *Flavobacterium* and *Pseudomonas* are bacterial genera associated with the degradation of plastics and polymers. Studies have shown that *Pseudomonas* species possess the ability to degrade and metabolize synthetic plastics such as polystyrene and polyethylene [20]. Furthermore, *Pseudomonas*, together with *Flavobacterium*, has been identified as a dominant genus involved in the degradation of polypropylene carbonate (PPC) plastic films, further emphasizing their relevance in plastic degradation processes [21]. Three fungal isolates were also obtained: *Sporobolomyces roseus* and *Bullera alba* from soil samples, and *Scedosporium boydii* from the ORAS sample.

Table 1. DNA identification of isolates and their enzymatic properties. The isolates used in the biodegradation experiment are shown in bold.

Sample	Isolate	DNA identification, sequence similarity	Lipases assay	H. D. assay	Cutinases assay
Soil	S1	HQ634928 <i>Acinetobacter</i> sp. 99.49%	+	-	-
	S2	LT547828 <i>Flavobacterium oncorhynchi</i> 100%	+	-	-
	S3	MH512740 <i>Flavobacterium succinicans</i> 100%	+	+	-
	S4	MK965149 <i>Flavobacterium</i> sp. 100%	+	-	++
	S5	MK368666 <i>Rahnella</i> sp. 99.84%	+	++	++
	S6	KY117494 <i>Stenotrophomonas</i> sp. 100%	+	++	++
	S7	MN193369 <i>Luteibacter</i> sp. 99.69%	+	-	-
	S8	OP811704 <i>Luteibacter pinisoli</i> 99.84%	+	-	-
	S9	MT502791 <i>Sporobolomyces roseus</i> 99.62%	+	++	-
	S10	MH595326 <i>Bullera alba</i> 100%	+	++	+
Oil Refinery Activated Sludge	ORAS1	JN378750 <i>Pseudomonas stutzeri</i> 100%	+	-	-
	ORAS2	ON688707 <i>Pseudomonas stutzeri</i> 100%	+	-	-
	ORAS3	ON706951 <i>Pseudomonas linyingensis</i> 99.37%	+	-	-
	ORAS4	OL348500 <i>Scedosporium boydii</i> 100%	+	-	+
	ORAS5	LT629780 <i>Pseudomonas guangdongensis</i> 99.84%	+	-	-
	ORAS6	OL445008 <i>Bacillus tropicus</i> 99.69%	+	++	++
	ORAS7	AF094741 <i>Pseudomonas putida</i> 99.83%	+	-	+
	ORAS8	MZ519906 <i>Acinetobacter</i> sp. 100%	+	-	-

All microorganisms tested using the Spirit Blue agar (SBA) assay exhibited positive responses (Table 1), indicating widespread lipolytic activity among the selected isolates. This assay primarily targets the presence of lipases and esterases, and the uniform positivity suggests that these enzymes, or closely related enzymatic functions, are commonly expressed by the investigated microorganisms. Lipases are well known for their ability to catalyze the hydrolysis of ester bonds, which are prevalent in both thermoplastic and thermosetting polymer structures. Previous studies have demonstrated that bacterial lipases preferentially hydrolyze primary ester bonds in substrates such as triacylglycerols [22,23]. In addition, esterases have been reported to participate in the degradation of natural polymers such as cellulose, suggesting that analogous enzymatic pathways may also be involved in the breakdown of synthetic polymers containing similar ester functionalities, including polyethylene terephthalate (PET) [24]. Furthermore, several investigations have highlighted the effectiveness of enzymes belonging to the esterase subclass, particularly cutinases and lipases, in PET degradation, where ester bond cleavage leads to progressive depolymerization of the polymer chains [25].

From a practical standpoint, SBA plates initially display a dark greenish-blue coloration. During incubation, microorganisms producing lipolytic enzymes hydrolyze tributyrin present in the agar, resulting in a visible transition from the original greenish-blue background to a clear halo surrounding the colonies (Figure S1). This halo formation reflects tributyrin hydrolysis and the associated local acidification of the medium.

Haloalkane dehalogenases (HLDs) constitute a class of enzymes capable of catalyzing the cleavage of carbon–halogen bonds in a wide range of halogenated organic compounds, thereby playing an important role in biodegradation processes [26]. Although their direct involvement in polymer degradation is highly dependent on substrate specificity and polymer chemistry, HLD activity could potentially contribute to the modification or partial breakdown of certain thermoplastic or thermosetting materials containing halogenated moieties [26]. In the present study, the indicator plates initially exhibited a violet coloration (Figure S2). Following the transfer of nitrocellulose filters bearing overnight-grown microorganisms, distinct color changes

were observed after incubation. Some isolates induced a clear shift toward pink or orange tones, indicating HLD expression, whereas others produced no visible change, suggesting the absence of detectable dehalogenase activity (Figure S2). The qualitative outcomes of the HLD assay for each isolate are summarized in Table 1.

Cutinases represent a heterogeneous group of enzymes involved in the degradation of cutin, a natural polyester forming the protective cuticular layer of plant surfaces [27]. Due to structural similarities between cutin and synthetic polyesters, certain microorganisms are also capable of degrading polymers such as polycaprolactone (PCL), which is widely used in polyurethane production [28]. In this assay, PCL-containing indicator plates initially appeared milky white (Figure S3a). To enhance visualization of enzymatic activity, the plates were subsequently stained with an aqueous safranin solution (Figure S3b). Only a limited number of microorganisms demonstrated growth-associated clear zone formation on PCL plates, indicative of cutinolytic activity. Among these, *Flavobacterium* sp. (S4), *Rahnella* sp. (S5), *Stenotrophomonas* sp. (S6), and *Bacillus tropicus* (ORAS6) exhibited the most pronounced responses (Table 1). Previous studies have shown that certain cutinases possess the ability to degrade a broad range of polyesters and polyamides through enzymatic attack on ester bonds within polymer chains [27]. In the specific case of PET, cutinases have been demonstrated to cleave ester linkages effectively, leading to polymer depolymerization and the release of monomeric products [29].

Based on the combined results of the colorimetric agar assays, five microorganisms were selected for subsequent biodegradation experiments. These included the bacterial isolates *Rahnella* sp. (S5), *Stenotrophomonas* sp. (S6), and *Bacillus tropicus* (ORAS6), as well as the fungal isolates *Sporobolomyces roseus* (S9) and *Bullera alba* (S10). These microorganisms were subsequently employed to evaluate the biodegradation behavior of PET, polystyrene (PS), and the two epoxy resin systems (RS1 and RS2).

3.3. Characterization of Degradation of Plastic Materials by Selected Microorganisms

3.3.1. Polyethylene Terephthalate (PET)

Figure 1 reports the FTIR spectra in the “fingerprint” region (below 2000 cm^{-1}), where most of the characteristic PET bands are located [30]. The black line represents the pristine PET sample. The main peaks include: 727 cm^{-1} , associated with the interaction of polar ester groups and benzene rings; 1018 cm^{-1} , gauche bending of the oxymethylene group; 1099 cm^{-1} , C–O–C stretching; 1261 cm^{-1} , asymmetric C–C–O stretching bonded to the aromatic ring; 1340 cm^{-1} , trans wagging band of $-\text{CH}_2$; 1370 cm^{-1} , gauche wagging band of $-\text{CH}_2$; 1410 cm^{-1} , $-\text{CH}_2$ bending; and $1710\text{--}1740\text{ cm}^{-1}$, $-\text{C}=\text{O}$ stretching.

When comparing the FTIR spectra of PET samples subjected to microbial degradation with the PET control, the most significant bands did not shift in wavelength. However, the absorbance of several bands in the degraded samples was lower than that of the control. This may indicate that the degraded samples retained their original chemical structure but experienced a reduction in mass. To better compare the data, the FTIR spectra were normalized to the carbonyl stretching band at 1720 cm^{-1} .

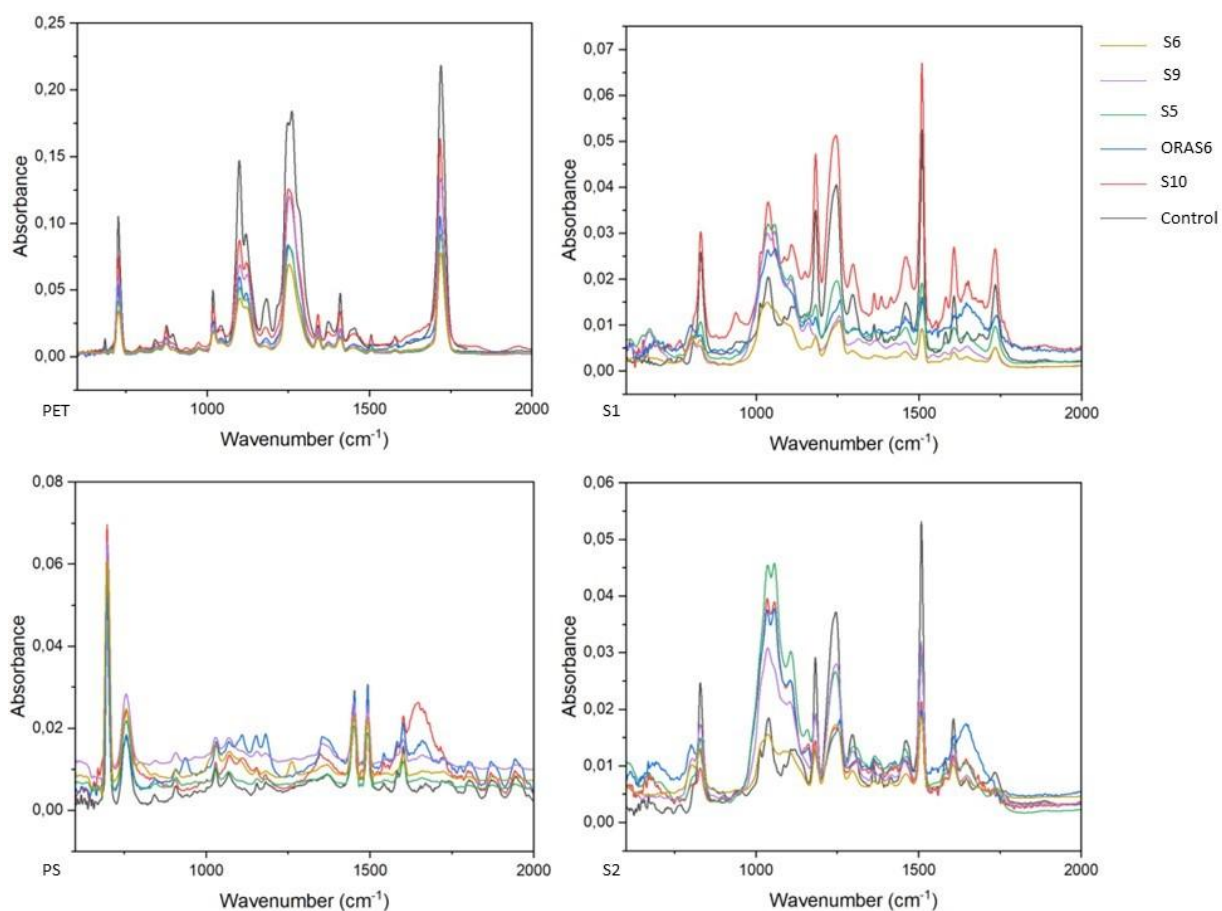


Figure 1. Overlapped mode of PET (a), PS (b), RS1 (c), and RS2 (d) after 30-day microbial degradation carried out by 5 microorganisms. With control representing pristine PET sample, *Rahnella* sp. (S5), *Stenotrophomonas* sp. (S6), *Sporobolomyces roseus* (S9), *Bullera alba* (S10), and *Bacillus tropicus* (ORAS6).

SEM images of the PET control surface, captured at magnifications of 500× and 1400×, reveal a generally smooth surface with minor irregularities resulting from the sample preparation process. Among the five microorganisms tested for their degradative activity on PET, notable effects were observed for *Rahnella* sp. and *Stenotrophomonas* sp. *Rahnella* sp. appears to have induced the formation of crystalline structures, visible at 1400× magnification. In contrast, *Stenotrophomonas* sp. seems to have caused the formation of folds on the material's surface (Figure 2), a phenomenon previously documented in studies on the biodegradation of low-density polyethylene [31].

These findings suggest that *Rahnella* sp. and *Stenotrophomonas* sp. exhibit a strong affinity for PET surfaces, likely through the expression of specialized esterases, such as cutinases, which facilitate surface binding and cleavage of ester bonds. In the literature, there is limited direct evidence regarding the PET-degrading capabilities of these specific genera. However, several studies have demonstrated enzymatic PET degradation by other bacterial species. For example, Müller [32] reported rapid hydrolysis of PET by a hydrolase from *Thermobifida fusca*, highlighting the influence of factors such as crystallinity, melting point, and glass transition temperature on enzymatic breakdown. These insights contribute to a broader understanding of the mechanisms involved in PET degradation and the catalytic potential of certain enzymes.

Additionally, *Stenotrophomonas* sp. has been associated with the degradation of various complex compounds. Santos [33] identified *Stenotrophomonas* sp. among bacteria capable of efficiently degrading the fungicide carbendazim. Although carbendazim is not a plastic, its structure contains aromatic components, including a benzene ring and an imidazole ring, along with nitrogen and oxygen atoms. These features share similarities with certain aromatic polyesters and polyimides. Such metabolic versatility suggests that *Stenotrophomonas* sp. possesses the enzymatic adaptability required to break down structurally complex organic molecules, indicating its potential applicability in the degradation of thermoplastic and thermosetting materials.

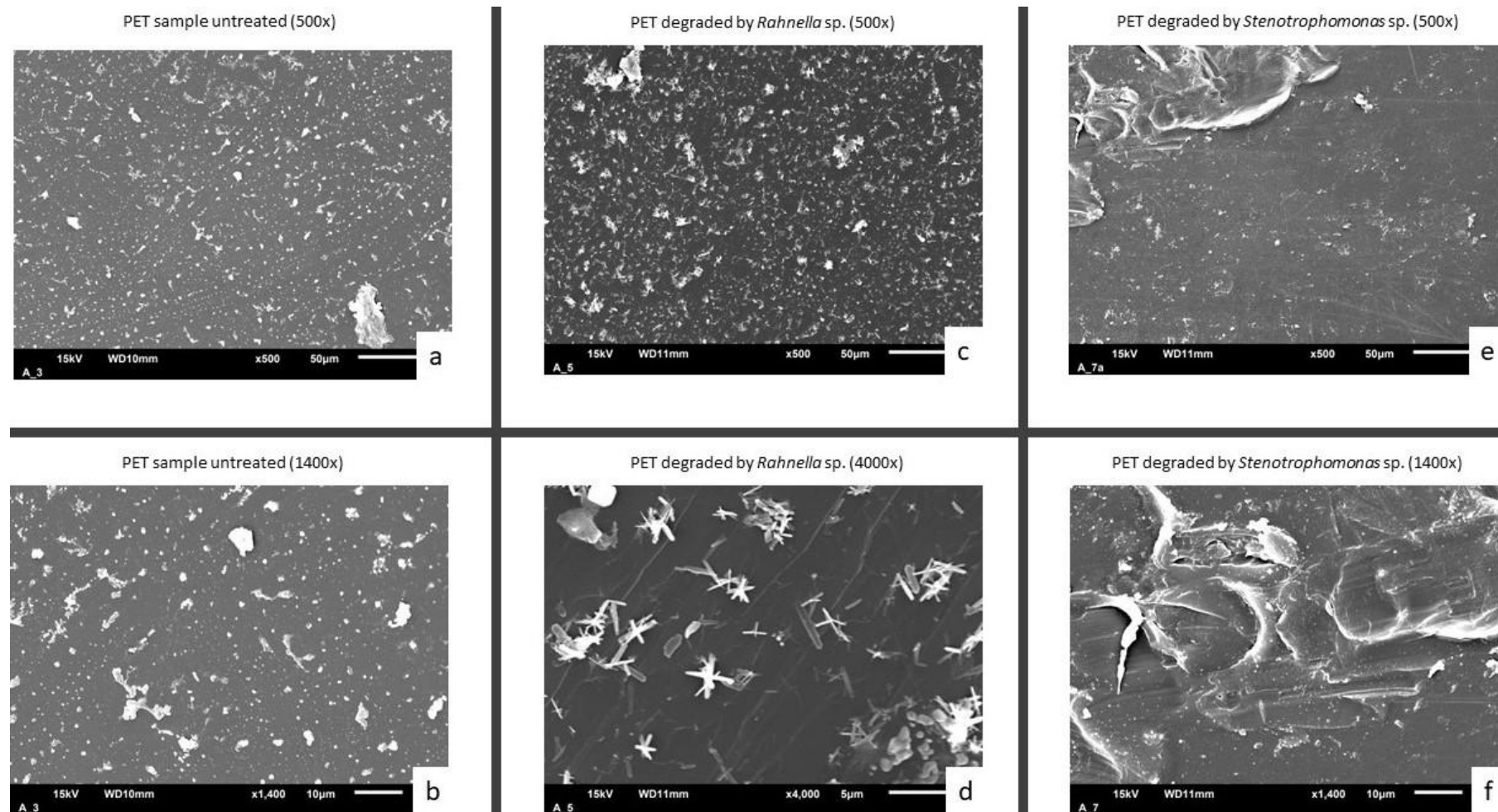


Figure 2. Images acquired through SEM. Sample of PET control (a,b), and samples of PET degraded by *Rahnella* sp. (c,d) and *Stenotrophomonas* sp. (e,f) observed under different magnifications.

3.3.1.1. Comparison of the Area Under the Curve in PET FTIR Spectra Following Biodegradation

The absorption bands of both pristine PET and PET subjected to microbial degradation exhibit overlapping features without any observable shifts. Consequently, the area under the curve of the six principal absorption bands was calculated and found to be comparable across samples. Specifically, the analyzed absorption bands correspond to: the interaction of polar ester groups and benzene rings (P1), gauche bending of the oxymethylene group (P2), C–O–C stretching (P3), asymmetric C–C–O stretching bonded to the aromatic ring (P4), –CH₂ bending (P5), and –C=O stretching (P6), as shown in Figure 3.

The area under the curve for each peak was determined using OriginPro software (OriginLab Corporation) and subsequently compared between the control sample and the PET samples exposed to microbial degradation. This analysis was performed for each microorganism included in the study (Figure 3). A tabulated dataset was then compiled to illustrate the percentage reduction in peak area relative to the PET control after a 30-day degradation period (Table 2). Finally, the mean percentage reduction in area under the curve for each peak was calculated.

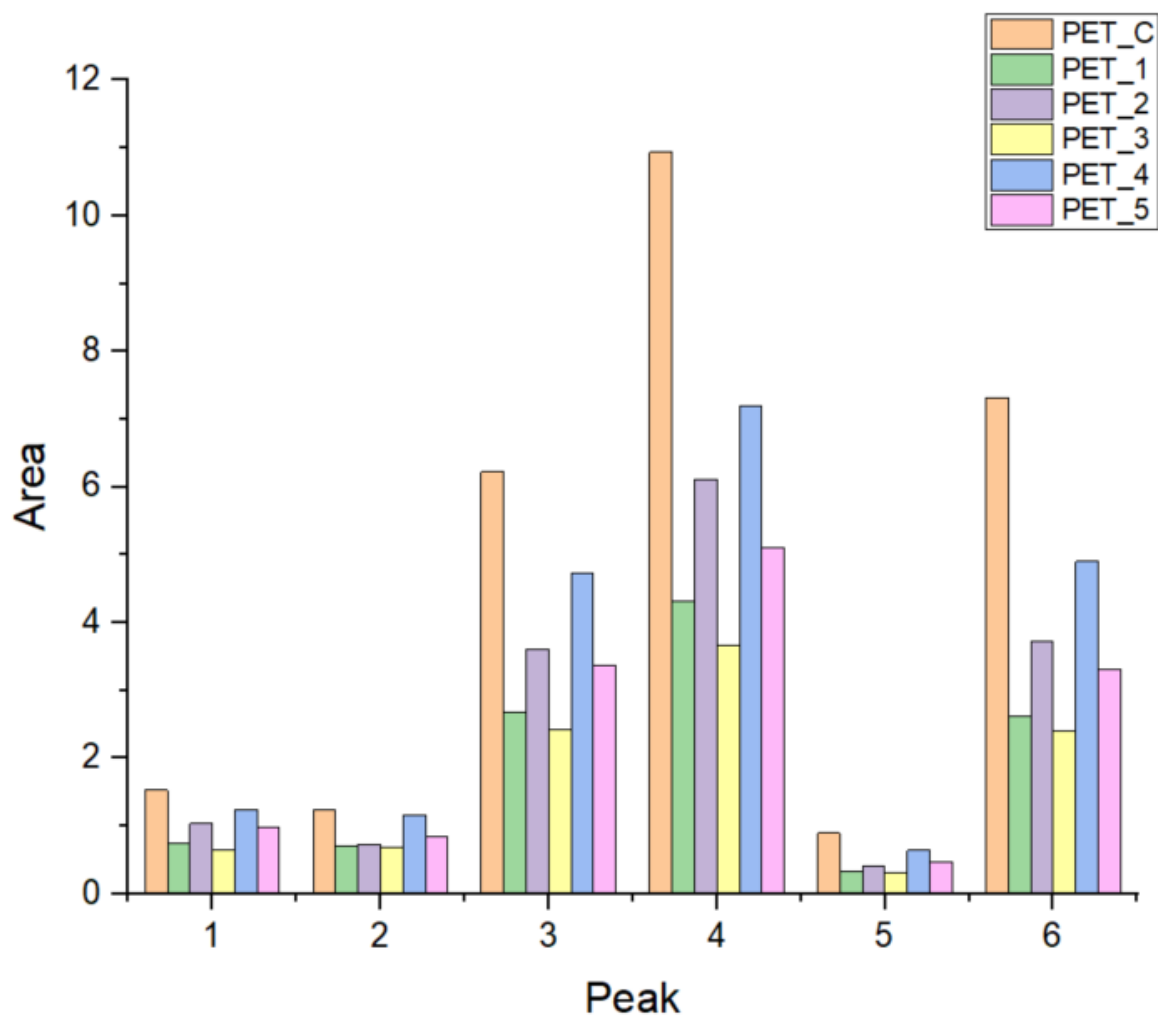


Figure 3. The area under the curve for six absorption peaks characteristic of PET, with PET_C representing the pristine sample. *Rahnella* sp. (PET_S5), *Stenotrophomonas* sp. (PET_S6), *Sporobolomyces roseus* (PET_S9), *Bullera alba* (PET_S10), and *Bacillus tropicus* (PET_ORAS6).

Table 2 Comparison of inoculating PET samples by a single microorganism [*Rahnella* sp. (PET_S5), *Stenotrophomonas* sp. (PET_S6), *Sporobolomyces roseus* (PET_S9), *Bullera alba* (PET_S10), *Bacillus tropicus* (PET_ORAS6)] with PET_C representing the pristine sample.

N° peak	PET_S5	PET_S6	PET_S9	PET_S10	PET_ORAS6
	reduced area compared to PET_C after 30 days	reduced area compared to PET_C after 30 days	reduced area compared to PET_C after 30 days	reduced area compared to PET_C after 30 days	reduced area compared to PET_C after 30 days
1	50,66 %	57,24 %	31,58 %	18,42 %	35,53 %
2	44,00 %	44,80 %	42,40 %	7,20 %	32,80 %
3	57,15 %	61,16 %	42,22 %	24,24 %	45,91 %
4	60,57 %	66,52 %	44,10 %	34,22 %	53,34 %
5	64,05 %	64,05 %	52,81 %	28,09 %	47,20 %
6	64,03 %	67,31 %	49,12 %	32,97 %	54,72 %
mean ± standard error	56,74 ± 3,26	60,18 % ± 3,42	43,70 ± 2,96	24,19 ± 4,14	44,91 ± 3,68

Notably, our findings reveal a consistent reduction in the absorption peaks of all bands following microbial degradation. Among the microorganisms studied, *Stenotrophomonas* sp. exhibited the highest efficacy, achieving a 60.18% reduction in the area under the curve, with a standard error of ± 3.42 (Table 2). This significant result indicates the potential alteration in the mass of the samples undergoing degradation.

3.3.2. *Polystyrene (PS)*

The PS spectrum (Figure 1) exhibits dominant peaks at 2926 and 2851 cm^{-1} corresponding to methylene stretching vibrations; intense out-of-plane C–H bending of the aromatic ring at 697 and 755 cm^{-1} (mono-substituted); aromatic ring breathing modes at 1601, 1493, and 1452 cm^{-1} ; and C–H wagging bands at 1028 and 1068 cm^{-1} . Peaks at 3082, 3061, and 3027 cm^{-1} are associated with aromatic C–H stretching absorptions [34]. Characteristic C=C stretching bands of the vinyl group are found at 1630–1660 cm^{-1} [35], particularly in PS samples degraded by *Rahnella* sp. and *Bacillus tropicus*. This may indicate a possible alteration of the polymer chains and structure, as further supported by the SEM images of PS (Figure 4).

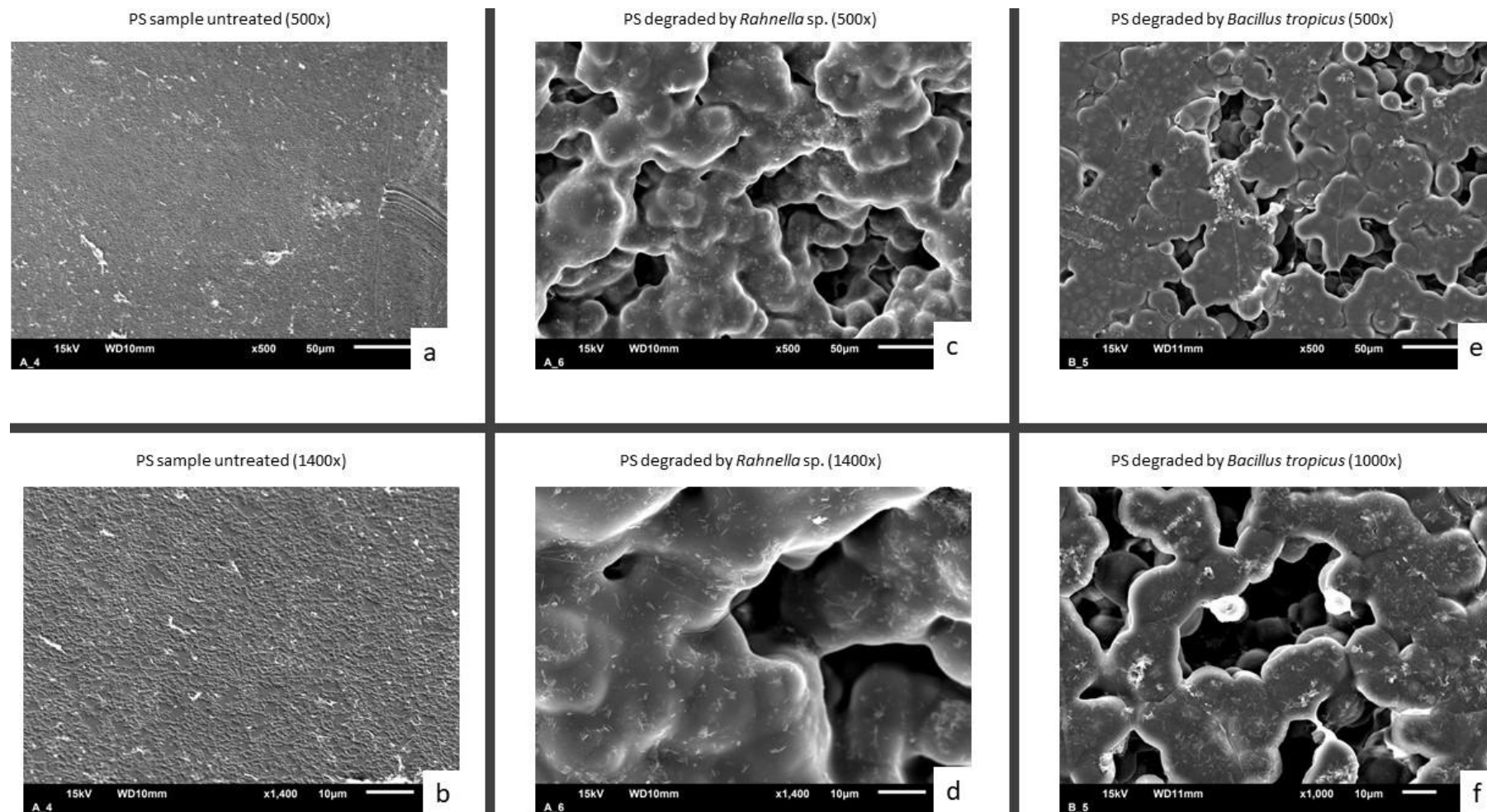


Figure 4. Images acquired through SEM. Sample of PS control (a,b) and samples of PS degraded by *Rahnella* sp. (c,d) and *Bacillus tropicus* (e,f) observed under different magnifications.

The acquired SEM images of the PS samples show a surface that appears relatively smooth at 500× magnification but is noticeably rough at 1400×. Among the five microorganisms tested for degradative activity, *Rahnella* sp. and *Bacillus tropicus* exhibited particularly pronounced effects. Both microorganisms induced a form of material remodeling, characterized by the formation of fenestrations of various sizes, as shown in Figure 4. *Rahnella* sp. and *Bacillus tropicus* may demonstrate enhanced degradation activity on PS due to their potential to express specific enzymes such as esterases or cutinases. These enzymes could interact with and cleave ester bonds associated with the polymer structure, facilitating the breakdown and structural modifications observed in the SEM images. Currently, evidence regarding the PS-degrading capabilities of *Rahnella* sp. and *Bacillus tropicus* remains limited. However, an interesting study investigated the biodegradation efficacy of *Bacillus tropicus* on a low-density polyethylene (LDPE) matrix. That research, which employed analytical methods such as FTIR and SEM—also used in the present study—reported a loss of mechanical properties and a reduction in sample weight as indicators of degradation [36].

3.3.3. Epoxy System 1 (RS1)

RS1 shows peaks at 829 cm^{-1} , associated with the C–O–C stretching of the oxirane group; 1037 cm^{-1} , C–O–C stretching of ethers; 1182 cm^{-1} , asymmetric C–O stretching of esters (absent in RS2); 1246 cm^{-1} , asymmetric aliphatic C–O stretching; 1296 cm^{-1} , asymmetric CH_2 deformation; 1458, 1508, and 1607 cm^{-1} , corresponding to C–C stretching vibrations in aromatic rings and N–H bending of primary amines; 1734 cm^{-1} , bending of primary amines; 2871 cm^{-1} , symmetric C–H stretching of the $-\text{CH}_3$ group; 2931 cm^{-1} , asymmetric C–H stretching of the $-\text{CH}_2$ group; and 2961 cm^{-1} , asymmetric C–H stretching of the $-\text{CH}_3$ group [34] (Figure 1).

When comparing the SEM images of the degraded samples with those of the control, it is evident that only minimal degradation occurred. The majority of the sample surfaces exposed to microbial treatment did not exhibit consistent alterations and appeared as smooth as the control. However, in the sample subjected to degradation by *Bullera*

alba, the presence of a concave area containing fragments and crystalline structures was observed. This concave region—absent in the control sample—provides an important, albeit subtle, indication of degradative activity induced by *Bullera alba* (Figure 5).

In epoxy resins, microorganisms may target specific chemical bonds during degradation. These include oxirane (epoxide) groups (-C-O-C-), ester bonds, aromatic C–C bonds within aromatic rings, and aliphatic C–O bonds. Enzymatic activity on these bonds, as indicated by characteristic FTIR peaks, suggests the potential for biodegradation, with implications for material performance and recycling strategies.

To date, no evidence in the literature directly associates *Bullera alba* or *Bacillus tropicus* with the ability to degrade epoxy resins. This reflects the inherent complexity of identifying microorganisms capable of attacking and breaking down epoxy systems, highlighting the challenges in this area. Nonetheless, the findings of this study may represent an initial step toward future research focused on microbial degradation of epoxy materials.

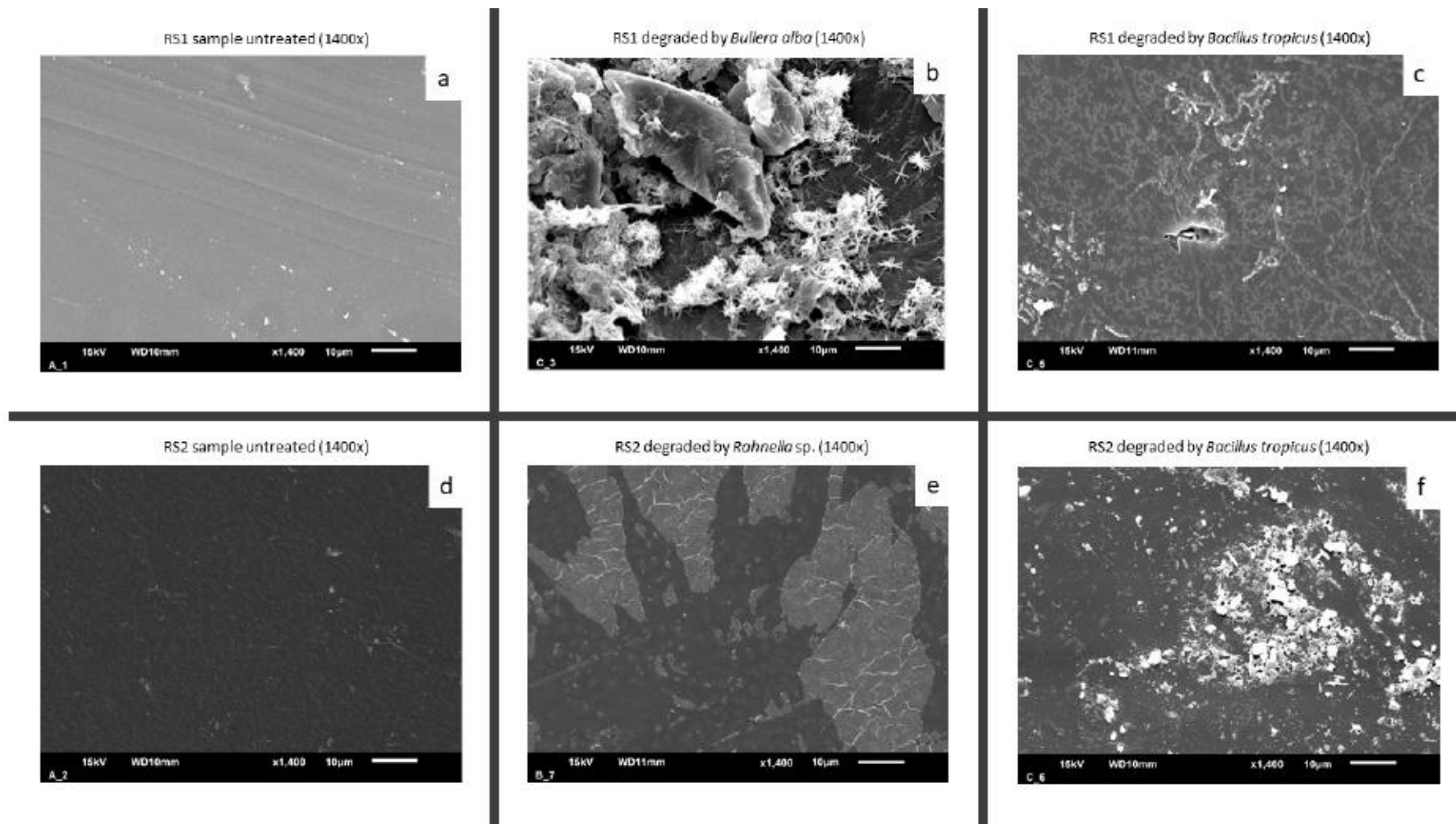


Figure 5. Images acquired through SEM. Sample of RS1 control observed at 1400× (a). Samples of RS1 degraded by *Bullera alba* observed at 1400× (b) and by *Bacillus tropicus* observed at 1400× (c). Sample of RS2 control observed at 1400× (d). Samples of RS2 degraded by *Rahnella* sp. observed at 1400× (e) and by *Bacillus tropicus* observed at 1400× (f).

3.3.4. Epoxy System 2 (RS2)

Given that both systems are epoxy-based, RS2 exhibits peaks resembling those of RS1. However, minor differences can be observed at 1734 cm^{-1} , corresponding to the bending of primary amines. As shown in the plot, this absorption band is almost absent in RS2 (Figure 1).

The SEM images of the degraded samples, compared with the control, indicate minimal degradation activity. A subtle alteration was observed on the surface of the sample exposed to *Rahnella* sp., manifested as variations in color intensity, appearing as both lighter and darker patches. Additionally, surface irregularities were detected in the sample subjected to *Bacillus tropicus*. At $1400\times$ magnification, these irregularities resembled clusters of microorganisms that appeared to elevate the surface, leading to the detachment of small material flakes (Figure 5).

No evidence was found in the literature directly associating *Rahnella* sp. or *Bacillus tropicus* with the degradation of epoxy resins. However, the study by Eliaz [37] demonstrated the ability of specific microorganisms to utilize epoxy resin as their primary carbon source. By isolating bacteria capable of metabolizing epoxy resin, that study highlights potential avenues for sustainable waste management and recycling strategies. Notably, the use of an epoxy resin from the Araldite® family—similar to the resin used in the present study—underscores the relevance of this research to industrial applications and environmental concerns surrounding epoxy-based materials.

4. Conclusions

The integrated analytical approach adopted in this study—combining DNA sequencing, enzymatic screening, and material degradation analyses—provides insight into the adaptive potential of microorganisms isolated from heterogeneous environments, including fresh soil and oil refinery activated sludge. The selected isolates demonstrated the capacity to survive and proliferate under nutrient-limited conditions in which thermoplastic and thermosetting polymers represented the sole carbon source. This capability is associated with the expression of specific enzymatic activities, including lipases, esterases, cutinases, and haloalkane dehalogenases, which enable the microorganisms to interact with and metabolize polymeric substrates.

ATR-FTIR analyses revealed measurable changes in the biodegraded materials, particularly in polyethylene terephthalate (PET), where a significant reduction in infrared absorbance intensity was observed without appreciable shifts in peak positions, indicating chemical modification without major alteration of the polymer backbone. The decrease in absorbance ranged from $24.19 \pm 4.14\%$ to $60.18 \pm 3.42\%$ across the evaluated vibrational bands. Scanning electron microscopy further supported these findings, revealing surface fenestrations and morphological irregularities in PET and polystyrene (PS), consistent with structural alteration and potential material loss.

In contrast, the epoxy resin systems exhibited a markedly lower susceptibility to microbial attack. Although microbial growth was observed when epoxy resins served as the sole carbon source, the associated material modifications were limited. ATR-FTIR spectra of RS1 and RS2 showed a reduction in infrared absorbance intensity, suggesting surface-level interactions, while SEM imaging revealed minimal or no detectable changes in surface morphology, highlighting the inherent resistance of cross-linked thermosetting polymers to biodegradation.

Despite the modest extent of degradation observed for epoxy systems, these results provide valuable preliminary evidence of microorganism–polymer interactions and establish a baseline for further investigation. Future studies will incorporate additional quantitative parameters, such as precise mass variation measurements, to better

elucidate degradation mechanisms. Moreover, the application of chemical or physico-chemical pre-treatments aimed at enhancing surface accessibility and microbial activity on epoxy resins will be explored, with the goal of improving biodegradation efficiency and supporting advances in environmental remediation and sustainable polymer waste management.

The findings presented in this chapter therefore serve as the foundation for the additional experimental investigations described in Chapter 3.

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Chapter 3: Experimental Evaluation of Chemical Pre-Treatments for Enhancing the Microbial Degradation of Epoxy Resins

1 Introduction

The results presented in the previous chapter demonstrated the strong resistance of epoxy resins to microbial degradation. Despite the ability of selected microorganisms to interact effectively with thermoplastic materials, their impact on epoxy systems remained limited, indicating that the intrinsic cross-linked structure of thermosets presents a substantial barrier to microbial colonization and enzymatic attack [1]. These observations led to the hypothesis that introducing controlled surface modifications could improve microbial interaction with epoxy resins and potentially increase their susceptibility to biodegradation.

Rather than revisiting the general chemistry and curing mechanisms of epoxy systems, already discussed in the literature review, this chapter focuses on the influence of surface characteristics on microbial attachment and early biofilm establishment. Previous studies have shown that an increase in surface hydrophilicity, the introduction of oxygen-containing functional groups, and modifications to micro- or nanoscale roughness can enhance bacterial adhesion and promote polymer–microbe interactions [2-4]. Plasma-based surface treatments, in particular, are widely employed for this purpose, as they can alter surface chemistry and wettability while preserving the bulk properties of the material [5].

In this context, two epoxy systems (RS1 and RS2) were subjected to plasma etching under two different atmospheric conditions, 100% O₂ and a 50:50 O₂/CF₄ mixture, to assess whether these treatments could enhance surface wettability and thus support subsequent microbial colonization. Surface modifications were quantified through contact angle measurements and further examined through morphological and chemical characterization techniques, including SEM, EDX, and XPS. Following functionalization, each resin–treatment combination was exposed individually to microbial isolates previously identified as exhibiting relevant enzymatic activities [6], allowing the evaluation of whether surface activation could facilitate the onset of biodegradative processes.

In this study, biodegradation experiments were intended to evaluate the influence of plasma-induced surface modification on microbial adhesion and early-stage

degradation phenomena, rather than to assess complete chemical breakdown of the polymer matrix.

2. Materials and Methods

2.1. Target Materials, Sample Preparation and Curing

Epoxy samples were prepared using two different three-component resin systems, both supplied by COMEC Innovative s.r.l. (Chieti, Italy). The first resin system (RS1) was based on Araldite®, while the second resin system (RS2) utilized Epikote™ as its primary component. Each of these resin systems consisted of three essential components: the resin itself, a hardener, and an accelerator, which were carefully selected to achieve the desired mechanical and chemical properties.

For both RS1 and RS2 systems, the components were mixed according to the specific ratios provided by the manufacturer to ensure optimal cross-linking and mechanical performance. Once mixed, the resins were subjected to curing conditions as outlined in the respective technical data sheets, which included specific temperature profiles and curing times.

The curing process was performed in silicone molds, allowing for the preparation of samples with precise dimensions of 5 mm × 5 mm × 5 mm. The use of silicone molds was essential for ensuring dimensional accuracy and easy demolding of the cured samples. The molds allowed the epoxy to solidify in a controlled environment, resulting in well-formed, defect-free samples suitable for subsequent testing and analysis.

2.2. Polishing Procedure

The two set of epoxy samples, RS1 and RS2, were then subjected to a multi-step polishing process to achieve a smooth surface suitable for further analysis. The polishing procedure was carried out manually using a series of progressively finer silicon carbide abrasive sheets. The following grit sizes were employed in sequence: P280, P600, P1000, P2000, and P3000. Each sample was polished in a controlled environment to minimize contamination and ensure consistent results across both sets of samples.

For each grit level, the polishing technique involved first making uniform circular motions across the entire surface of the sample. These circular motions ensured even material removal and avoided uneven wear on the sample surface. After achieving an initial smoothness, the polishing direction was changed to a series of "8-shape" motions. This figure eight technique is known for producing a more uniform finish and eliminating any potential directional scratches introduced during the circular polishing phase [7].

Each step of the polishing process was carefully monitored using calipers to ensure that the entire surface of the sample was plan-parallel and uniformly treated. The final step with P3000 grit resulted in a highly smooth surface, free of visible scratches or defects, making the samples ready for subsequent surface characterization and testing.

Water was applied as a lubricant during the polishing process to prevent excessive heat build-up and to wash away debris. After each polishing stage, the samples were thoroughly rinsed with distilled water and dried using compressed air to ensure that no abrasive particles from the previous grit remained on the surface before moving on to the next step.

2.3. Surface Functionalization by Plasma Etching

To enhance the surface hydrophilicity of the RS1 and RS2 epoxy samples, plasma etching was performed using a low-pressure plasma system (Femto, Diener electronic, GmbH + Co. KG). The primary objective of this treatment was to improve surface wettability and facilitate microbial attachment in subsequent biofilm experiments, which is critical for assessing biodegradation potential [5].

Two distinct atmospheric conditions were employed for the plasma treatment: the first atmosphere consisted of 100% oxygen (O_2), while the second atmosphere was a mixture of 50% oxygen (O_2) and 50% tetrafluoromethane (CF_4). For both conditions, the samples were placed in the plasma chamber, where the pressure was reduced to a low-pressure environment suitable for plasma generation. The treatment duration and power settings were optimized to 10 minutes, based on preliminary trials and literature

evidence indicating enhanced surface activation under comparable plasma conditions [8, 9].

Following plasma treatment, the samples were analyzed to verify changes in surface properties. Enhanced hydrophilicity was anticipated to promote better interaction between the treated surfaces and microorganisms, thereby facilitating biofilm formation in subsequent microbial adhesion tests.

2.4 Wettability Measurements

To prepare the samples for contact angle measurement, plan parallel surfaces were visually observed to ensure no contaminants that could affect the wettability results were present. Each sample was then placed on a flat, stable platform and a 5 μ L droplet of distilled water placed on the plan-parallel sample surface. The experimental setup involved a digital microscope equipped with a high-resolution camera [Dino-Lite AM7915MZT, AnMo Electronics Corp., Taiwan] to capture images of the droplet at various time intervals. The droplet was allowed to stabilize for a few seconds before capturing images for analysis [10].

The contact angle was determined by analyzing the profile of the droplet using the ImageJ software Ver. 1.8.0., which is freely available. (LOCI, University of Wisconsin, Madison, WI, USA). The results were recorded, and the average contact angle was calculated from three measurements taken on different faces of the samples to ensure statistical reliability [11].

2.5 Surface and Chemical Characterization after Plasma Treatment

2.5.1. Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDX)

Surface morphology and elemental composition of the epoxy samples subjected to microbial exposure were analyzed using scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM/EDX). Imaging was performed using a JSM JEOL 6610 SEM (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 15 kV. Prior to analysis, all samples were sputter-coated with a thin gold layer using a Balzers SCD 040 sputter coater (Balzers Union Limited, Balzers, Liechtenstein) to enhance surface conductivity and prevent charging during electron bombardment. SEM micrographs were acquired with AzTec software, Version 2.1 (Oxford Instruments, UK), and later processed using ImageJ software, Version 1.8.0 (LOCI, University of Wisconsin–Madison, USA). Elemental analysis was performed using the EDX detector integrated into the SEM system, enabling qualitative identification of elements present on the sample surface. EDX spectra and maps were collected from selected regions of interest to assess potential compositional changes associated with plasma treatment or microbial degradation. Morphological and elemental comparisons were carried out using secondary electron images acquired at magnifications up to 10,000 $\times$.

2.5.2. Fourier Transform Infrared Spectrometry Analysis (FTIR)

FTIR analysis was performed to characterize the bulk chemical structure of the epoxy resin samples following plasma treatment and prior to biodegradation experiments. Spectra were acquired in the mid-infrared range (4000–400 cm^{-1}) using a NICOLET 8700TM spectrophotometer (ThermoFisher Scientific, Madison, WI, USA). For each sample, spectra were collected at three different surface locations to account for potential heterogeneity, and the arithmetic mean spectrum was calculated.

The spectral resolution was set to 4 cm^{-1} , with 100–200 scans averaged per measurement. All spectra were baseline-corrected and automatically smoothed using OMNIC software (version 8.1.0.10, ThermoFisher Scientific). FTIR analysis was

employed to identify the characteristic functional groups of the epoxy resins and to evaluate potential bulk-level chemical changes induced by plasma etching, while acknowledging the limited sensitivity of this technique to surface-confined modifications.

2.5.3. X-ray Photoelectron Spectroscopy (XPS)

X-ray Photoelectron Spectroscopy (XPS) was performed to investigate plasma-induced surface chemical modifications of the epoxy resin samples prior to biodegradation experiments. Surface chemical composition and functional group analysis were performed using a Thermo Scientific Nexsa G2 Surface Analysis System (Thermo Fisher Scientific, UK), equipped with a micro-focused monochromatic Al K α X-ray source (1486.68 eV). A 400 μ m X-ray spot size was used for all measurements. Survey spectra were acquired in constant analyzer energy mode at a pass energy of 200 eV, while high-resolution spectra were collected at a pass energy of 50 eV to resolve specific bonding environments.

Charge compensation was achieved using the instrument's dual-beam flood-gun system. Data acquisition and processing were performed using Thermo Scientific Avantage software, Version 6.5.1. Calibration was carried out using the automated internal routine referencing Au 4f, Ag 3d, and Cu 2p peak positions. Elemental atomic percentages (at.%) were determined based on the integrated peak areas and manufacturer-provided sensitivity factors. Peaks were fitted using a standard Shirley background and mixed Gaussian–Lorentzian functions, following conventional analysis procedures.

2.6. Biodegradation Experiment

The etched RS1 and RS2 samples, treated under both atmospheric conditions, were used in the biodegradation experiment. Prior to microbial exposure, each sample was carefully weighed to determine its initial mass, which was later compared with the final mass after a 30-day incubation period. Monitoring weight variation is a commonly adopted approach for assessing degradation-related mass changes in polymeric materials [12].

Each treated sample was individually exposed to three different microorganisms, with separate inoculations performed for every treatment–microorganism combination. The selection of microbial strains was based on a previous study that evaluated their enzymatic activity and reactivity toward thermosetting and thermoplastic substrates, including epoxy-based systems [5]. This approach ensured that both resin systems (RS1 and RS2) and both plasma treatments were systematically tested with each microorganism in independent Erlenmeyer flasks, enabling a comparative assessment under controlled and standardized conditions [13].

The flasks contained minimal medium (MM), the epoxy samples, and the selected microorganisms, and were placed on an orbital shaker operating at 100 rpm at room temperature for 30 days. Continuous shaking promoted adequate aeration and nutrient dispersion, supporting microbial metabolic activity throughout the incubation period. At the end of the experiment, samples were retrieved and subjected to a sterilization procedure to inhibit further microbial activity prior to subsequent analyses. The incubation time of 30 days was selected to investigate early-stage biodegradation phenomena within a time frame compatible with potential industrial applications [14].

2.7. Weight loss measurements

Weight measurements of RS1 and RS2 samples were conducted at two distinct time points: baseline (t_0), prior to microbial exposure, and follow-up (t_1), after the biodegradation experiment. At both time points, sample weights were recorded using the same calibrated analytical balance (Mettler Toledo AE240, Mettler Toledo, Switzerland), providing an accuracy of 0.1 mg.

Prior to follow-up weighing, samples were subjected to a standardized cleaning procedure to remove residual microbial biomass and loosely attached surface deposits. Specifically, samples were gently rinsed with distilled water, followed by wiping of the surface with lint-free wipes moistened with ethanol (70% v/v) to remove biofilm residues without inducing chemical modification of the epoxy matrix. Subsequently, samples were thoroughly rinsed with distilled water, dried under controlled conditions, and allowed to equilibrate to room temperature in a desiccator to ensure complete removal of moisture before final weighing. Follow-up measurements were performed under controlled environmental conditions to minimize variability related to temperature and humidity. The change in weight for each sample was calculated as:

$$\Delta P = P_{t1} - P_{t0}$$

Where:

- ΔP represents the change in weight,
- P_{t1} is the weight at the follow-up time point, and
- P_{t0} is the weight at baseline.

The average weight change across all samples was computed as:

$$\overline{\{\Delta P\}} = \left(\frac{1}{n}\right) \sum_{i=1}^n \Delta P_i$$

Where n is the total number of replicates ($n = 3$).

To estimate the variability of the measurements, the standard deviation (SD) was calculated as:

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (\Delta P_i - \overline{\Delta P})^2}$$

where s is the standard deviation of the individual weight changes. This series of calculations provides a precise quantification of the effectiveness of the weight loss intervention in the studied sample.

2.8. SEM/EDX Surface Analysis after Biodegradation

SEM and EDX analyses after biodegradation were performed using the same instrumentation and operating conditions described in Section 2.5.1. Following completion of the biodegradation experiments, selected samples were analyzed to qualitatively assess surface morphological alterations and to semi-quantitatively evaluate elemental surface changes induced by microbial exposure.

This post-biodegradation analysis was intended to provide a comparative assessment with the plasma-treated surfaces prior to biological exposure, with the aim of identifying surface alterations associated with microbial interaction rather than demonstrating complete chemical degradation of the epoxy polymer matrix.

3. Results and discussion

3.1. Target Materials Resin Systems and Chemical Considerations

Prior to any surface functionalization, both RS1 and RS2 samples were subjected to the same multi-step polishing protocol, resulting in smooth, plan-parallel surfaces and minimizing roughness-related contributions to subsequent wettability measurements [7].

The two epoxy resin systems examined in this study, designated as RS1 and RS2, are both protected by industrial patents, which complicates the precise identification of their chemical compositions. Due to these patents, only limited information about the detailed formulation of these resins is publicly accessible. Nevertheless, some key characteristics of the components are known.

RS1 comprises three main elements: Araldite LY 3508, Aradur 1571, and Accelerator 1573. Araldite LY 3508 is an epoxy resin derived from bisphenol-A, notable for its high molecular weight and exceptional thermal stability, making it well-suited for applications requiring robust performance under heat stress [15]. The curing agent, Aradur 1571, contains dicyandiamide (DICY), a latent hardener that remains inactive at room temperature, thereby offering an extended working time. Upon exposure to heat, however, DICY reacts to form a highly cross-linked polymer network, which enhances the structural integrity of the cured resin [16]. Complementing this, Accelerator 1573, also DICY-based, functions to speed up the curing process, ensuring that polymerization occurs uniformly and fully, even under time-constrained conditions [17].

RS2, on the other hand, utilizes a different set of components, namely Epikote 05910, Epikure Curing Agent 05900, and Epikure Catalyst 05900. Epikote 05910 is another bisphenol-A-based epoxy resin, recognized for its outstanding resistance to chemicals and durability, making it ideal for harsh environmental conditions [18]. The curing agent, Epikure Curing Agent 05900, shares similarities with Aradur 1571 as it also uses DICY as a latent hardener [19]. Epikure Catalyst 05900, a dispersion of DICY in liquid epoxy resin, acts as an accelerator, promoting efficient and consistent curing. This catalyst

ensures the formation of a uniform cross-linked structure, enhancing the overall performance of the material [20].

The differences in the chemical compositions and curing mechanisms of RS1 and RS2 highlight the importance of selecting appropriate resin systems for specific applications. The use of DICY in both systems underscores its versatility as a curing agent, providing the benefits of extended working time and enhanced thermal stability. Furthermore, the unique properties of each resin system can be leveraged to optimize performance in various environmental conditions, making them suitable for a wide range of applications in industries such as aerospace, automotive, and construction [21]. Given the small and uniform dimensions of the epoxy specimens ($5 \times 5 \times 5$ mm), plasma exposure can be reasonably assumed to be homogeneous across the treated surfaces, minimizing edge or shadowing effects.

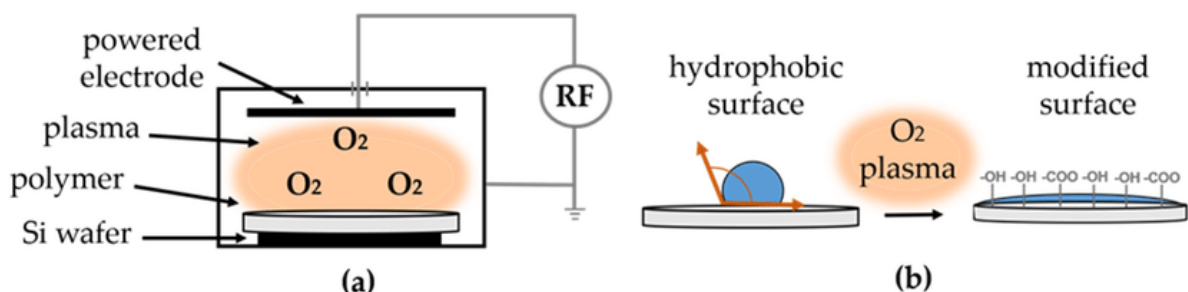


Figure 1. Schematic illustration of the oxygen plasma treatment process (a) and the resulting surface modification (b). Reproduced from [14].

3.2. Surface Wettability after plasma etching.

Oxygen plasma treatment is widely reported to increase polymer surface hydrophilicity through the incorporation of polar oxygen-containing functional groups, such as hydroxyl and carbonyl species, which enhance surface energy [9, 14]. Conversely, plasma treatment performed in an O₂/CF₄ atmosphere is expected to induce a more complex surface modification, combining oxidative processes with partial fluorination, thereby modulating wettability and surface reactivity in a different manner. The selected plasma power and exposure time were based on literature-reported optimized conditions that maximize surface activation while avoiding bulk polymer degradation, ensuring that the observed changes are primarily attributable to surface modification [8, 9].

The results of the contact angle measurements for the RS1 and RS2 resin samples, measured in triplicate under different treatment conditions, are summarized in Table 1, while representative droplet images are shown in Fig. 2. Untreated samples exhibited initial contact angles of 74.88° for RS1 and 64.12° for RS2, indicating a moderately hydrophobic surface. Following exposure to an O₂ atmosphere, a significant decrease in the contact angle was observed for both samples, with RS1 dropping to 8.60° and RS2 to 10.35°, demonstrating a substantial increase in surface hydrophilicity. This pronounced reduction in contact angle suggests that the O₂ treatment effectively oxidized the surface, in agreement with surface chemical analyses discussed in Section 3.3

Table 1. Static water contact angle values of RS1 and RS2 epoxy samples after plasma treatment (mean ± SD, n = 3).

Treatment	RS1 (°)	RS2 (°)
Untreated	74.88 ± 0.67	64.12 ± 0.69
O ₂ Plasma	8.60 ± 0.30	10.35 ± 0.31
O ₂ /CF ₄ Plasma	34.09 ± 0.70	24.65 ± 0.61

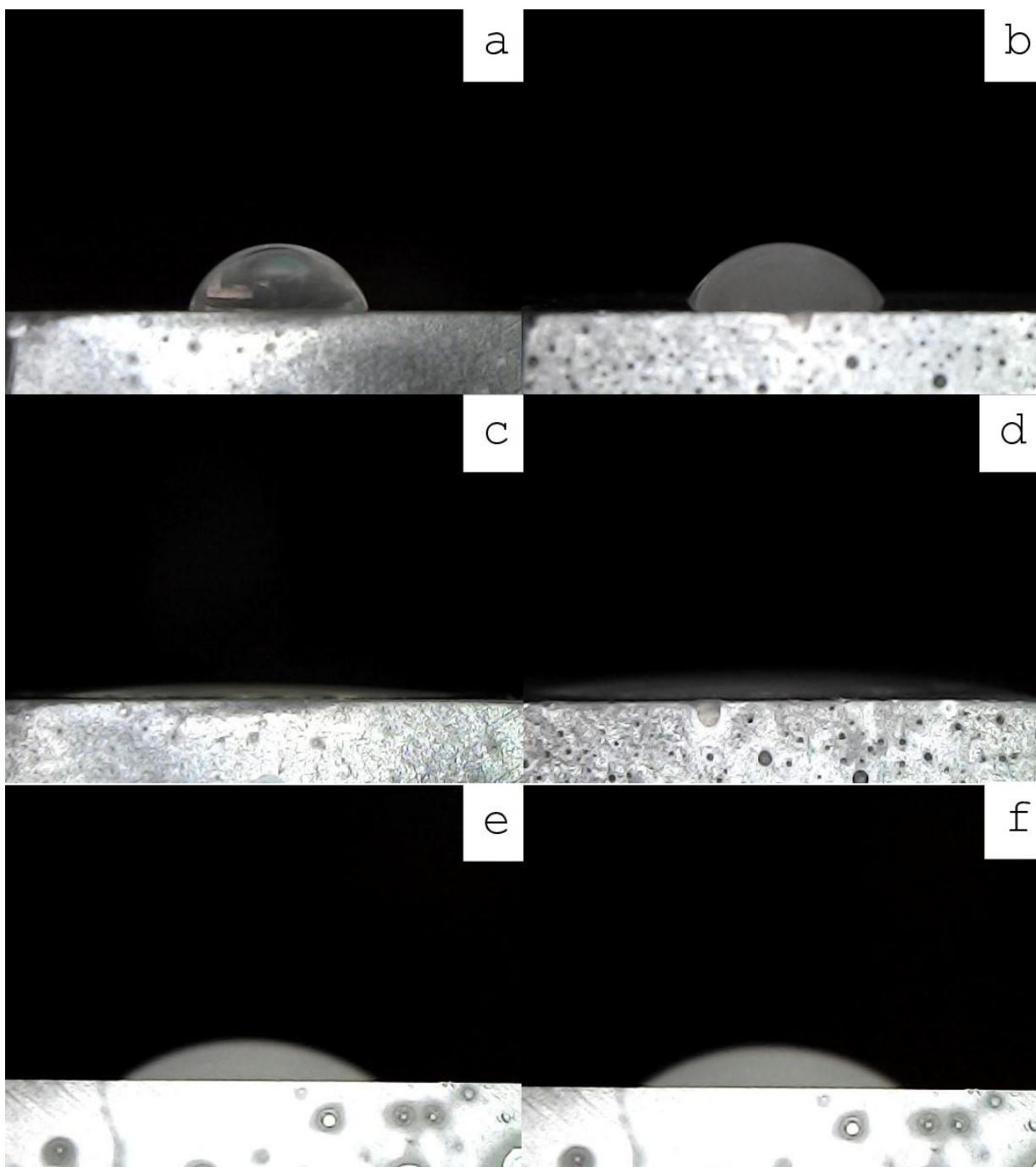


Figure 2. Representative static water contact angle images of RS1 and RS2 epoxy resin samples under different plasma treatment conditions: (A) RS1 untreated, (B) RS2 untreated, (C) RS1 O₂ plasma, (D) RS2 O₂ plasma, (E) RS1 O₂/CF₄ plasma, and (F) RS2 O₂/CF₄ plasma. Contact angle values are reported in Table 1.

In contrast, treatment with a 50% O₂ and 50% CF₄ atmosphere led to a less pronounced reduction in contact angles. The values measured for RS1 and RS2 after this treatment were 34.09° and 24.65°, respectively. Although the surfaces still exhibited an increased hydrophilicity compared to the untreated samples, the CF₄/O₂ mixture appeared to inhibit the full oxidation effect seen with pure O₂, likely due to the fluorination introduced by CF₄, which can contribute to maintaining some hydrophobic character.

Overall, these results indicate that while both O₂ and CF₄/O₂ treatments increase the hydrophilicity of the resin surfaces, pure O₂ is more effective in achieving a highly hydrophilic surface, whereas the CF₄/O₂ treatment results in a more moderate modification. The differences observed between RS1 and RS2 may be attributed to variations in their initial surface chemistry and crosslinked structure, which influence their response to plasma exposure.

3.3. Surface Morphology and Chemistry after Plasma Treatment

3.3.1. SEM Surface Morphology after Plasma Treatment

RS1 and RS2 resin samples, both untreated and after O₂ or O₂/CF₄ plasma treatments, were examined using SEM equipped with EDX to evaluate surface morphology and elemental composition. Gold signals detected in the EDX spectra originate from the sputter-coating layer applied prior to SEM analysis and are not related to the intrinsic chemical composition of the epoxy resins. Elemental quantification is reported in Table 2, while Figure 3 shows the corresponding SEM images, revealing the morphological evolution of the surfaces following plasma exposure. Together, SEM and EDX provide complementary information on how plasma treatments alter both the chemical composition and the physical structure of the resin surfaces.

Table 2. Surface elemental composition (wt.%) of RS1 and RS2 epoxy resin samples before and after plasma treatment, as determined by EDX.

Condition	C (wt.%)	O (wt.%)	N (wt.%)	Au (wt.%)
RS1 untreated	43.21	16.47	9.48	30.84
RS2 untreated	51.73	24.10	10.64	13.53
RS1 O ₂ plasma	51.11	25.23	11.75	11.91
RS2 O ₂ plasma	45.70	22.91	9.83	21.56
RS1 O ₂ /CF ₄ plasma	43.50	20.76	11.35	24.39
RS2 O ₂ /CF ₄ plasma	46.67	21.71	10.15	21.47

Note: Au originates from the sputter-coating step

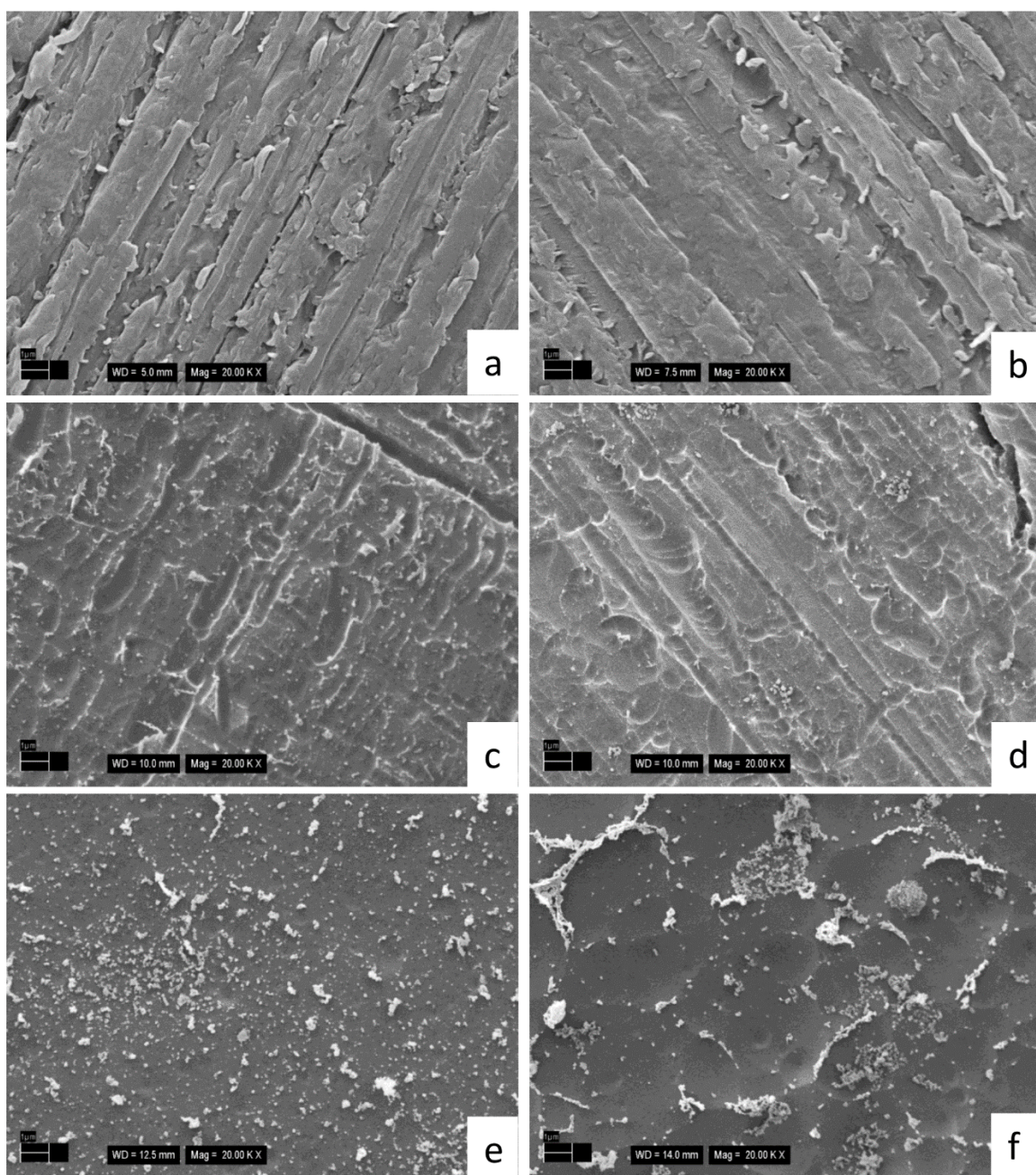


Figure 3. SEM micrographs of RS1 and RS2 epoxy resin samples before and after plasma treatments: (A) RS1 pristine, (B) RS2 pristine, (C) RS1 treated with O₂ plasma, (D) RS2 treated with O₂ plasma, (E) RS1 treated with 50% O₂ / 50% CF₄ plasma, and (F) RS2 treated with 50% O₂ / 50% CF₄ plasma. Plasma-treated samples exhibit surface etching and morphological modifications compared to pristine samples, with more pronounced effects observed for RS2.

3.3.1.1. *Untreated RS1 and RS2 Samples (SEM + EDX)*

SEM observations:

Pristine RS1 and RS2 samples (Fig. 3) display relatively smooth and uniform surfaces with faint striations originating from the manufacturing or cutting process. No micro-cracks, pits, or significant surface roughness are visible. RS1 tends to exhibit slightly more defined linear features, while RS2 appears smoother overall.

EDX Observations:

The untreated RS1 sample exhibited a carbon content of 43.21 wt.-% and an oxygen content of 16.47 wt.-%, with nitrogen contributing 9.48 wt.-%. These values are typical for polymeric resins, where carbon constitutes the principal backbone and nitrogen and oxygen originate from curing agents and functional groups within the resin structure. In comparison, the untreated RS2 sample showed higher carbon (51.73 wt.-%) and oxygen (24.10 wt.-%) contents, while nitrogen was slightly higher at 10.64 wt.-%. The higher oxygen suggests a larger proportion of oxygenated functionalities, which may contribute to the slightly more hydrophilic behavior observed in contact angle measurements.

3.3.1.2. *Effect of O₂ Plasma Treatment (SEM + EDX)*

SEM observations:

O₂-treated RS1 and RS2 samples (Fig. 3) exhibit clear signs of surface etching. The originally smooth textures evolve into more irregular topographies, with micro-scale grooves and roughened domains becoming more pronounced. RS1 shows a stronger increase in roughness, with visible erosion along the original striations, while RS2 displays a combination of shallow pits and more uniform surface disruption. These features are characteristic of oxidative plasma etching, where polymer chains near the surface are broken and volatilized, leaving behind a more textured surface.

EDX observations:

Both RS1 and RS2 samples showed an increase in oxygen content after O₂ plasma treatment, confirming surface oxidation. For RS1, oxygen increased from 16.47 wt.-% to 25.23 wt.-%, accompanied by a reduction in carbon from 43.21 wt.-% to 39.11 wt.-%. Nitrogen increased slightly from 9.48 wt.-% to 11.75 wt.-%. The substantial incorporation of oxygen is consistent with the formation of polar functionalities such as hydroxyl and carbonyl groups, aligning with the observed decrease in contact angle.

RS2 also exhibited compositional changes after O₂ plasma treatment; however, the increase in oxygen content was less pronounced compared to RS1, suggesting a more limited extent of surface oxidation. Carbon decreased from 51.73 wt.-% to 45.70 wt.-%, while nitrogen showed only minor variation (from 10.64 wt.-% to 9.83 wt.-%). These compositional changes are consistent with the marked decrease in contact angle observed for both resins after O₂ plasma treatment (Section 3.2).

3.3.1.3. *Effect of O₂/CF₄ Plasma Treatment (SEM + EDX)*

SEM observations:

The O₂/CF₄-treated samples (Fig. 3) show morphological changes distinct from pure O₂ plasma. RS1 exhibits fine particulate deposits and localized roughness rather than deep etching. RS2 displays a more heterogeneous surface with clusters of etched

material and partially eroded regions, but the overall roughness is less pronounced than in the O₂-treated samples. The presence of CF₄ tends to compete with oxidative etching, often introducing fluorinated fragments or generating polymer fragments that re-deposit on the surface, explaining the more granular appearance.

EDX Observations:

In samples treated with a 50% O₂ and 50% CF₄ atmosphere, the oxidation process was less pronounced, as indicated by the smaller increase in oxygen content. For RS1, oxygen content reached 20.76 wt.-%, lower than the value obtained after pure O₂ treatment. Carbon remained relatively stable (43.50 wt.-%), while nitrogen increased slightly to 11.35 wt.-%. The presence of fluorinated groups may inhibit oxygen incorporation and partially preserve hydrophobic character, consistent with the higher contact angles measured.

For RS2, the O₂/CF₄ treatment resulted in an oxygen content of 21.71 wt.-%, slightly lower than the 22.91 wt.-% observed in the O₂-treated sample. The carbon content was reduced to 46.67 wt.-%, and nitrogen showed a slight decrease to 10.15 wt.-%. These results align with the contact angle measurements, where the O₂/CF₄ treatment produced more moderate changes in surface wettability compared to pure O₂, suggesting that the fluorine introduced by CF₄ imparts some resistance to further oxidation. Representative EDX spectra supporting the elemental trends discussed above are shown in Fig. 4.

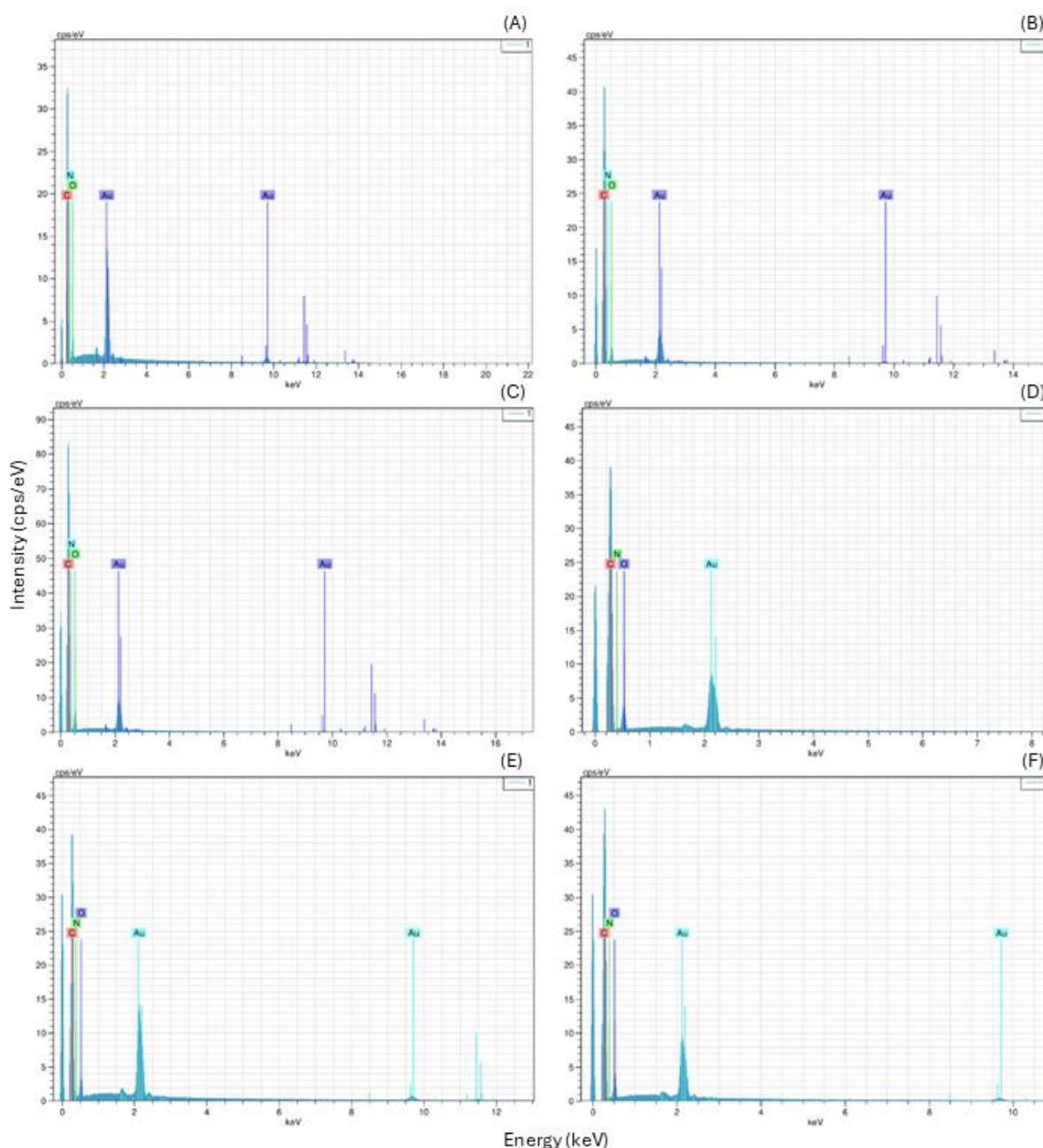


Figure 4. Representative EDX spectra of RS1 and RS2 epoxy resin samples before and after plasma treatments. (A) RS1 untreated, (B) RS2 untreated, (C) RS1 treated with O₂ plasma, (D) RS2 treated with O₂ plasma, (E) RS1 treated with O₂/CF₄ plasma, and (F) RS2 treated with O₂/CF₄ plasma. The spectra highlight plasma-induced changes in surface elemental composition, including increased oxygen after O₂ plasma treatment and the appearance of fluorine after O₂/CF₄ plasma exposure. Gold peaks originate from sputter coating. Spectra are shown as exported (x-axis limits may vary); comparisons focus on the low-energy C, N, O, and F peaks.

3.3.2. *FTIR Analysis after Plasma Treatment*

FTIR spectroscopy was employed to assess whether plasma treatments induced detectable changes in the bulk chemical structure of the epoxy resins. As expected for a surface-limited modification, the FTIR spectra of untreated and plasma-treated samples showed no significant differences in the characteristic absorption bands associated with the epoxy network, including the aromatic C=C stretching, ether C–O–C vibrations, and epoxy-related bands. The absence of measurable spectral changes confirms that plasma etching selectively modifies the outermost surface layers without affecting the bulk polymer matrix. Therefore, surface-sensitive techniques such as XPS and wettability measurements provide the primary evidence of plasma-induced chemical modification.

3.3.3. XPS Surface Chemical Analysis after Plasma Treatment

The surface chemical composition of RS1 and RS2 epoxy resins before and after plasma treatments, as determined by XPS, is summarized in Table 3.

Table 3. Surface chemical composition of RS1 and RS2 epoxy resins before and after plasma treatments determined by XPS (atomic %).

Sample	C–C / C–H (%)	C–O (%)	C=O + O–C=O (%)	O total (%)	F total (%)
RS1 untreated	75.1	3.2	7.0	10.2	1.8
RS2 untreated	71.3	7.2	6.6	11.6	—
RS1 O ₂ plasma	39.4	18.8	8.2	25.4	0.4
RS2 O ₂ plasma	56.9	13.0	5.9	19.3	—
RS1 O ₂ /CF ₄ plasma	38.1	23.9	5.7	23.4	1.7
RS2 O ₂ /CF ₄ plasma	66.4	9.2	6.8	14.1	0.9

Notes: In XPS analysis, different chemical bonds involving carbon produce signals at slightly different binding energies. In this table, carbon bonded to carbon or hydrogen (C–C/C–H) represents the hydrocarbon backbone of the epoxy resin. Signals assigned to C–O and C=O/O–C=O correspond to oxygen-containing functional groups introduced by plasma treatment. The total oxygen and fluorine contents were obtained by summing the respective O1s and F1s contributions.

Untreated RS1 and RS2 samples

The XPS spectra of untreated RS1 and RS2 samples reveal surfaces dominated by carbon, with oxygen and nitrogen present as minor components, consistent with the chemical structure of epoxy resin networks cured with nitrogen-containing hardeners.

For RS1 untreated, the C1s envelope is largely dominated by the C–C/C–H component (≈ 75 at.%), with smaller contributions from C–O (≈ 3.2 at.%), C=O (≈ 5.3 at.%), and O–C=O (≈ 1.7 at.%). The O1s signal is relatively low and mainly associated with oxidized carbon species, while nitrogen is detected primarily as C–N bonds (≈ 0.9 at.%), indicating limited surface oxidation in the pristine state.

RS2 untreated exhibits a similar chemical fingerprint but with a slightly higher contribution of oxygenated carbon species. The relative intensity of C–O groups

increases (≈ 7.2 at.%), while the C–C component decreases to ≈ 71 at.%. Nitrogen content is also higher (≈ 1.8 at.% as C–N), suggesting intrinsic differences in resin formulation or curing chemistry. These compositional differences are consistent with, and likely contribute to, the slightly higher wettability observed for RS2 prior to plasma treatment (Section 3.2).

Overall, both untreated surfaces are characterized by low surface oxidation and a predominance of hydrocarbon-like functionalities, resulting in limited surface polarity.

Effect of O₂ plasma treatment

Oxygen plasma treatment induces marked chemical modifications on both resin systems, as evidenced by substantial changes in the relative contributions of carbon and oxygen-containing functional groups.

In RS1 treated with O₂ plasma, the C–C/C–H contribution decreases sharply from ≈ 75 at.% to ≈ 39 at.%, while oxygen-containing functionalities increase significantly. In particular, the C–O component rises to ≈ 18.8 at.% and the O–C=O contribution to ≈ 6.4 at.%. This is accompanied by a strong increase in total oxygen, especially the higher-binding-energy O1s component associated with oxidized species (O1s III ≈ 18.3 at.%). Nitrogen content also increases (≈ 4.5 at.% as C–N), possibly due to increased surface exposure of nitrogen-containing groups after etching.

Similarly, RS2 O₂-treated samples show a pronounced reduction of the C–C component (to ≈ 56.9 at.%) and a concomitant increase in C–O (≈ 13.0 at.%) and O–C=O (≈ 4.4 at.%). The O1s signal intensifies, confirming effective surface oxidation. Compared to RS1, the extent of oxidation in RS2 appears slightly lower, suggesting a more moderate response to oxygen plasma, potentially related to differences in crosslink density or chemical composition.

These XPS results confirm that O₂ plasma effectively introduces polar oxygen-containing functional groups on both resin surfaces, in agreement with the strong decrease in contact angle reported in Section 3.2 and the increased surface roughness observed by SEM (Section 3.3.1).

Effect of O₂/CF₄ plasma treatment

Treatment with an O₂/CF₄ plasma results in a more complex surface chemistry, reflecting the competition between oxidative processes and fluorination.

For RS1 treated with O₂/CF₄ plasma, the C–C contribution remains low (≈38.1 at.%), while the C–O component reaches ≈23.9 at.%, indicating that oxidation still occurs. However, fluorine is clearly detected in multiple chemical environments (F–, C–F, and CF₂), with a total fluorine content exceeding that of the O₂-treated surface. The presence of C–F species (≈0.8 at.%) and fluorinated F1s components confirms successful fluorine incorporation at the surface.

RS2 treated with O₂/CF₄ plasma exhibits a similar trend, though with slightly lower overall oxidation compared to RS1. The C–C component remains relatively high (≈66.4 at.%), and oxygenated carbon species are present at levels comparable to the untreated surface. Fluorine is again detected, confirming surface fluorination, but its relative abundance is modest compared to RS1. This suggests that RS2 is less susceptible to fluorine grafting or that fluorinated species are more weakly bound.

The coexistence of oxygenated and fluorinated functionalities indicates that O₂/CF₄ plasma produces surfaces with intermediate polarity. This chemical balance is consistent with the moderate wettability observed after O₂/CF₄ treatment and supports the reduced microbial attachment and apparent mass change discussed in Sections 3.4 and 3.5.

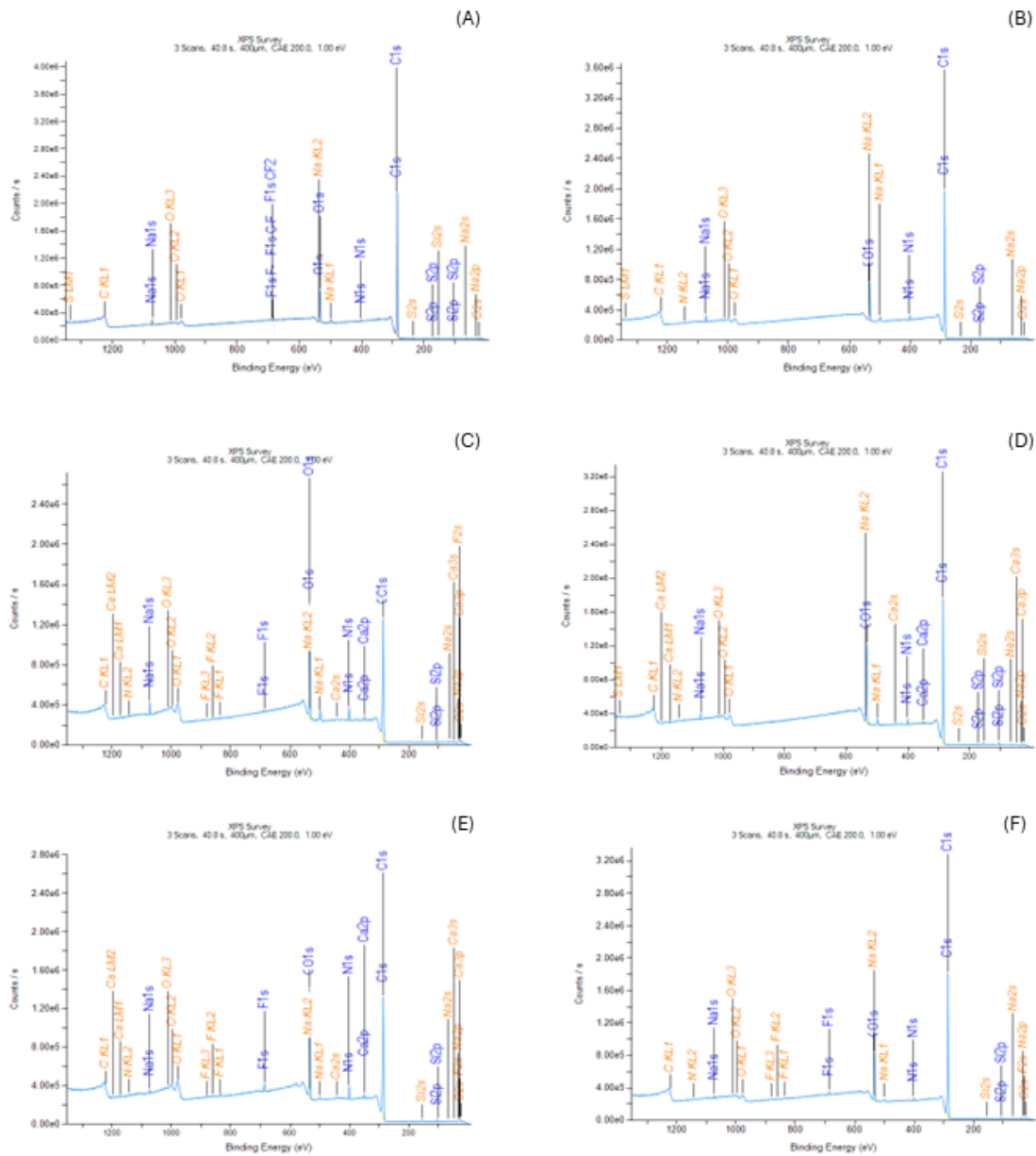


Figure 6. XPS survey spectra of RS1 and RS2 epoxy resin samples before and after plasma treatments. The spectra highlight changes in surface chemical composition induced by O_2 plasma and O_2/CF_4 plasma treatments, including increased oxygen contributions after O_2 plasma exposure and the presence of fluorine signals following O_2/CF_4 plasma treatment. Gold-related peaks arise from the conductive coating applied prior to analysis.

3.4. Microorganism Growth and Surface Colonization

The growth behavior of three microorganisms—*Rahnella* sp., *Bullera alba*, and *Bacillus tropicus*—was investigated to evaluate their ability to colonize plasma-treated epoxy resin surfaces and to assess the influence of surface chemistry on microbial proliferation. Four experimental conditions were considered, corresponding to RS1 and RS2 samples treated by O₂ plasma or O₂/CF₄ plasma. Microbial growth was monitored by quantifying colony-forming units (CFUs) over time, allowing a comparative analysis of microbial adaptation, persistence, and surface-dependent proliferation on plasma-treated surfaces (Figure 7).

The selected microorganisms represent different metabolic and structural characteristics, enabling a broader evaluation of surface–microorganism interactions. Differences in growth trends among treatments were therefore interpreted in relation to plasma-induced modifications of surface chemistry and wettability, as discussed in Sections 3.2 and 3.3.

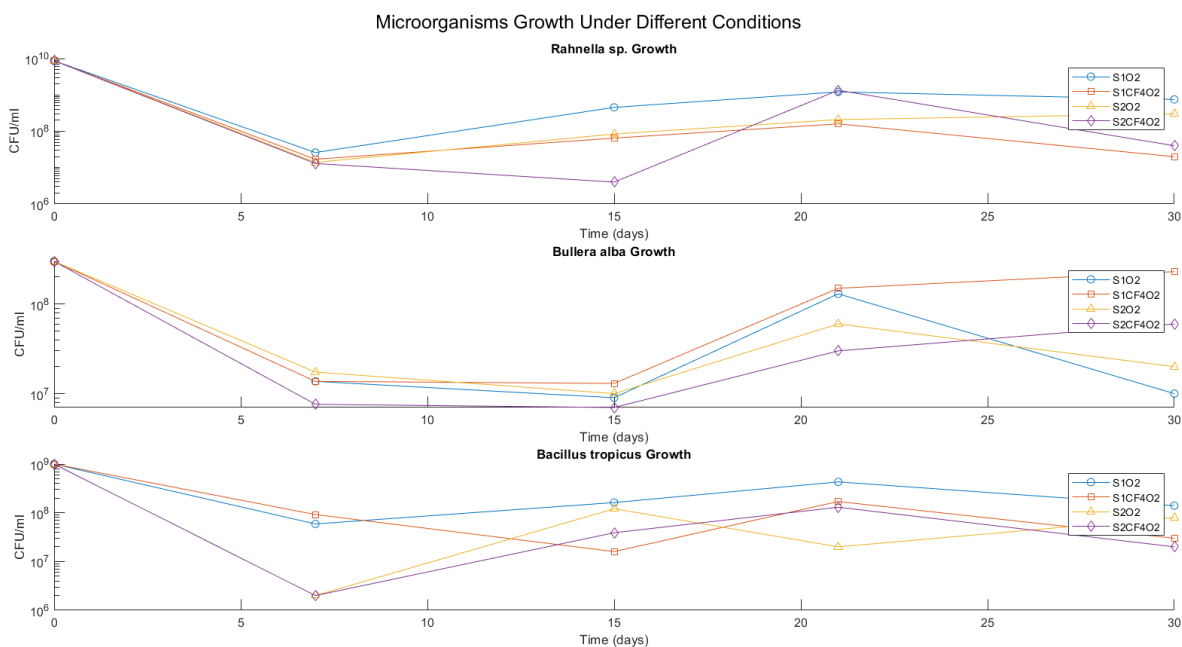


Figure 7. Growth curves of *Rahnella* sp., *Bullera alba*, and *Bacillus tropicus* on RS1 and RS2 epoxy resin samples treated with O₂ plasma and O₂/CF₄ plasma, expressed as CFU/mL over time. Data are shown on a logarithmic scale to highlight differences in microbial proliferation under different surface conditions.

3.4.1. RS1 - O₂ Plasma Treatment

On RS1 samples treated with O₂ plasma, all three microorganisms exhibited an initial adaptation phase followed by sustained growth, indicating that the oxidized and hydrophilic surface promoted microbial attachment and proliferation. *Rahnella* sp. showed the most pronounced response, reaching a clear growth maximum before a moderate decline at the final time point. This behavior is consistent with the strong affinity of *Rahnella* species for oxygen-rich and hydrophilic environments, which facilitate adhesion and metabolic activity.

Bullera alba displayed moderate growth on RS1–O₂ plasma-treated surfaces, characterized by an initial decrease followed by partial recovery. This trend suggests a slower adaptation to the substrate, potentially related to the yeast-like nature of the organism and its different surface interaction mechanisms compared to bacteria. *Bacillus tropicus* exhibited a more dynamic growth profile, with a marked increase during intermediate incubation times and a subsequent decline, possibly reflecting nutrient depletion or competition effects at later stages.

3.4.2. RS2 - O₂ Plasma Treatment

Microbial growth on RS2 samples treated with O₂ plasma differed from RS1, highlighting the role of resin formulation in governing surface–microorganism interactions. Although O₂ plasma treatment enhanced surface oxidation, the growth of *Rahnella* sp. on RS2 remained lower than that observed on RS1, indicating that intrinsic differences in resin chemistry or crosslink density may influence microbial accessibility and adhesion.

Bullera alba exhibited limited but sustained growth on RS2–O₂ plasma-treated surfaces, while *Bacillus tropicus* showed a variable growth pattern characterized by an initial increase followed by partial decline. These trends suggest that, while O₂ plasma treatment generally promotes microbial activity, the response is strongly dependent on the underlying polymer matrix.

3.4.3. RS1 - O₂/CF₄ Plasma Treatment

In contrast, RS1 samples treated with O₂/CF₄ plasma showed a more selective microbial response. *Rahnella* sp. exhibited reduced and less stable growth compared to the O₂ plasma condition, indicating that the presence of fluorinated functionalities may hinder sustained bacterial proliferation. The initial adaptation was followed by a transient increase and a sharp decline at later stages, suggesting limited long-term compatibility with the fluorinated surface.

Interestingly, *Bullera alba* demonstrated comparatively higher persistence under O₂/CF₄ plasma treatment, maintaining detectable growth throughout the incubation period. This behavior may be attributed to the ability of yeast-like microorganisms to tolerate or adapt to surfaces with lower surface energy and reduced polarity. *Bacillus tropicus* showed fluctuating growth under this condition, with a temporary increase followed by a pronounced decrease, indicating that fluorination may negatively affect stable bacterial attachment.

3.4.4. RS2 - O₂/CF₄ Plasma Treatment

The combination of RS2 resin chemistry with O₂/CF₄ plasma treatment resulted in the most heterogeneous microbial responses. *Rahnella* sp. displayed a pronounced but transient growth peak during intermediate incubation times, followed by a sharp decline, suggesting a short-term metabolic response rather than stable surface colonization. This behavior may reflect an initial utilization of accessible surface functionalities, followed by inhibition due to fluorinated surface regions.

Bullera alba showed limited growth under this condition, reaching only modest levels by the end of the incubation period. *Bacillus tropicus* exhibited moderate proliferation with a peak at intermediate times and subsequent decline, indicating partial compatibility with the surface but reduced long-term persistence.

3.5. Apparent Mass Change after Biodegradation

The influence of plasma-functionalized surface chemistry on the biodegradation behavior of epoxy resin samples was assessed by measuring the change in dry sample mass after 30 days of microbial exposure. For each resin system and treatment condition, the apparent mass change (ΔW) was calculated as the difference between the final dry mass (W_{t30}) and the initial dry mass (W_{t0}). Contrary to an absolute mass loss, all conditions exhibited a small positive mass change, indicating an apparent increase in sample weight following incubation and standardized rinsing and drying procedures (Figure 8).

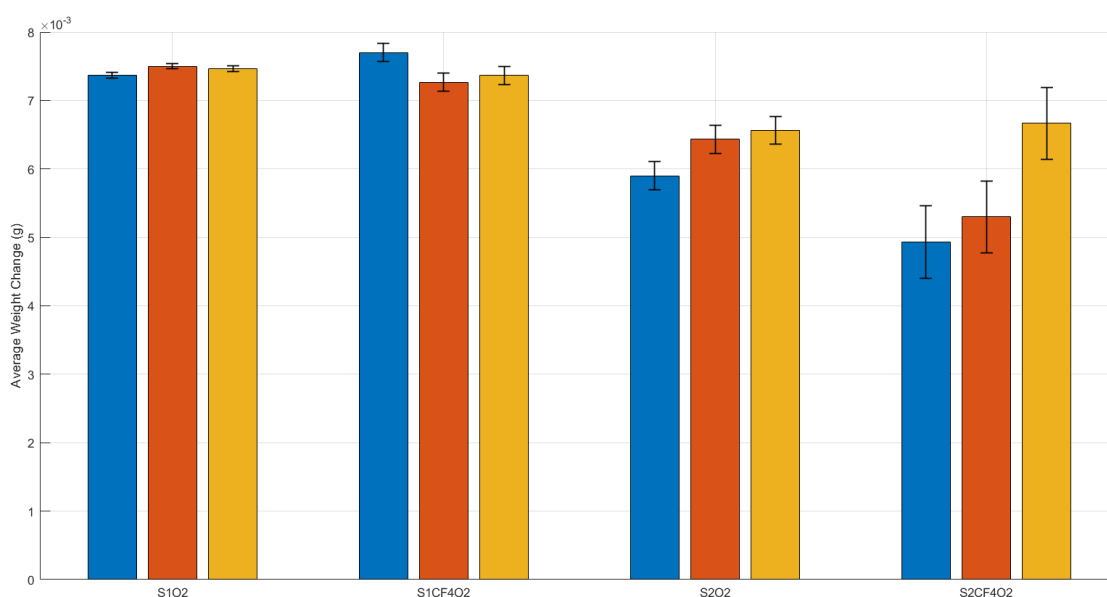


Figure 8. Apparent mass change ($\Delta W = W_{t30} - W_{t0}$) of RS1 and RS2 epoxy resin samples after 30 days of exposure to *Rahnella sp.*, *Bullera alba*, and *Bacillus tropicus* under different plasma treatment conditions. Values are reported as mean $\pm$ SD ($n = 3$).

This apparent mass gain likely arises at least in part from a combination of factors associated with microbial colonization and surface chemistry. Plasma treatment introduces polar oxygen-containing functional groups (e.g., hydroxyl, carbonyl) at the polymer surface, increasing surface energy and hydrophilicity, as widely reported in plasma surface functionalization of polymers [9]. Such effects have been shown to significantly enhance wettability through incorporation of polar groups and alteration of surface free energy. Enhanced wettability favors water adsorption and intimate interface contact between microorganisms, extracellular polymeric substances (EPS), and the resin surface. The presence of residual moisture, tightly bound water, and microbial byproducts on or within the surface can contribute to measured mass increases, even after rinsing [1].

In addition, the formation of microbial biofilms and EPS, which can penetrate surface irregularities and remain attached after conventional rinsing, could contribute to elevated apparent mass. Biofilm formation and the accumulation of extracellular polymeric substances (EPS) on solid substrates are well documented and can contribute to measurable increases in apparent mass after biological exposure [1]. While rigorous desiccation until constant mass was not performed, the observed trends are consistent with comparative rather than absolute differences in biodegradation-related interactions.

Consistent with these considerations, RS1 samples treated with O_2 plasma generally exhibited the largest apparent mass changes, suggesting more extensive microbial engagement with the highly oxidized and hydrophilic surfaces. In contrast, samples treated with O_2/CF_4 plasma showed smaller mass changes, indicating reduced microbial attachment and/or water adsorption, in line with the presence of fluorinated functionalities that tend to lower surface energy and partially inhibit hydrophilic interactions [22].

Differences between the two resin systems were also evident. RS1 displayed greater apparent mass change under comparable treatments than RS2, which may be related to inherent differences in resin chemistry affecting surface reactivity and microbial affinity. Across treatments, these comparative patterns align with observations from

surface characterization (Sections 3.2–3.3), where increased surface oxidation corresponded to enhanced wettability and microbial adhesion.

The relatively low standard deviations confirm the reproducibility of the measurements across triplicates, reinforcing that the observed mass changes, though small, reflect systematic differences in surface–microorganism interactions rather than random variation. Taken together, these results indicate that plasma-induced surface functionalization modulates the degree of microbial colonization and associated apparent mass change, and that the apparent mass gain observed here can serve as a comparative indicator of biodegradation-related processes rather than absolute material loss within the investigated timeframe.

3.6. Surface Morphology after Biodegradation (SEM/EDX)

Based on the results of the biodegradation experiments and the apparent mass change analysis (Section 3.5), a subset of samples was selected for post-biodegradation surface characterization by SEM coupled with EDX. Specifically, four microorganism–surface combinations exhibiting the most pronounced mass changes were chosen for further investigation, as these conditions were considered the most representative of effective surface–microorganism interactions.

The microorganisms selected for this analysis—*Rahnella* sp., *Bullera alba*, and *Bacillus tropicus*—were identified as those inducing the highest apparent mass variations under specific plasma treatment conditions. This targeted selection allowed a focused evaluation of surface morphological and compositional changes associated with enhanced microbial colonization, while avoiding redundant analysis of conditions showing minimal interaction.

SEM observations (Figure 9) reveal clear differences between plasma-treated surfaces before and after biodegradation. In contrast to the relatively uniform morphologies observed prior to microbial exposure (Section 3.3), post-biodegradation surfaces display heterogeneous features, including irregular deposits, localized agglomerates, and surface-adhered layers. These features are consistent with the presence of microbial residues, extracellular polymeric substances (EPS), and degradation-related byproducts, rather than bulk erosion of the epoxy matrix. The extent and distribution of these features vary depending on both the plasma treatment and the microorganism involved, indicating a strong dependence on surface chemistry and microbial metabolism.

EDX spectra acquired from the same regions (Figure 10) provide complementary information on surface elemental composition following biodegradation. Compared to pre-degradation analyses, post-biodegradation spectra show increased variability and the presence of elements not intrinsic to the epoxy resin, such as potassium, calcium, chlorine, and sodium. These elements are commonly associated with microbial cells, culture media residues, and EPS, and their detection supports the SEM evidence of

surface-associated biological material. The persistence of strong carbon and oxygen signals confirms that the epoxy matrix remains the dominant substrate, while the absence of major compositional shifts indicates that biodegradation primarily affects the surface interface rather than the bulk polymer.

Notably, samples previously treated with O₂ plasma generally exhibit more extensive surface coverage by biological residues compared to those treated with O₂/CF₄ plasma. This observation aligns with earlier findings on surface wettability and chemical composition (Sections 3.2 and 3.3), where oxygen plasma treatment produced highly hydrophilic, oxidized surfaces that favor microbial adhesion. Instead, O₂/CF₄-treated surfaces display more localized and discontinuous deposits, consistent with the reduced microbial attachment and lower apparent mass changes observed under these conditions.

Overall, the combined SEM/EDX analysis after biodegradation confirms that plasma-induced surface functionalization strongly influences microbial colonization behavior. The observed surface features and elemental signatures support the interpretation that the apparent mass changes measured after biodegradation arise predominantly from surface-associated biological material and retained moisture, rather than from significant polymer matrix degradation. These observations confirm that surface chemistry plays a key role in microorganism–polymer interactions and help relate plasma treatment to the observed microbial growth patterns and apparent mass changes.

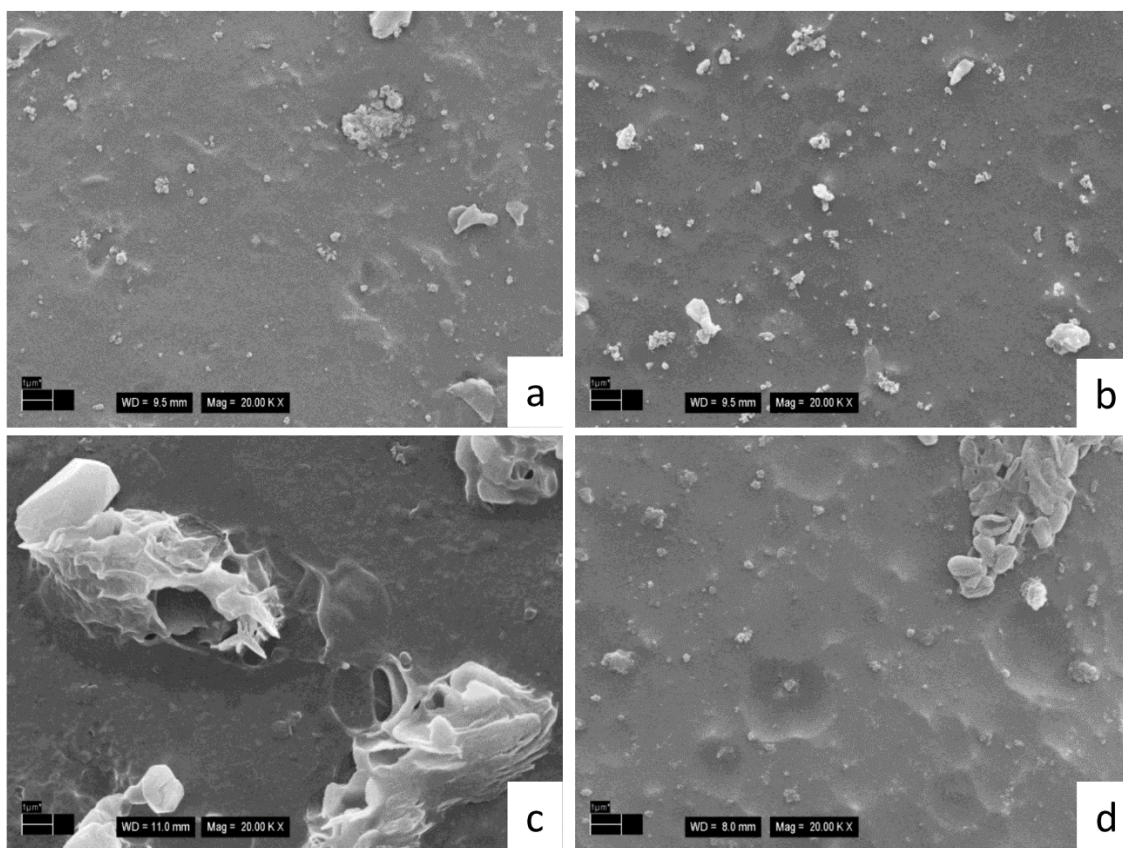


Figure 9. SEM micrographs of RS2 epoxy resin surfaces after biodegradation under different plasma treatment and microorganism conditions. (A) RS2 treated with O₂ plasma after biodegradation with *Rahnella* sp.; (B) RS2 treated with O₂/CF₄ plasma after biodegradation with *Rahnella* sp.; (C) RS2 treated with O₂ plasma after biodegradation with *Bullera alba*; (D) RS2 treated with O₂/CF₄ plasma after biodegradation with *Bacillus tropicus*. The images highlight microorganism- and treatment-dependent surface features following biological exposure.

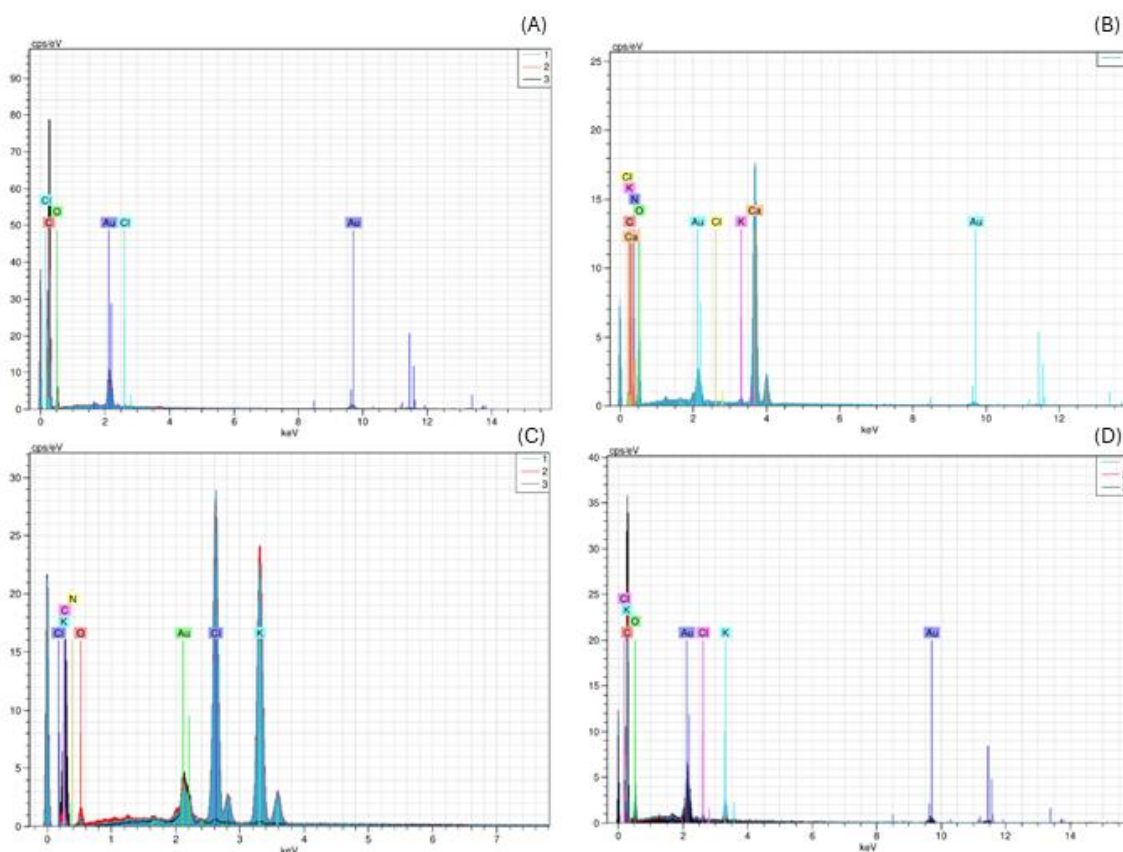


Figure 10. Representative EDX spectra of RS2 epoxy resin surfaces after biodegradation under different plasma treatment and microorganism conditions. (A) RS2 treated with O_2 plasma after biodegradation with *Rahnella* sp.; (B) RS2 treated with O_2/CF_4 plasma after biodegradation with *Rahnella* sp.; (C) RS2 treated with O_2 plasma after biodegradation with *Bullera alba*; (D) RS2 treated with O_2/CF_4 plasma after biodegradation with *Bacillus tropicus*. The spectra provide qualitative information on elemental surface composition following microbial exposure, supporting the SEM observations of surface-associated residues and deposits.

4. Conclusions

This chapter investigated the effects of plasma surface functionalization on epoxy resin systems and their subsequent interaction with microorganisms under biodegradation conditions. The combined use of surface-sensitive techniques (contact angle measurements, SEM, EDX, and XPS) enabled a comprehensive assessment of the chemical, morphological, and biological responses induced by O_2 and O_2/CF_4 plasma treatments.

Plasma etching effectively modified the outermost surface layers of both resin systems without altering their bulk chemical structure, as confirmed by FTIR analysis. Oxygen plasma treatment led to a pronounced increase in surface oxidation, with a significant incorporation of polar oxygen-containing functional groups and a corresponding enhancement in surface wettability. In contrast, O_2/CF_4 plasma treatment resulted in mixed surface chemistries, combining oxidation with fluorination and producing surfaces with intermediate polarity.

These plasma-induced surface modifications strongly influenced microbial behavior. Increased hydrophilicity and surface oxidation promoted microbial adhesion and proliferation, particularly on O_2 plasma-treated samples, while fluorinated surfaces exhibited reduced and less stable microbial colonization. The apparent mass gain observed after biodegradation was attributed to surface-associated phenomena, including water adsorption, biofilm formation, and retention of extracellular polymeric substances, rather than bulk material degradation.

Post-biodegradation SEM and EDX analyses further confirmed the presence of organic and inorganic residues on the resin surfaces, supporting the interpretation of surface-mediated interactions rather than extensive polymer breakdown. Overall, the results demonstrate that plasma surface functionalization is an effective strategy for tuning epoxy resin surface chemistry and controlling microorganism–surface interactions, providing a foundation for the design of polymer systems with tailored biodegradation or biofouling behavior.

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4. General Conclusions and Outlook

This thesis investigated the effects of plasma surface functionalization on epoxy resin systems and their interaction with microorganisms under biodegradation conditions. By combining surface modification techniques with physicochemical and biological characterization, this work provides insight into how tailored surface chemistry influences wettability, microbial colonization, and apparent biodegradation-related phenomena.

O₂ plasma treatment was shown to be highly effective in introducing polar oxygen-containing functional groups at the resin surface, leading to a substantial increase in surface hydrophilicity and enhanced microbial adhesion. In contrast, O₂/CF₄ plasma treatment produced surfaces with mixed chemical functionalities, where fluorinated groups partially counterbalanced oxidation effects, resulting in intermediate wettability and reduced microbial persistence.

Surface-sensitive analytical techniques, particularly XPS and SEM/EDX, proved essential for capturing plasma-induced modifications and post-biodegradation surface changes, while FTIR confirmed that these treatments remained confined to the surface without affecting the bulk polymer matrix. Microbial growth experiments demonstrated that surface chemistry plays a decisive role in governing microbial adaptation and colonization dynamics. The apparent mass increase observed after biodegradation was attributed to surface-associated processes such as water retention and biofilm formation rather than true polymer mass loss.

Overall, this work highlights plasma surface functionalization as a versatile and controllable approach to modulate epoxy resin–microorganism interactions. The findings contribute to a deeper understanding of surface-driven biodegradation mechanisms and may inform the design of advanced polymer materials for applications where microbial interaction, environmental exposure, or surface performance are critical considerations.