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Biodegradability of Polyethylene Plastics by Individual and Co-cultured Bacterial Isolates

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Abstract

The extensive dependence on polyethylene-based products has contributed to their persistence in the environment, accounting for about 64% of global plastic waste and posing serious ecological and public health risks. Conventional disposal methods such as incineration and landfilling further intensify pollution, emphasizing the need for eco-friendly alternatives. This study explores the biodegradation of polyethylene plastics using bacteria isolates obtained from soil samples collected across three sites in Kumasi, Ghana. Degradation efficiency was assessed through weight loss analysis and Fourier Transform Infrared Spectroscopy (FTIR) to determine changes in the polymer functional groups. Response Surface Methodology (RSM) was employed to evaluate the effect of pH, temperature, and incubation time on bacterial growth and degradation process. A total of eleven bacterial isolates were identified using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF), with *Bacillus cereus* being the most prevalent. Optimization analysis revealed that temperature, pH and incubation time significantly influenced degradation, with optimal conditions predicted at 25°C, pH 9- and 21-days incubation respectively. Co-cultures demonstrated higher degradation efficiency than single isolates, supported by elevated laccase activity indicating synergistic enzymatic interactions. FTIR analysis showed polymer modification, showing changes in asymmetric and symmetric C-H stretching peaks in all treatments except *Bacillus cereus.* In conclusion, the results demonstrate that co-cultured microbes enhance polyethylene degradation through metabolic cooperation and increased enzyme activity, highlighting the potential of microbial synergy as a sustainable strategy for plastic waste management.

Keywords: Polyethylene, Biodegradation, Microbial consortia, Oxidative enzymes, Plastics

1.0 Introduction

Polyethylene, which consists of alkene monomers that are made up of carbon and hydrogen atoms, is the world's most widely produced synthetic polymer [1]. On its own, it accounts for roughly one-third of overall plastics produced [2]. The high dependence on polyethylene-based products has contributed to its accumulation in the environment. It accounts for about 64% of plastic waste globally, significantly adding to environmental pollution and ecological harm [3,4]The defiant nature of polyethylene, with its long carbon chain and hydrophobic nature, contributes to its persistence in the environment, as it makes it difficult to manage using traditional waste disposal methods [5].

Managing plastic waste remains a major challenge in many African countries and this can be credited to rapid population growth, poor waste management practices, limited waste disposal infrastructure, and weak enforcement. In Ghana, plastic consumption has increased significantly, leading to the production of around 840,000 tonnes of plastic waste yearly. Despite bans on single-use plastics, only 25% of Ghana's plastic is reused. The majority are landfilled (58%) or incinerated (17%) [6,7]. Common plastic waste disposal methods, such as incineration and landfilling, pose serious drawbacks, including toxic emissions, hazardous by-products, and contamination of land and water sources. These drawbacks greatly affect the environment and pose various public health concerns. These issues highlight just how important it is to find sustainable, effective, and environmentally friendly methods to deal with the increasing plastic waste problem [8–11]
Biodegradation, which involves the use of microorganisms and their enzymes, has emerged as a promising and more eco-friendly approach to plastic waste management [12–14]. Plastic biodegradation is a process in which microorganisms alter the properties of plastics by breaking down their molecular structure, reducing molecular weight, and modifying physical characteristics such as shape, colour, and tensile strength. This occurs through enzymatic and non-enzymatic hydrolysis, primarily carried out by bacteria and fungi [15]. Since microorganisms exhibit diverse metabolic capabilities, degradation efficiency varies among species, with polymer composition, microbial traits, and treatment conditions serving as the key influencing factors [16].

Although biodegradation is a promising solution to polyethylene pollution, using a microbial consortium is more advantageous than monocultures.[17] Microbial consortia offer greater metabolic diversity and a wider range of enzyme allowing them to effectively degrade polyethylene as compared to the dependence on monocultures, which raises the concern of metabolic limitations and enzymatic restrictions [18,19] . Despite studies having shown that microorganisms and their enzymes can be used in polymer degradation, there is limited research on the biodegradation of polyethylene by microbial consortia under Ghanaian environmental conditions. This study aims to address this gap by comparing polyethylene degrading capabilities of individual and co-cultured bacterial isolates derived

from Ghana's environment. By exploring the interactions among the microorganisms and their related enzymes, the findings from this study will add to valuable, localized data and will inform the best strategy for handling polyethylene within the Ghanaian community.

2. Methods

2.1 Sample Collection

Nine soil samples were collected in triplicate from three actively used dumpsites in the Ashanti region of Ghana: Pramso, Jachie, and Manhyia. Surface soil and soil at a depth of 10cm beneath identified polyethylene bags were aseptically collected into sterile Ziplock bags. The selection criteria for dumpsites were based on several key factors, specifically locations with prolonged accumulation of polyethylene waste, dumpsites that are actively in use, and dumpsites that have not been recorded in previous studies.

2.2 Primary Screening and Isolation of Polyethylene-Degrading Bacteria

The isolation was modified from previous methods [20,21] to meet the objectives of this investigation. To obtain polyethylene-degrading bacteria, 5 g of soil collected from each waste dumpsite was weighed into basal media, consisting of yeast extract (Thermo Fisher Scientific Oxoid, USA) (0.05 g), $MgSO_4$ (0.125 g), KH_2PO_4 (2.9 g), and K_2HPO_4 (1.85 g), in a total of 50 ml for each sample. To each sample, 30 mg of shredded polyethylene plastic was added to serve as the carbon source. Incubation was done at 37°C for eight weeks to impose selective pressure that enriches bacterial strains capable of utilizing polyethylene, thereby facilitating effective isolation of polyethylene-degrading bacteria. The bacteria obtained from this inoculation process were first cultured through pour plating on nutrient agar (Thermo Fisher Scientific Oxoid, USA) and incubated at 37°C for 24 hours. Colonies of microorganisms obtained from this step were streaked on nutrient agar plates.

2.3 Optimization of Degradation Conditions

A full factorial design under the Response surface Methodology (RSM) framework was employed to systematically evaluated the influence of pH, temperature, and incubated time on bacterial growth and polyethylene degradation efficiency [22]. The parameter ranges tested were as follows: pH levels of 5,7, and 9; temperatures of 25 °C, 35°C, and 45°C; and incubation times of 7, 14,21 and 28days. The selected ranges facilitate the assessment of diverse microbial responses to varying conditions, enabling identification of conditions under which bacteria demonstrate optimal efficiency in degrading polyethylene. Prior to identification, isolates obtained from the pre-screening process were treated as "working strains" and used to optimize culture conditions. Each isolate was initially evaluated individually, after which pairwise combinations were systematically generated to produce all possible mixed culture consortia based on the total number of isolates. Both individual isolates and mixed cultures were assessed for degradation potential across the defined process viable. All experimental setups were conducted in replicates to ensure the reliability and reproducibility of the results. Each experimental

setup consisted of 20ml of nutrient broth adjusted to the desired pH, containing pre-weighed polyethylene pieces. The polyethylene materials used in this study were locally sourced and manufactured by Duraplast LTD. The plastic samples used were low density polyethylene (LDPE) and they were obtained in sealed packaging and handled aseptically prior to use. The polyethylene pieces together with the nutrient broth were sterilized by autoclaving before beginning the inoculation process. Each flask was then inoculated with bacterial isolates and incubated at the corresponding temperature in a shaker at 120rpm for the specified incubation periods. After incubation, the polyethylene pieces were carefully retrieved, rinsed with distilled water followed by 70% ethanol to remove adhering biomass, and dried at 90 °C for 60 minutes. The dried films were reweighed, and percentage degradation was calculated. Bacterial growth and weight loss data for each treatment were recorded and analyzed to determine the individual and interaction effects of the selected parameters on polyethylene degradation.

2.4 Degradation assay

Polyethylene biodegradation by bacterial isolates was evaluated in nutrient broth under controlled incubation conditions. Nutrient broth was used to maintain viable bacterial growth during incubation, while degradation potential was assessed through weight loss measurements and FTIR analysis of polyethylene films. The degradation experiment was conducted using both monocultures and mixed culture consortia. Mixed cultures were designed to include all possible combinations of the selected isolates. Isolates recovered only once across the sampling were considered unique representatives of their type and were included directly in the consortia design. Where the same isolate was recovered multiple times, the single best-performing representative determined by polyethylene degradation efficiency during the optimization phase was selected for inclusion in the mixed cultures.

Sterilized, pre-weighed low-density polyethylene (LDPE) samples (locally produced by Duraplast Ltd, Kumasi, Ghana and obtained from a local market) were aseptically introduced into 20ml of autoclaved nutrient broth. Bacterial isolates obtained from the pre-screening stage (Section2.2) were cultured for 12 hours at 25°C with agitation at 120 rpm. Following incubation, cell suspension was standardized to $OD_{600} \approx 0.5$ before being used for the inoculation. After inoculation, the experimental setup was incubated for 28days at 25°C under alkaline conditions(pH9) with continuous agitation at 120 rpm. The procedure was adapted from ([23,24]). After the incubation period, polyethylene samples were removed from each flask and rinsed with distilled water and ethanol (70%) and dried at 90°C for 30 minutes (Asmita, Shubhamsingh, and Tejashree, 2015). The percentage weight loss was calculated by following the formula

Percentage (%) degradation = $\frac{(Initial\ weight - Final\ weight)}{Initial\ weight} \times 100$

2.5 Identification of Bacterial Isolates

Pure isolates of polyethylene degradation bacteria obtained from (section 2.2) were identified using Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) at the Noguchi Memorial Institute for Medical Research, Accra, Ghana. Before identification, all isolates were purified. Identification was performed using the extended direct transfer sample preparation method on the Microflex LT MALDI-TOF mass spectrometer (Bruker, Germany) following the standard operating procedure outlined by the institute. Representative colonies of the polyethylene-degrading isolates were smeared thinly onto designated wells of the MALDI target plate. Each spot was overlaid with 1µL of 70% (v/v) formic acid and allowed to dry at room temperature, followed by the addition of 1 µL Bruker HCCA solution (10mg/mL). The matrix overlaid sample spot was allowed to dry at room temperature. Using the MALDI-TOF equipment for Biotyping of microorganisms, the resulting spectra were compared with reference spectra using the Bruker MALDI Biotyper Compass (MBT) software (v 4.1.100) for microbial identification.

2.6 **Partial Purification Process**

The cell-free supernatant (crude enzyme extract) obtained after centrifugation of the bacterial culture was subjected to partial purification by cold acetone precipitation. Pre-chilled acetone (-20°C) was added to the supernatant at a ratio of 4:1 (v/v, acetone: supernatant). The mixture was incubated at -20 °C for 1 hour to allow proteins to precipitate, followed by centrifugation at 5,000 rpm for 10 mins at 4°C. The supernatant was discarded, and the resulting pellet was air-dried at room temperature for 30 min to remove residual acetone [25]. The precipitate was then resuspended in 0.1M sodium acetate buffer (pH 5.0) for further analysis.

2.6.1 Protein Concentration Assay

The levels of enzyme produced after the degradation process were determined by the Bradford assay following the manufacturer's protocol [26]. A 1.0 mg/ml bovine serum albumin (BSA) stock was prepared and serially diluted twofold across eight concentrations in a 96-well microtiter plate, with each standard prepared in a 10 µL volume. Purified enzyme samples (10 µL) were loaded in triplicate on the same plate. To each well, 200 µL of Bradford reagent was added and mixed thoroughly by pipetting. Plates were incubated at room temperature in the dark for 5–10 min, and absorbance was read at 595 nm using a microplate reader. Sample protein concentrations were calculated from the BSA standard curve by linear regression ($Y = mX + c$), where Y is absorbance at 595 nm, X is protein concentration (mg/ml), m is the slope, and c is the intercept.

2.6.2 Laccase Activity Assay Protocol

The enzymatic reaction mixture (200 µL) consisted of 80 µL 0.5mM ABTS solution, 20 µL of 0.1 M sodium acetate buffer (pH 5.0), and 100 µL of crude enzyme extract. The mixtures were incubated for 15 minutes at room temperature, and absorbance was measured at 420nm. Non-enzymatic blanks were subtracted from the enzymatic readings.

Laccase activity was calculated from the standard curve and expressed as U/mL. One unit (U) of laccase activity was defined as the amount of enzyme that oxidizes 1 µmol of ABTS per minute under the assay conditions. Protein concentration was determined by the Bradford method using bovine serum albumin (BSA) as the standard (section 2.6.1). Specific laccase activity (U/mg protein) was calculated by dividing the volumetric laccase activity (U/mL) by the protein concentration (mg/mL) [27,28].

2.7 Fourier Transform Infrared Spectroscopy

Polyethylene samples were analyzed using a BRUKER ALPHA Fourier Transform Infrared (FTIR) spectrometer (Germany) equipped with an Attenuated Total Reflectance (ATR) accessory featuring a platinum crystal on a BRUKER instrument. Analyses were conducted at the Central Laboratory, Kwame Nkrumah University of Science and Technology, following the standard operating procedures. Before each run, the ATR crystal was cleaned with isopropanol, and a background spectrum was recorded. Spectra were collected using 24 scans per sample over a wavenumber range of 4000-500cm^{-1} at a resolution of 4cm^{-1}.

3.0 Results

Polyethylene-degrading bacterial isolates were successfully obtained and identified, serving as the basis for subsequent comparative analyses. Each isolate's degradation efficiency and enzyme production were assessed in both monoculture and co-culture. Results revealed differences in polyethylene degradation performances and enzymatic activity across single strains and combined consortia, suggesting that co- cultivation may enhance polymer breakdown.

3.1 Optimization of Degradation Parameters

A second-order Response Surface Methodology model was fitted to evaluate the effect of temperature, pH, and incubation time on microbial growth (OD change). The model was statistically ($F=17.08$, $p < 0.001$) with moderate explanatory power ($R^2 = 0.3487$, adjusted $R^2 = 0.3283$). However, only linear terms contributed significantly to the model ($F = 49.03$, $p < 0.001$), while interaction ($p=0.192$) and quadratic terms ($p= 0.612$) were not significant. The lack-of-fit test was significant ($F = 2.85$, $p < 0.001$), indicating that the second-order model did not adequately describe the system. A simplified first-order model was therefore adopted and found to be highly significant ($F= 48.93$, $p < 0.001$), explaining

33.38% of the variability in Od change (adjusted $R^2 = 0.327$). All three factors exhibited statistically significant effects on microbial growth. Temperature showed a significant negative effect ($\beta = -0.0185$, $p < 0.001$), indicating that increasing temperature reduced growth. In contrast, both pH ($\beta = 0.0310$, $p = 0.018$) and incubation time ($\beta = 0.0358$, $p < 0.001$) had significant positive effects, with incubation time exerting the strongest influence. Steepest ascent analysis further indicated that optimal growth conditions could be approached by decreasing temperature while increasing pH and incubation time.

However, to Change in Optical density (Odchange), the response variable weight change exhibited limited sensitivity to the studied process variables. The second-order model was not statistically significant ($F= 1.226$, $p=0.278$) with neither linear, interaction, nor quadratic terms showing significance ($p > 0.05$). A centered interaction model produced similar results ($F=1.425$, $p=0.205$, $R^2 = 0.0286$), confirming the weak dependence of weight change on temperature, pH, and incubation time. Notably, a single interaction between pH and interaction time was statistically significant ($\beta = 0.00547$, $p = 0.0198$). This points to a possible combined effect of pH and time at specific conditions.

The predicted optimal conditions for polyethylene biodegradation based on multiple response analysis are presented in Table 1. The model suggests that biodegradation is favored under moderately alkaline conditions, relatively low temperature, and intermediate incubation time.

Table 1: Predicted Optimal Conditions for Polyethylene Biodegradation Based on Multiple Response Analysis.

Variable	Predicted optimal conditions
Temperature (°C)	25
pH	9
Incubation (days)	21

The contour plot (Fig.1) illustrates the combined effects of pH and temperature on cell growth, measured as Optical density (OD_{600}). The model indicates that higher OD_{600} values are associated with moderately alkaline conditions and lower temperatures. The highest response was observed at approximately pH 9 and 25°C.

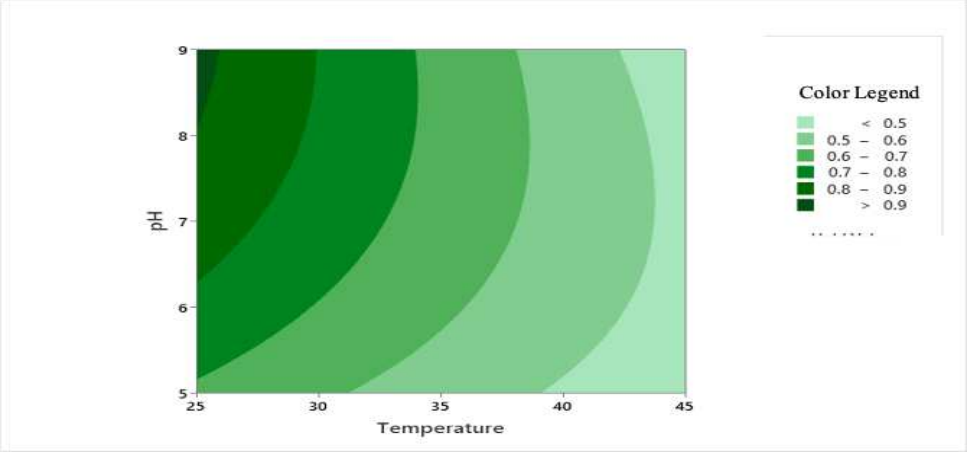


Fig.1 Response surface contour plot showing the combined influence of temperature (25–45°C) and pH (5–9) on bacterial growth, with colour intensity representing growth response values ranging from 0.5 to 0.9.

The effects of temperature and pH on polyethylene weight loss (mg) are presented in Fig.2. The contour plot indicates that weight loss was greatest at higher pH (approximately pH 9) and moderate temperatures around (around 25 °C). In contrast, weight loss remains low at pH values below 6.5 across all temperatures. At elevated temperatures (> 35°C), increases in pH were associated with only marginal increases in weight loss.

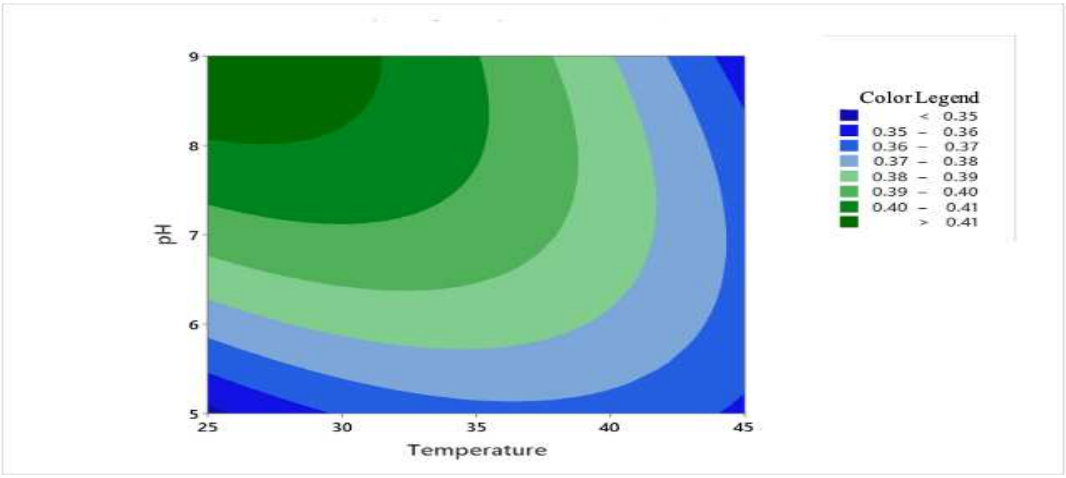


Fig.2 Response surface contour plot illustrating the combined effects of pH and temperature on predicted LDPE weight loss(mg) based on fitted RSM model.

3.2 Identification of Polyethylene Degrading Bacteria Isolates

Bacterial isolates obtained from the pre-screening process were identified using MALDI-TOF mass spectrometry. All isolates yielded identification scores above 2.0, indicating reliable species level identification. Most isolates were identified as *Bacillus cereus*, while *Achromobacter insolitus* and *Alcaligenes faecalis* were also detected (Table 2).

The isolates originated from three sampling sites: JS1-JS3 from Jachie, PS1-PS3 from Pramso, and MS1- MS3i from Manhyia.

Table 2: Identification of bacterial isolates using MALDI-TOF Mass Spectrometry.

Sample ID	Organisms	MALDI-TOF Score value
JS1	*Bacillus cereus*	2.04
JS2	*Bacillus cereus*	2.20
JS3	*Bacillus cereus*	2.17
PS1	*Bacillus cereus*	2.30
PS2	*Bacillus cereus*	2.21
PS3	*Bacillus cereus*	2.17
MS1	*Bacillus cereus*	2.16
MS2	*Bacillus cereus*	2.15
MS2i	*Achromobacter insolitus*	2.37
MS3	*Alcaligenes faecalis*	2.15
MS3i	*Bacillus cereus*	2.31

Key: Score of ≥ 2.0 indicates High confidence identification to the species level, 1.70 - 1.99
(Low confidence identification), and 0.00 - 1.69 (No organism identification possible).

3.3 Polyethylene Degradation Assay

Plastic weight measurements taken before and after treatment showed reductions across all experimental groups, though the magnitude of reduction varied by treatment. Mean initial weights ranged from 4.80 mg to 6.00 mg, while mean final weights ranged from 4.75 mg to 5.87 mg. The control group exhibited the smallest mean reduction (0.05 mg), corresponding to a mean percentage weight loss of 1.09%. Among the treatment groups, the greatest mean weight loss was observed in the BC-AI group (8.04%), followed by AI (5.66%), AI-AF (5.18%), and BC-AI-AF (4.75%). Lower mean losses were observed in AF (2.27%), BC (2.36%), and BC-AF (2.86%)

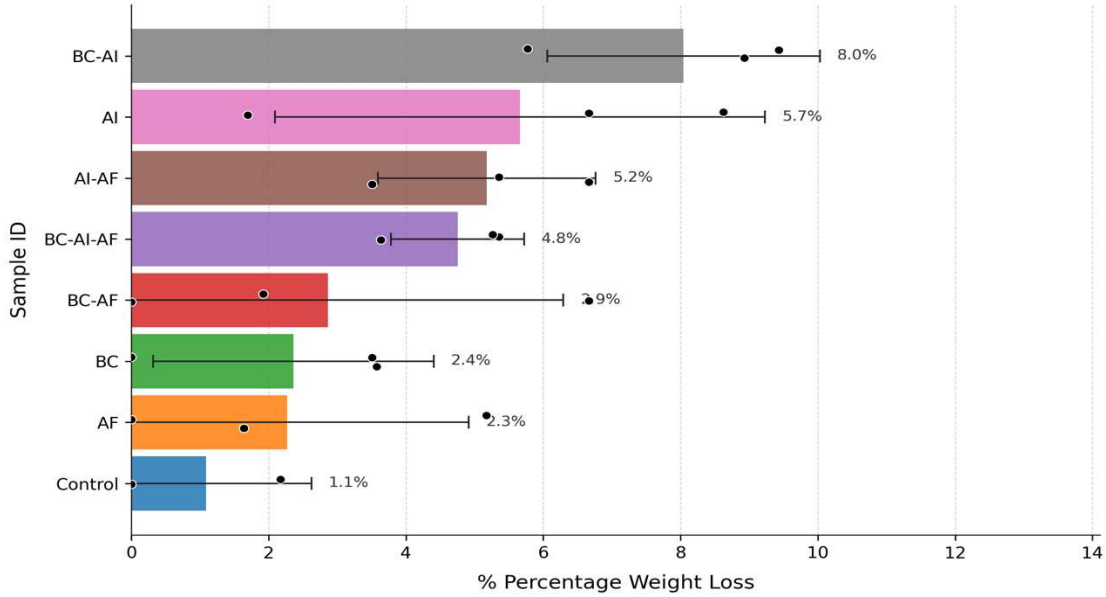


Fig.3 Evaluation of polyethylene biodegradation via percentage weight loss following incubation.

To determine whether weight reductions were statistically meaningful, a Wilcoxon signed-rank test was performed comparing initial and final weights for each treatment. No group demonstrated a statistically significant within-group reduction at the $\alpha = 0.05$ level. Specifically, p-values were 0.1855 for AF, BC, and BC-AF; 0.1225 for AI and AI-AF; 0.0868 for BC-AI and BC-AI-AF; and 0.5000 for the control group. Furthermore, to compare weight loss among groups, a Kruskal–Wallis rank-sum test was employed, which revealed no statistically significant differences across the treatments ($\chi^2 = 6.6051$, df = 7, $p = 0.4771$). As a result, under the current experimental conditions, the observed weight changes cannot be confidently linked to treatment-specific effects.

3.4. Laccase Enzyme Activity

Beyond weight-loss measurements, the biochemical potential of the isolates to degrade polyethylene was assessed by measuring specific laccase activity following the 28-day incubation period (Fig.4). The BC-AI consortium exhibited the highest specific laccase activity (6.62 ± 0.12 U/mg protein), followed by the AI monoculture (5.65 ± 0.21 U/mg protein). In contrast, the BC-AF consortium showed the lowest specific activity (1.74 ± 0.38 U/mg protein).

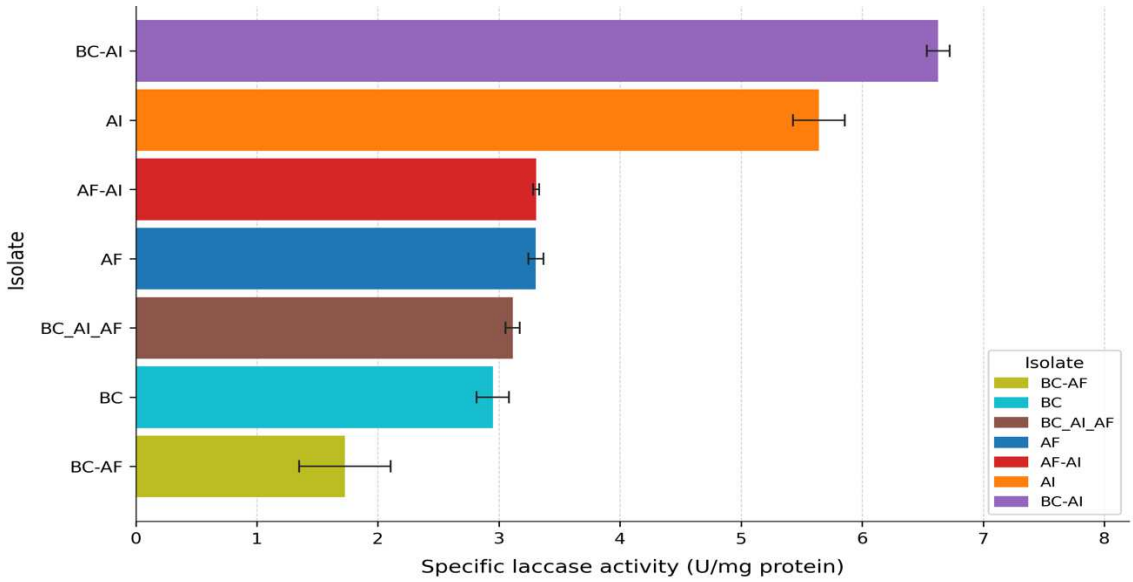


Fig.4 Enzyme Activity Profile of Laccase in Individual and Co-Culture Bacterial Consortia After Polyethylene Degradation. Data represent the mean ± SD (n = 3).

3.5 Structural Characterisation via FTIR

3.5.1 Normalization and Crystallization Analysis

To evaluate structural alterations in low-density polyethylene (PE) films following a 28-day microbial incubation, qualitative Attenuated Total Reflectance (ATR) FTIR spectra were acquired for the untreated reference, the sterile abiotic control (CTRL), and the biologically treated samples (AF, AI, AI-AF, BC, BC-AF, BC-AI-AF, and BC-AI). The spectrum of the untreated polyethylene exhibited the characteristic, highly symmetric aliphatic vibrational doublets expected of unmodified PE. The dominant absorbance bands were resolved at ~2915 and ~2847 cm^{-1}, corresponding to the asymmetric and symmetric CH_2 stretching vibrations, respectively. A bending/scissoring doublet reflecting crystalline and amorphous chain packing was identified at ~1473 and ~1462 cm^{-1}. Additionally, a rocking deformation doublet at ~730 and ~720 cm^{-1} was attributed to the orthorhombic crystalline and amorphous phases of the polyethylene backbone. Comparative overlay of the spectra across all experimental groups showed that these core aliphatic stretching and bending doublets remained the most prominent features in every treated and untreated sample, indicating that the underlying polyethylene backbone was broadly preserved across all conditions. However, a qualitative difference emerged in the region between 1000 and 1150 cm^{-1}, where a broad, heterogeneous absorption band centered around 1080–1100 cm^{-1} was evident in several of the biologically treated samples. This feature was most pronounced in the individual isolate AF and the triple co-culture BC-AI-AF, while it remained flat and close to baseline in the untreated reference, the abiotic control (CTRL), and the AI isolate.

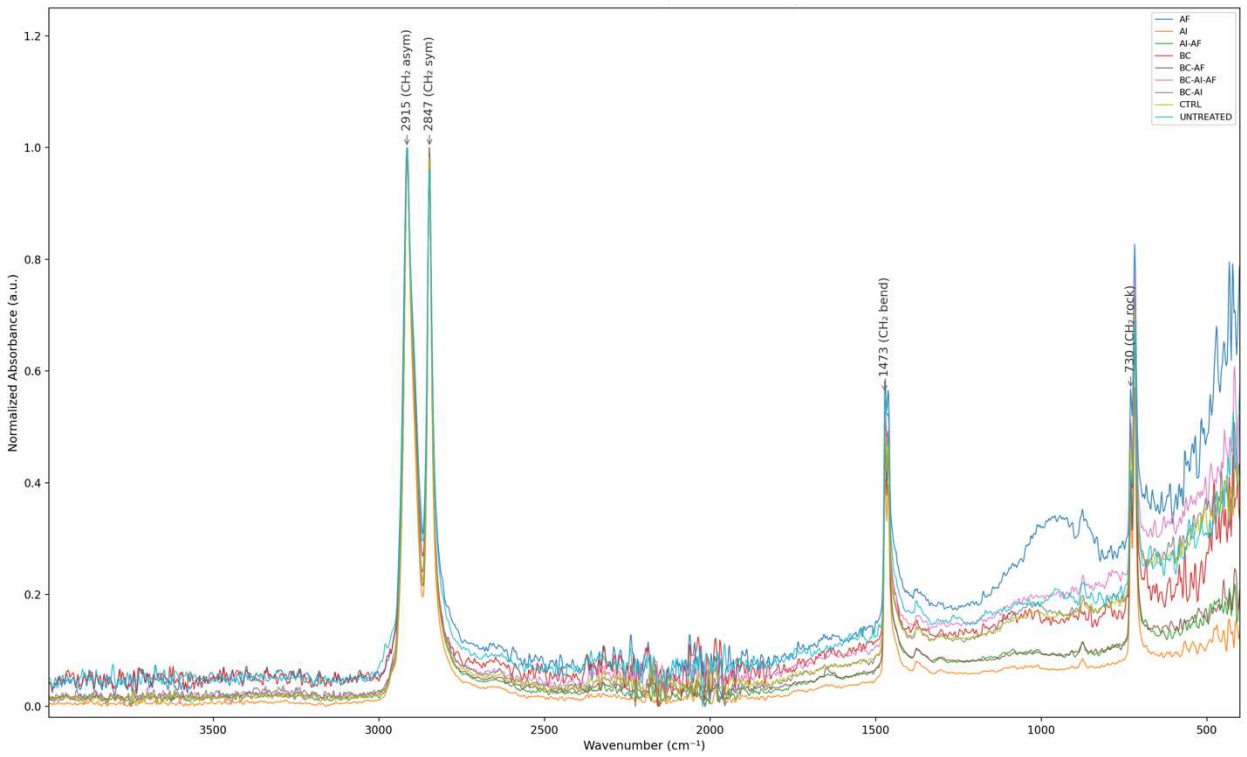


Fig.5: Min-max normalized ATR-FTIR spectra of low-density polyethylene films following 28 days of incubation with individual bacterial isolates and co-cultures. The characteristic aliphatic vibrational doublets of polyethylene are indicated, comprising the asymmetric and symmetric CH_2 stretching bands (~2915 and ~2847 cm^{-1}), the CH_2 bending/scissoring band (~1473 cm^{-1}), and the CH_2 rocking deformation doublet (~730 and ~720 cm^{-1}). A broad absorption feature in the 1000–1150 cm^{-1} region, most prominent in the AF-treated and BC-AI-AF-treated samples and absent in the untreated reference and abiotic control (CTRL), is consistent with C–O and C–O–C stretching vibrations associated with surface oxidation products and/or the deposition of biofilm-derived extracellular polymeric substances (EPS); these two contributions cannot be unambiguously distinguished on the basis of FTIR data alone.

3.5.2 Deconvolution and Fitting

To isolate overlapping vibrational contributions within the C-H stretching envelope (2800–2960 cm^{-1}), Gaussian-Lorentzian curve fitting and peak deconvolution were performed. Across all nine experimental conditions, the mathematical fit demonstrated high reliability, with coefficients of determination ($R^2 \geq 0.938$). The deconvolution protocol successfully resolved three sub-component bands within the C-H envelope: a high-wavenumber shoulder centered at ~2952 cm^{-1}, corresponding to methyl (CH_3) asymmetric stretching or chain branching; the dominant asymmetric CH_2 stretching band centered at ~2915 cm^{-1}; and the symmetric CH_2 stretching band centered at ~2847 cm^{-1}.

The resulting integrated peak areas and the calculated asymmetric-to-symmetric stretching ratio (A_{2915}/A_{2847}) are summarized in Fig.6, reflecting microbial-induced changes in chain conformation and crystallinity across the various consortia treatments.

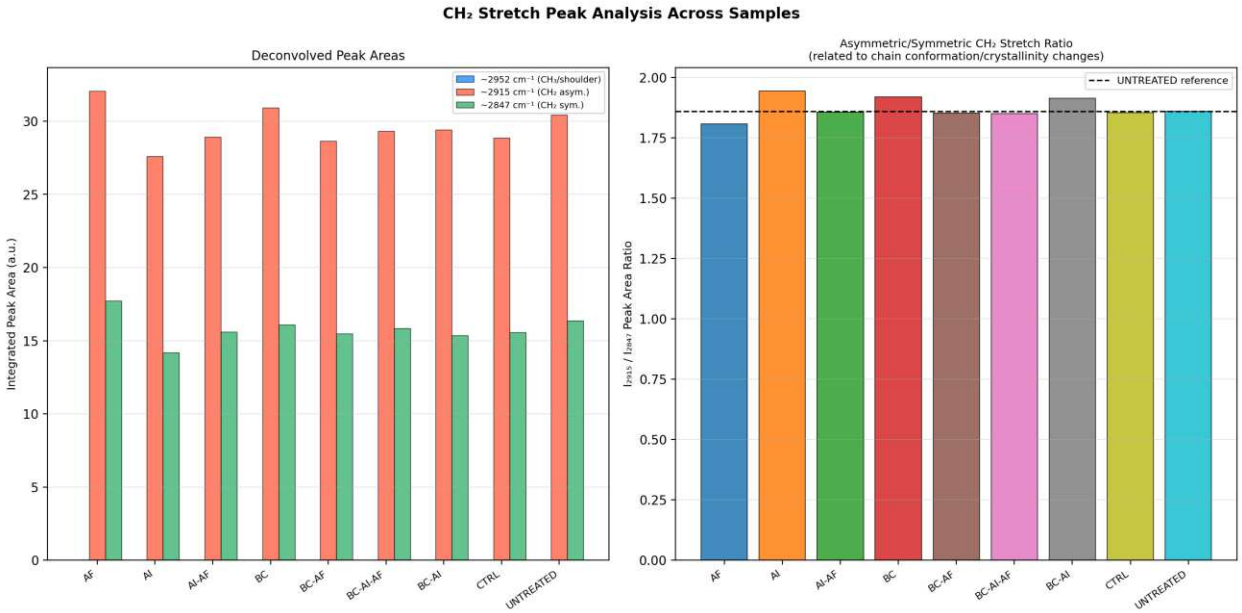


Fig.6 Quantitative ATR-FTIR deconvolution and chain conformation analysis of low-density polyethylene (LDPE) films following a 28-day microbial incubation, showing the integrated peak areas of the deconvolved CH_2 asymmetric (~2915 cm^{-1}), symmetric (~2847 cm^{-1}), and terminal methyl/shoulder (~2952 cm^{-1}) stretching bands, together with the calculated asymmetric-to-symmetric stretching ratio (A_{2915}/A_{2847}) reflecting microbial-induced changes in chain conformation and crystallinity. The horizontal dashed line represents the A_{2915}/A_{2847} value of the untreated reference film (~1.86). Experimental groups correspond to the untreated reference, sterile abiotic control (CTRL), individual microbial isolates (AF, AI, BC), dual co-cultures (AI-AF, BC-AF, BC-AI), and the triple co-culture (BC-AI-AF).

4.0 Discussion

The primary objective of this study was to determine whether co-cultured bacterial consortia enhance low-density polyethylene (LDPE) degradation efficiency compared to individual microbial isolates. Although the quantitative differences observed across our experimental endpoints did not reach statistical significance within the 28-day incubation period, the combined dataset shows clear directional trends. In particular, the numerical alignment between physical mass loss, enzymatic activity, and structural polymer modification suggests that microbial co-cultivation may influence early-stage degradation dynamics. Together, these patterns provide an exploratory framework for understanding how inter-isolate interactions shape the initial stages of polymer breakdown, as described below.

4.1 Identification of polyethylene-Degrading Bacteria and optimization of Biodegradation conditions.

MALDI-TOF mass spectrometry reliably identified all bacterial isolates with high confidence (score values > 2.0) [29], revealing *Bacillus cereus* as the predominant species across samples from Jachie, Prasmo, and parts of Manhyia consistent with its widely reported ability to degrade low- density polyethylene through enzymatic oxidation and surface erosion [30,31] . Additionally isolates, including *Achromobacter insolitus* and *Alcaligenes faecalis* detected in the Manhyia samples, further demonstrated the microbial diversity associated with polyethylene biodegradation, as both species have been linked to polymer oxidation, carbonyl group formation, and measurable weight loss [32,33]. Building on this characterization of the microbial community, a full factorial design based on Response Surface Methodology (RSM) in Minitab 22.2.1 was used to evaluate how pH, temperature, and incubation time influence both bacterial growth and polyethylene degradation. The statistical model revealed significant linear effects of temperature and incubation time on degradation, and multiple response optimization identified ~25°C, pH 9, and 21 days as the optimal conditions which is consistent with mesophilic and alkaline environments known to enhance microbial metabolism and degradation activity [34–37]. Contour plots (Fig.1 and 2) supported these results, showing that microbial growth peaked under alkaline pH (8-9) and moderate to low temperature (15-25°C), while optimal polyethylene degradation occurred at higher pH (8-9) and moderate temperatures (25-32°C). The identification of potent degraders and determination of their optimal operating condition highlight the strong biodegradation potential of the environmental bacterial communities examined in this study.

4.2 Analysis of Polyethylene Weight Loss

The present study evaluated the potential for bacterial consortia to degrade polyethylene more efficiently than individual cultures. While our results showed a clear numerical trend where co-cultured isolates, particularly BC-AI, achieved higher mean weight loss than individual isolates, these differences did not reach statistical significance.

Nonetheless, the consistent trends in weight loss, paired with the spectral changes we saw in our FTIR data, suggest that these bacteria might be working together to modify the plastic surface, an observation that warrants further investigation under extended incubation conditions and with greater replication. This aligns with findings [38], who noted that microbial consortia often outperform monocultures through metabolic complementarity, where different species contribute to the breakdown of polymer chains or associated additives.

However, the lack of statistical significance is likely influenced by the inherent heterogeneity in the microbial degradation of recalcitrant polymers and the constraints of the current sample size, which may have limited the power of the statistical tests to detect subtle differences.

As established by [39,40], the inherent recalcitrance of high-crystallinity polyethylene makes significant degradation a slow, variable process that is often difficult to capture in small-scale laboratory experiments.

4.4.1 Laccase Enzyme Activity

A positive association was observed between enzymatic potential and degradation performance across the experimental treatments. In particular, the BC–AI consortium showed both the highest mean percentage weight loss (8.04%) and the highest specific laccase activity (6.62 U/mg protein). This suggests that the gravimetric trends seen in the co-cultured treatments may be driven by synergistic enzymatic activity. Laccases are well documented for their role in the oxidative degradation of synthetic polymers, including facilitating the introduction of oxygen-containing functional groups onto the hydrophobic polymer backbone as an initial step in biodegradation[38,41,42]. The link between laccase activity levels and weight-loss patterns therefore provides a plausible biochemical basis for the observed gravimetric results. While the differences in weight loss did not reach statistical significance, the alignment between enzymatic and gravimetric measurements supports the biological plausibility of these trends and warrants further investigation under extended incubation conditions and with increased replication.

4.5 **FTIR Analysis of Polyethylene Following Bacterial Treatment**

In the AF and BC–AI–AF treated samples; a broad band appears in the 1000–1150 cm^{-1} region. This is likely linked to two related surface changes. First, microbial oxidation can introduce oxygen-containing groups (such as C–O and C–O–C) onto the polyethylene chain, which makes the surface more hydrophilic. Second, biofilm material (especially EPS) can accumulate on the polymer surface. Because EPS contains polysaccharides and glycoproteins, it produces signals in this same spectral region. Since FTIR alone cannot clearly separate these overlapping contributions, both effects are treated as evidence of active microbial surface interaction and biofilm colonisation [43–45] .

The way the A_{2915}/A_{2847} ratio changes with treatment also points to different interaction patterns between the microbes and the polymer. Ratios are higher in AI (1.944), BC (1.919), and BC–AI (1.909) than in the untreated reference (1.854). This suggests that the amorphous parts of the polymer are being removed preferentially. Because amorphous regions are less tightly packed and more accessible, microbial enzymes are more likely to attack them. As these amorphous regions shrink, a larger fraction of the remaining material is crystalline, which increases the stretching ratio.

Supporting this, the absolute peak areas decrease for AI, which is consistent with actual loss of aliphatic hydrocarbon material (i.e., polymer breakdown and carbon utilisation), not just a change in how much material is present at the surface [46–48].

The AF isolate showed the biggest change compared with the untreated reference. Its lower A_{2915}/A_{2847} ratio (1.814) suggests that degradation is more advanced going beyond

erosion of the amorphous regions and beginning to affect the crystalline structure. This is likely driven by enzymatic cutting of polymer chains, together with the effect of oxygen-containing groups that interfere with normal chain rearrangement. At the same time, the higher overall peak area is best explained by strong surface colonisation. In other words, the biological material on the polymer surface (such as EPS and other biofilm macromolecules) contributes to the measured signal, so it does not necessarily mean that the polymer contains more methylene [47,49] .

In the co-cultures that included AF like AI-AF (1.853), BC-AF (1.845), and BC-AI-AF (1.854)—the ratios stayed very close to the untreated control. This suggests that when multiple strains work together, they may degrade the polymer in a more coordinated way, affecting both less-ordered (amorphous) and more-ordered (crystalline) regions more evenly. One possible explanation is that different strains play different roles: some may break down the more accessible amorphous parts first, while others quickly consume the small breakdown products that are released. By doing this, they may reduce the build-up of acidic compounds that could otherwise disrupt further degradation. Overall, this cooperation could help maintain a similar balance between crystalline and amorphous regions while still allowing more material to be broken down and used. However, to confirm the exact mechanism, you would need additional biochemical and molecular tests, which are outside the scope of this study [43,47,50] .

5.0 Conclusion

The collective physical, biochemical, and spectroscopic data generated in this study indicate that synthetic bacterial consortia alter early-stage low-density polyethylene (LDPE) degradation dynamics compared with single-strain treatments. Although the 28-day incubation period was insufficient to capture the full extent of polyethylene breakdown, the alignment between the highest gravimetric mass losses and the highest specific laccase activity in the BC–AI group supports a biologically likely mechanism of microbial cooperation. Importantly, quantitative ATR-FTIR deconvolution indicates a difference in degradation behavior between monoculture and consortium treatments in their interaction with the polymer matrix. Monocultures appear to preferentially target the more accessible, less-ordered amorphous regions, as reflected by shifts in the A_{2915}/A_{2847} ratio. By comparison, multispecies consortia exhibit a more balanced spectral profile, suggesting a coordinated mode of degradation involving both amorphous and crystalline domains, potentially driven by metabolic complementarity. Overall, these findings shift the emphasis from single-strain performance to the structural advantages of cooperative microbial systems, providing a justification for moving beyond monoculture designs and future bioremediation studies should use larger sample sizes and prolonged incubation time to better assess the synergistic potential of multispecies consortia.

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Clinical trial registration: Not applicable. This study is not a clinical trial.

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Supplementary Information

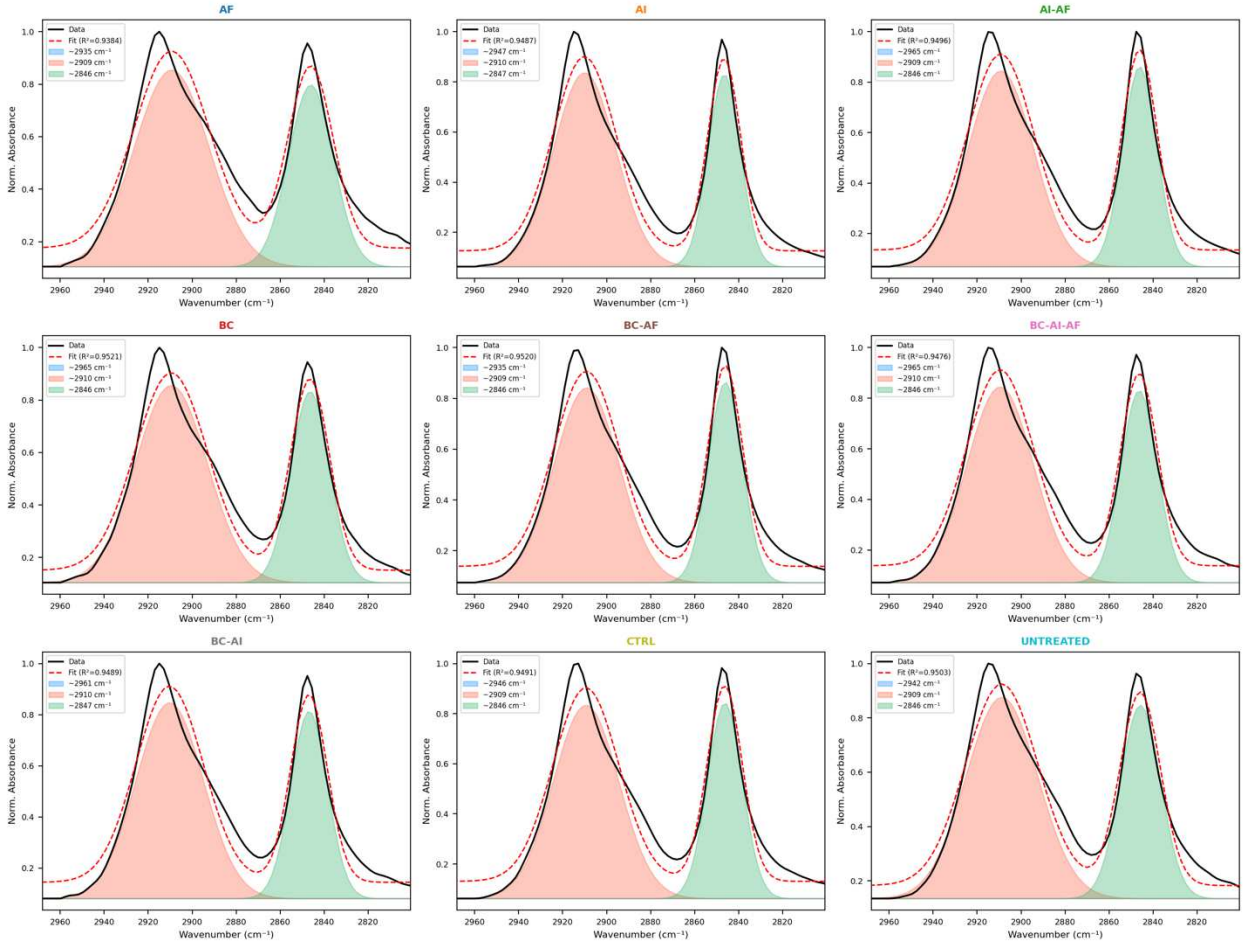


Fig.S1 Individual peak deconvolution profiles of the CH_2 stretching region (2800–2960 cm^{-1}) for low-density polyethylene (LDPE) films across experimental treatments. Deconvoluted sub-component bands are displayed for individual isolates (AF, AI, BC), dual co-cultures (AI-AF, BC-AF, BC-AI), the triple co-culture (BC-AI-AF), sterile abiotic control (CTRL), and the untreated reference film. Black solid lines represent the experimental Min-Max normalised ATR-FTIR absorbance data, while red dashed lines indicate the cumulative mathematical Gaussian-Lorentzian fit. Shaded areas resolve the independent structural sub-components: the high-wavenumber terminal methyl/shoulder (~2952 cm^{-1}), the dominant asymmetric CH_2 stretching band (~2915 cm^{-1}), and the symmetric CH_2 stretching band (~2847 cm^{-1}). Individual coefficients of determination (R^2 ≥ 0.938) confirming mathematical model reliability are reported within each respective panel legend.