

Report of Analysis



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Sample Number	16473265	Date of Sample Received:	06/04/2023
Date of Job started:	06/04/2023	Date of Job Completed:	27/09/2025

Test Method:

Evaluation of large microplastics, microplastics, and remnant analysis in resulting biomass of test sample Polyethylene films containing 1wt% Reverte BT 96638 additive masterbatch after testing for 450 days under ASTM D6954-24 - Standard Guide for Exposing and Testing Plastics that Degrade in the Environment by a Combination of Oxidation and Biodegradation.

* Currently, there is no standardized test method for assessing large microplastics, microplastics evaluation, or the remnant analysis of the original product following photo-oxidation or biodegradation processes. ISO 24187, which outlines various analytical methods and general guidelines for sampling, sample preparation, and data processing, for microplastics analysis was referred to design this protocol. The test protocol described herein has been specifically designed for the evaluation of microplastics, remnant analysis, and related assessments. While ISO 24187 has been referenced for analytical methodologies, sampling, and data processing, the protocol does not fully replicate or conform to the standard in its entirety. The modifications and adaptations made are intended to align with the specific objectives of this study and may not be directly comparable to results obtained under ISO 24187 compliant procedures. Specifically, it should be noted that only the test inoculum containing biodegradation residue was inspected and not a blank sample of the test inoculum prior to test sample biodegradation testing being performed.

Project Description:

The test sample Polyethylene films containing 1wt% Reverte BT 96638 additive masterbatch (Laboratory Reference No. 16473265) was submitted by WELLS PERFORMANCE MATERIALS LIMITED for testing under ASTM D6954-24 - Standard Guide for Exposing and Testing Plastics that Degrade in the Environment by a Combination of Oxidation and Biodegradation.

Sample Extraction and Analytical Methodology:

- The resulting biomass obtained after biodegradation testing as per ASTM D6954-24 study was dried at 40°C.
- The dried biomass was then transferred into petri dishes and examined under Light Microscopy for the presence of materials consistent with large microplastics, microplastics, or remnants of the original sample.

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- To ensure a comprehensive assessment, biomass was subjected to repeated agitation.
- Suspected particles resembling large microplastics, microplastics, or remnants of the original sample were isolated and placed onto separate sample holders for further analysis.
- A density separation method was performed, wherein a saturated Sodium chloride solution was added to the biomass and mixed thoroughly. This facilitated the separation of particles based on density, with less dense materials floating and denser materials sinking.
- The mixture was left undisturbed for 24 hours, after which the supernatant was carefully collected and filtered using a vacuum filtration assembly with Whatman Cellulose Nitrate 0.45 µm filter paper.
- This filter paper was air-dried and used to screen evidence of any material consistent of large microplastics/ microplastics or remnants of original samples
- Advanced analytical techniques were utilized for further characterization, including:
 1. Thermo Scientific Nicolet iN5 FTIR Microscopy for molecular identification.
 2. Thermo Scientific Axia Chemi Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX) for morphological and elemental analysis.
- All analytical procedures, including sampling, sample preparation, and detection, were conducted in plastic-free working environments to minimize the risk of contamination.

Observations:

Primary Screening under light microscopy

During the initial microscopic screening, several potential particles were identified and isolated for further analysis

- **Several irregularly shaped fragments** exhibiting transparent and whitish clusters.
- **Thin, flat, translucent particles** with a soft yet brittle texture, which exhibited characteristics indicative of a structurally and chemically non-polymeric material potentially formed from degraded polymeric substances.

All isolated particles were subjected to further screening to determine their classification as large microplastics, microplastics, or remnants of original sample if any.

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Optical Microscopy analysis:

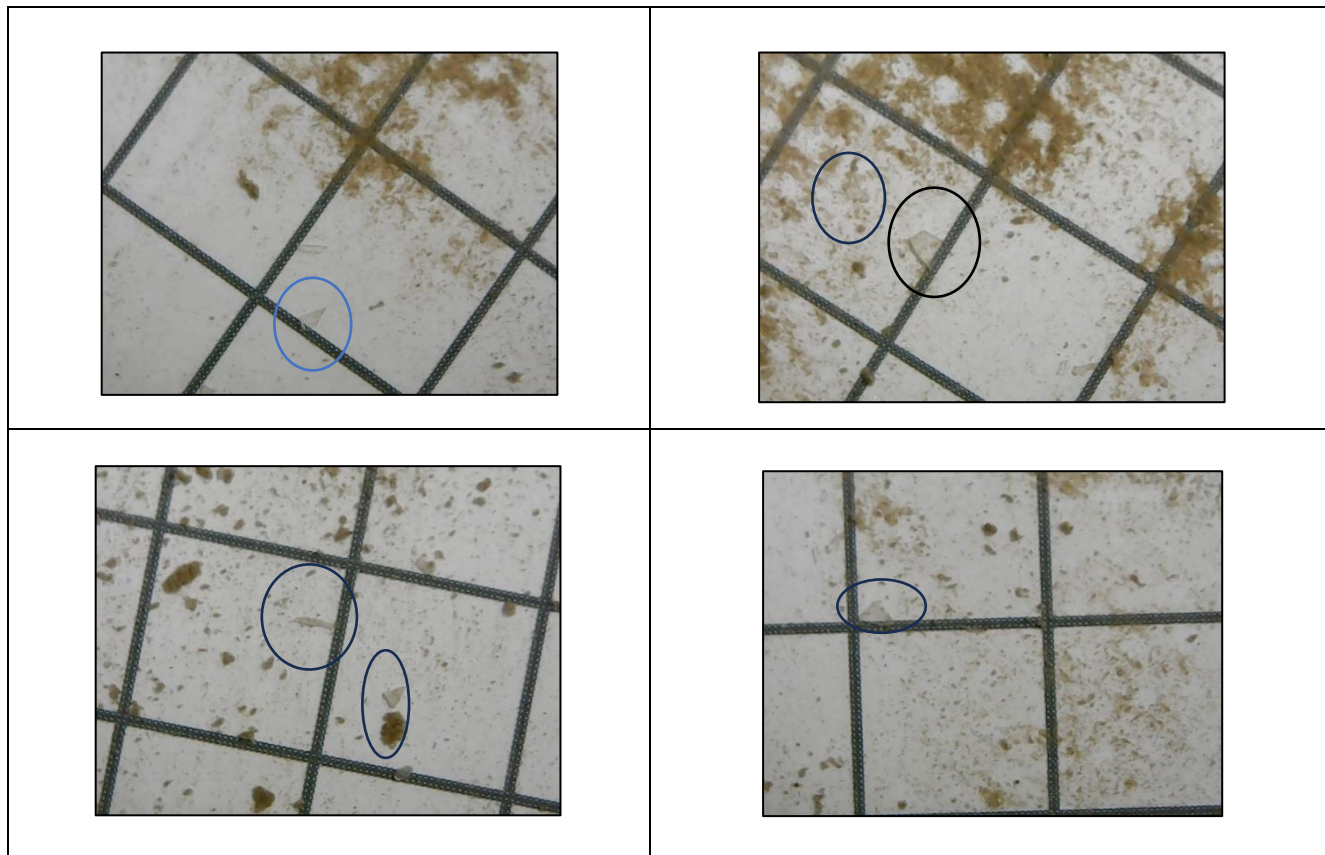


Figure 2: Microscopic images of primary screening under light microscopy

Scanning electron microscopy (SEM) images

Potential particles were scanned under Thermo Scientific Axia Chemi Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX) for morphological and particle size mapping.

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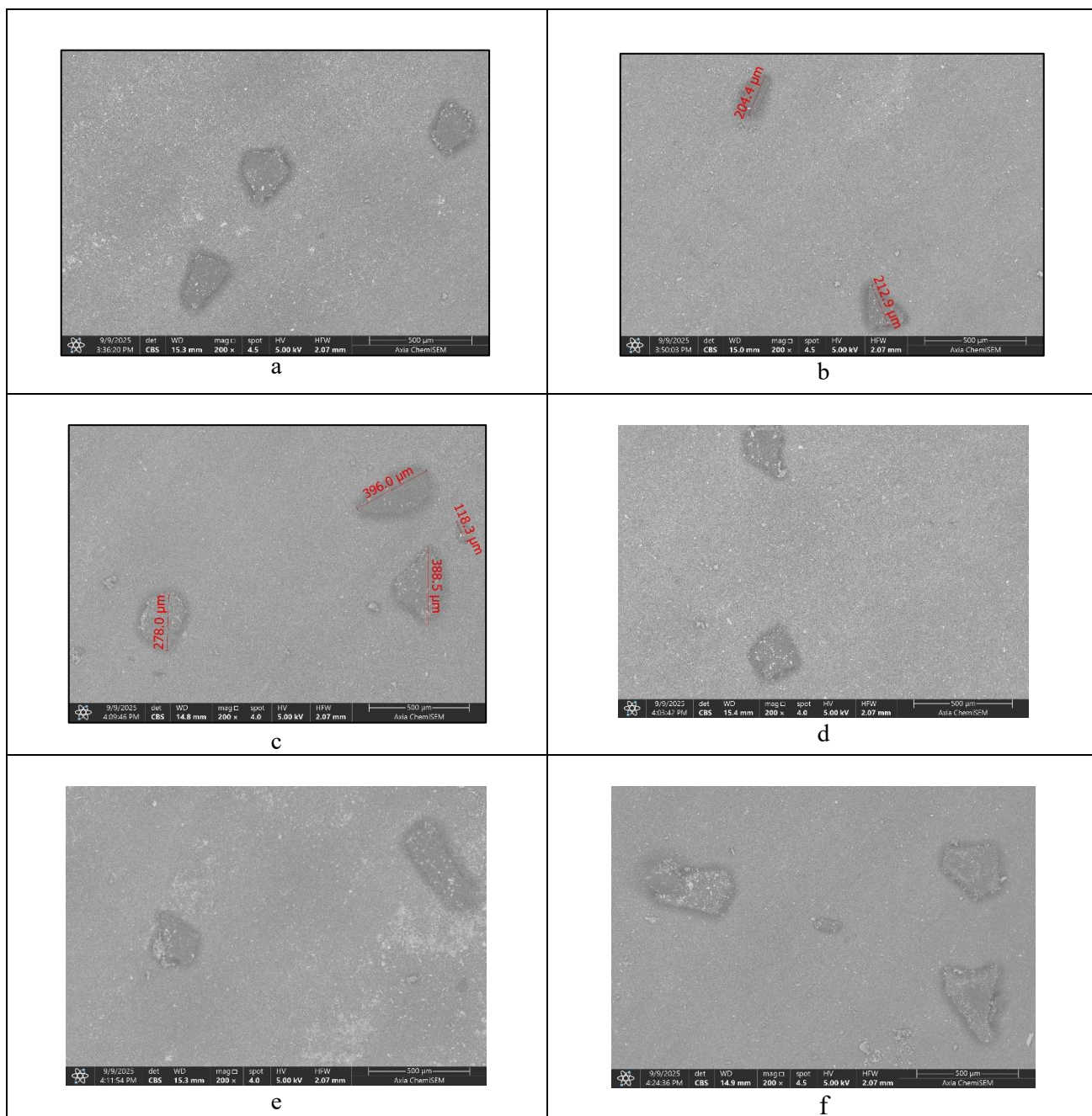


Figure - 3: SEM microscopy images potential particles with their size mapping

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IR Spectra

FTIR Spectra of original sample Polyethylene films containing 1wt% Reverte BT 96638 additive masterbatch (Laboratory Reference No. 16473265) fiber surface confirmed the material to be Polyethylene. This IR spectra were further used for ascertaining whether there is any evidence of potential remains of samples or any microplastics formed in resulting biomass obtained after 450 days testing under the ASTM D6954-24 study.

The following comparative FTIR spectra compares the original test sample PE film with PE wax and other PE based material, demonstrating that FTIR cannot determine molecular chain length nor nature of the material and thus confirming the limitations of the technique to determine whether a material is polymeric.

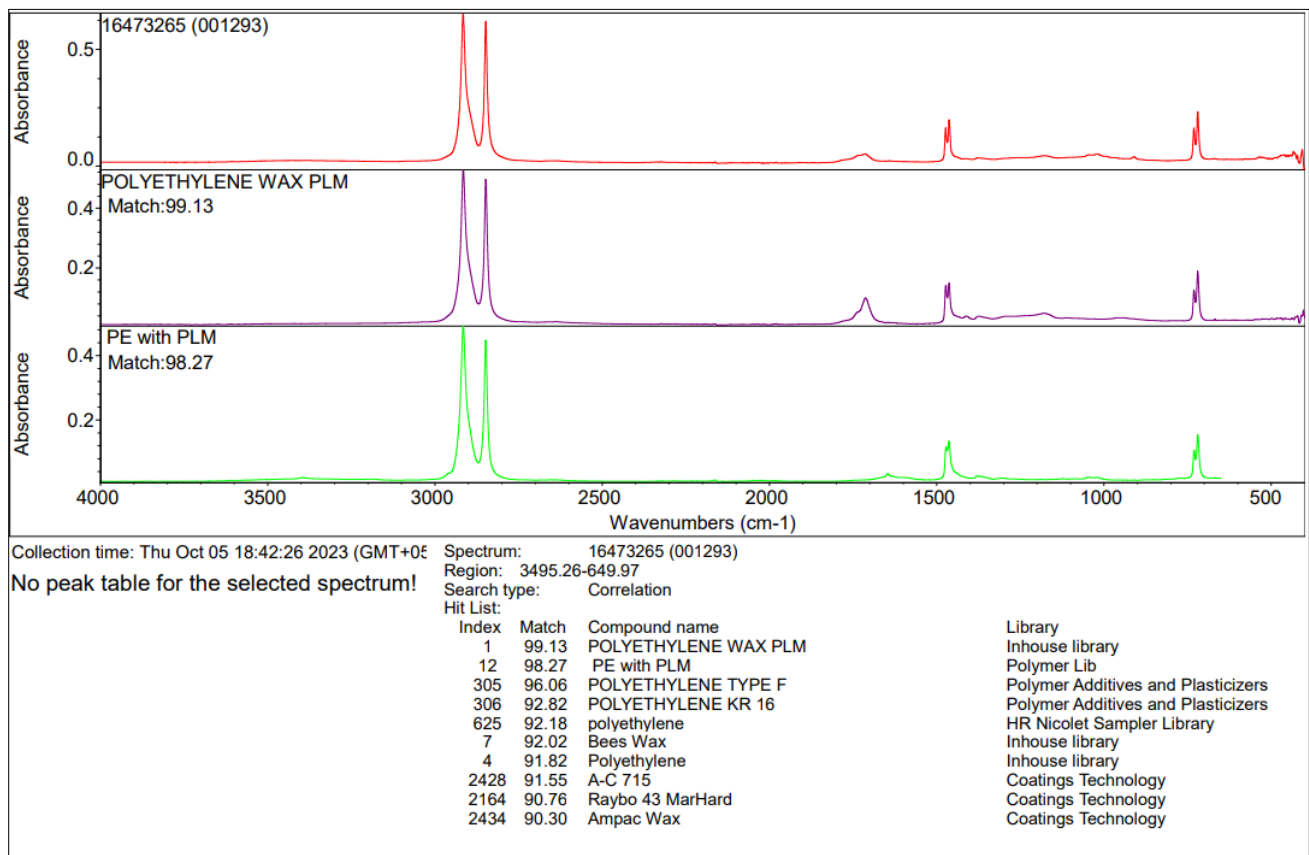


Figure 4: FTIR spectra of original sample Polyethylene films containing 1wt% Reverte BT 96638 additive masterbatch

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The following FTIR spectra compares 6 particles extracted from the test inoculum to the original, as received, test material.

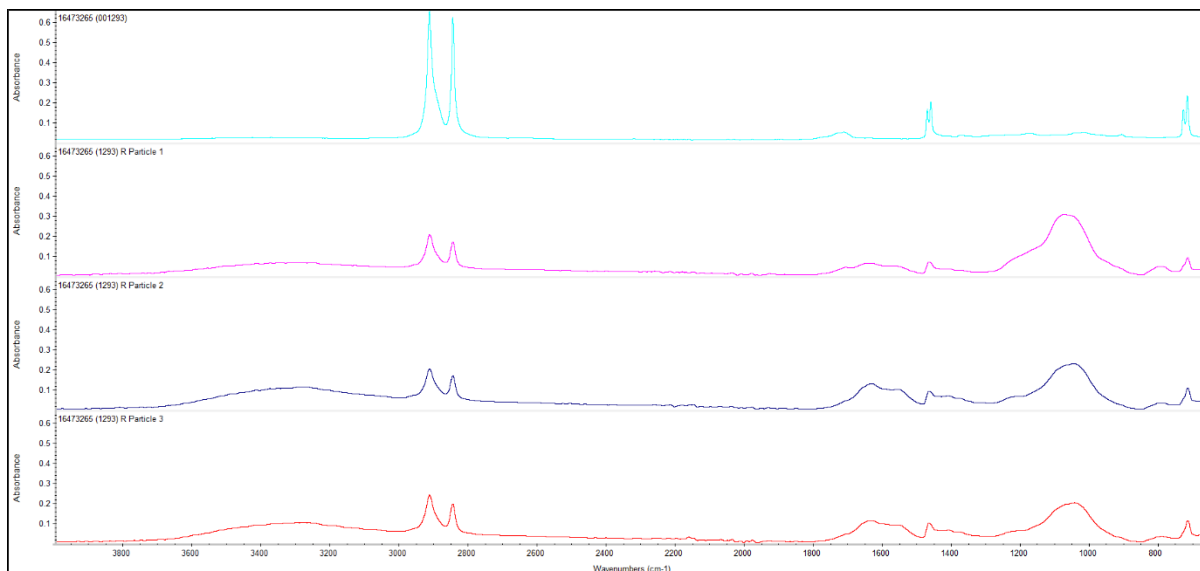


Figure - 5: FTIR spectra of Particle 1-3 from resultant biomass matched with the original test material

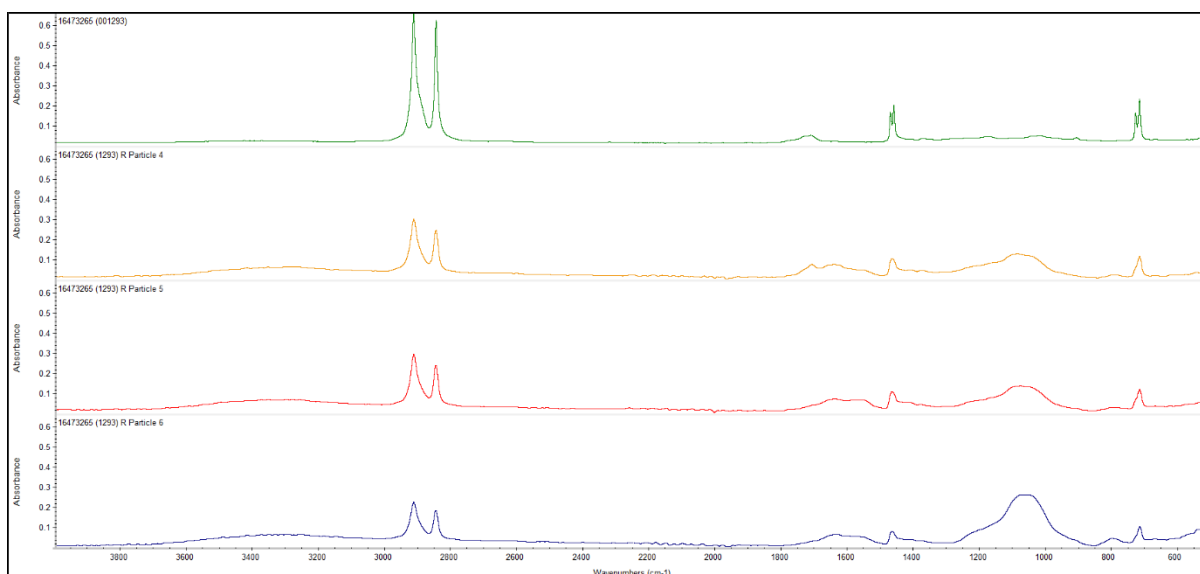


Figure - 6: FTIR spectra of Particle 4-6 from resultant biomass matched with the original test material

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The IR spectra of all particles matched with the library shows no resemblance to the IR spectra of the original sample Polyethylene films containing 1wt% Reverte BT 96638 additive masterbatch (Refer Figure 5 & 6).

Instead, we observed that the degraded particles exhibit carbonyl peaks around 1700 cm^{-1} , indicative of molecular oxidation influences from degradation. The sensitive fingerprint region between 900 and 1500 cm^{-1} are also significantly impacted, indicating significant change in functional groups of the material.

These differences in FTIR spectra between the original test material, as received, and the extracted particles from the biodegradation test inoculum, demonstrate (assuming the extracted material originated from the test material) that there has been significant chemical and associated structural changes to the test sample following Tier 1 abiotic degradation and Tier 2 biodegradation sequential testing in accordance with ASTM D6954-24.

These changes include; significant development of a very broad carbonyl peak, demonstrating significant introduction of oxygen into the material structure with the broadness of the peak indicating a mixture of positioning within the structure.

Additionally, there is a broad band at 3000 cm^{-1} indicating inclusion of OH groups and hydrogen bonding, probably associated with the formation of carboxylic acid groups. There is also significant change in the fingerprint region 1500-600 cm^{-1} demonstrating even more changes in the structure of the material such as branching, rotation etc.

Particle size mapping

The potential particles were sized on the basis below ranges, average particle size (μm) and number of particles observed in resulting biomass using SEM – EDX.

Table 1: Particle size classification

Particle size range (μm)	Average particle size (μm)	Number of Particles observed
<1.0	--	Not Detected
1 to < 5	--	Not Detected
5 to < 10	--	Not Detected
10 to < 50	42.98	39
50 to < 100	66.47	300
100 to < 500	292.2	456
500 to < 1,000	--	Not Detected
1,000 to 5,000	--	Not Detected

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Total 795 particles were observed based on the particle size (this definitive identification cannot be concluded from the FTIR). However, for inclusion in the test report, 6 representative particles were selected based on their size and representative spectra.

*The smallest detectable particle size for FTIR-ATR analysis is 5 µm; therefore, IR spectra could not be obtained for particles below this threshold. Particles smaller than 5 µm may not generate a reliable or interpretable spectrum due to inherent resolution limitations of the technique.

Interpretation:

- The interpretation provided here is based on the following:
 - It is assumed that the extracted particles originated from the original test sample material, though it should be noted that the test inoculum was not examined for particulate material prior to use in the associated biodegradation testing.
 - FTIR cannot prove that material is polymeric in nature.
- FTIR-ATR analysis of the original sample Polyethylene films containing 1wt% Reverte BT 96638 additive masterbatch (Laboratory Reference No. 16473265) confirmed its composition as Polyethylene. (Refer Figure 4).
- The FTIR spectra of selected particles were overlaid with the spectrum of the original sample, Polyethylene films containing 1wt% Reverte BT 96638 additive masterbatch (Laboratory Reference No. 16473265).
- Visual comparison illustrated that the materials only, essentially, had C-H characteristic peaks in common (Refer Figures 5 to 6).
- There are many significant differences illustrated by the FTIR between the original test material and the extracted material. These include:
 - Significant development of a very broad carbonyl peak, confirming significant introduction of oxygen into various positions within the molecular structure of the material as illustrated by the broadness of the peak.
 - A broad band at 3000 cm⁻¹ indicating inclusion of OH groups and hydrogen bonding, probably associated with the formation of carboxylic acid groups.
 - A significant change in the fingerprint region 1500-600 cm⁻¹ illustrating additional changes in the structure of the material such as branching and rotation.

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- The significant differences in the FTIR spectra between the original test sample and the extracted material confirm that the extracted material is substantially different in molecular structure, and chemical composition and nature, to the original test material following the degradation and biodegradation processes occurring during testing to the criteria of ASTM D6954-24.
- It can be considered that the significant structural and chemical differences between the original test sample and extracted material confirm that the extracted material cannot be polyethylene nor generally polymeric in nature and thus does not meet the generally accepted criteria for being classed as microplastic material

Authorized Signatory



Prasanth Babu
Assistant Manager – Biodegradability Services

----- **End of Report** -----

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