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Assessment of Foam Material Deterioration by Aspergillus terreus Using Scanning Electron Microscopy, EDX, and FTIR Analysis

Sanaa qasem badr[1]*, Raghad Jabbar Abdul Sahib[2], Zahraa Qasim Mousa[3]
[1] College of Education for Women University of Basrah , Iraq
[2,3] General Directorate of Education in Maysan, Ministry of Education, Iraq

تقييم تدهور المادة الرغوية بواسطة *Aspergillus terreus* باستخدام المجهر الإلكتروني الماسح، *EDX*، وتحليل *FTIR*

المؤلف الأول[1]*، المؤلف الثاني[2]، المؤلف الثالث[3]
[1] كلية التربية للبنات جامعة البصرة، العراق
[3،2] المديرية العامة لتربية ميسان، وزارة التربية، العراق

*Corresponding author: sanaa.badr@uobasrah.edu.iq

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Abstract:
The accumulation of foam waste in the environment has elevated interest in investigating microorganisms that may assist to the deterioration of polymer based materials. This study analyzed the effects of Aspergillus terreus isolated by water samples collected from the Al-Karma Ali River upon foam material under laboratory conditions. The fungal isolate was refined and identified using traditional mycological methods. Foam pieces weighing 0.1 g were incubated in 20 mL of potato dextrose broth (PDB) ,at 25°C for one and two months. Untreated standard samples and time matched controls absent fungal inoculation were contained for comparison. Aletrations in the material were assessed with scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR) derived carbonyl index measurements, dry weight determination, and medium pH measurements. The recorded dry weight loss in the fungus associated samples reached (10.64 ± 1.30)% after one month and (25.32 ± 2.60%) after two months, compared with (0.62 ± 0.18%) and (1.58 ± 0.42%) in turn, in the corresponding controls. The carbonyl index also raised during fungal incubation reaching (0.620 ± 0.016) after two months, as the final medium pH decreased to (5.471 ± 0.099). SEM observations revealed changes in surface morphology, whereas EDX showed differences in the relative elemental composition of the examined samples. These findings indicate that incubation with A. terreus was associated with measurable changes in foam mass, surface characteristics and selected analytical parameters. Yet, because the chemical identity of the foam was not established and complete mineralization was not measured, further investigations are required to confirm the extent and mechanism of fungal biodegradation.

Keywords: Aspergillus terreus; fungal treatment; foam waste; surface deterioration; SEM; EDX; FTIR.

الملخص
أدى تراكم نفايات الرغوة (الفوم) في البيئة إلى تزايد الاهتمام بدراسة الكائنات الدقيقة التي قد تساهم في تحلل المواد القائمة على البوليمرات. تناولت هذه الدراسة تأثير فطر *Aspergillus terreus* -الذي عُزل من عينات مياه جُمعت من نهر "الكرما علي"- على مادة الرغوة في ظروف مخبرية. جرى تنقية العزلة الفطرية وتحديد هويتها باستخدام الطرق الفطرية التقليدية. وُضعت قطع من الرغوة بوزن 0.1 غرام في 20 مل من مرق "البطاطس والدكستروز" (PDB) عند درجة حرارة

25 مئوية لمدة شهر وشهرين. كما أُعدت عينات قياسية غير معالجة ومجموعات ضابطة (مقارنة) مطابقة في المدة الزمنية ولكن دون تلقيح فطري. قُيمت التغيرات التي طرأت على المادة باستخدام المجهر الإلكتروني الماسح (SEM)، ومطيافية الأشعة السينية المشتتة للطاقة (EDX)، وقياسات "مؤشر الكربونيل" المستمدة من مطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR)، بالإضافة إلى تحديد الوزن الجاف وقياس درجة الحموضة (pH) للوسط الغذائي. بلغ فقدان الوزن الجاف في العينات المعرضة للفطر (10.64 ± 1.30)% بعد شهر واحد، و(25.32 ± 2.60)% بعد شهرين، مقارنة بـ (0.62 ± 0.18)% و(1.58 ± 0.42)% على التوالي في المجموعات الضابطة. كما ارتفع مؤشر الكربونيل خلال فترة التحضين مع الفطر ليصل إلى (0.620 ± 0.016) بعد شهرين، بينما انخفضت درجة الحموضة النهائية للوسط الغذائي إلى (5.471 ± 0.099). كشفت ملاحظات المجهر الإلكتروني الماسح (SEM) عن تغيرات في مورفولوجيا السطح، بينما أظهرت تقنية (EDX) فروقاً في التركيب العنصري النسبي للعينات المدروسة. تشير هذه النتائج إلى أن التحضين مع فطر *A. terreus* ارتبط بتغيرات ملموسة في كتلة الرغوة، وخصائص سطحها، وبعض المعايير التحليلية المختارة. ومع ذلك، ونظراً لعدم تحديد الهوية الكيميائية الدقيقة للرغوة وعدم قياس التمعدن الكامل، فإن هناك حاجة إلى إجراء المزيد من الدراسات لتأكيد مدى وآلية التحلل الحيوي الفطري..

الكلمات المفتاحية: *Aspergillus terreus*؛ المعالجة الفطرية؛ نفايات الرغوة؛ تدهور السطح؛ SEM؛ EDX؛ FTIR.

Introduction

The accumulation of plastic and foam waste has get an environmental concern because many artificial materials persist for long periods after disposal, their persistence can contribute to the gathering of solid waste in aquatic and earthly environments**.[1]** Conventional waste management methods, including collection, disposal and recycling, do not usually prevent the long term collection of these materials. Thus, biological approaches have obtained increasing attention as possible supporting methods for managing specific types of polymer containing waste**[2,3]**. Microorganisms are capable of attaching to producing extracellular and material surfaces substances which may influence surface properties. Some fungi have linked particular interest because of their capability to grow under different environmental conditions and yield enzymes and metabolites that can join in the transformation of organic compounds. Still, fungal growth on a material does not inevitably mean that the material itself is being used since a carbon source or completely degraded**[4,5,6]**. Changes in appearance, mass must or surface structure therefore be analyzed carefully. Several investigations have inspected fungal interactions with synthetic polymers. Investigated the biodeterioration of pre-treated polypropylene by Aspergillus terreus and Engyodontium album**[7].** A study showed that the mechanisms suggested to provide to fungal plastic degradation, emphasizing the importance of identifying surface-associated changes from proof of chemical transformation, depicted a screening approach for plastic-degrading fungi that joins different analytical methods, these studies support the use of supporting techniques when evaluating possible microbial impacts on polymeric materials**[8,9,10].** Scanning electron microscopy (SEM) provides precise images of surface morphology and can show features such as cracks, irregularities, cavities, and attached biological material**[11].** Energy-dispersive X-ray spectroscopy (EDX) supplies information on the relative elemental composition of the analyzed area**[12].** Fourier-transform infrared spectroscopy (FTIR) can provides information about changes in chemical functional groups, while dry-weight measurements offer a quantitative based on of material loss**[13].** Measuring the pH of the culture medium can also help identify changes in the surrounding environment during incubation. No single method, yet, is sufficient on its own to establish complete biodegradation or mineralization**[14].** Aspergillus terreus is a filamentous fungus that has been investigated in studies involving the deterioration of synthetic materials. Its interaction with foam waste may depend on the composition of the material, incubation conditions, and the characteristics of the fungal isolate. When the chemical identity of the tested material is unknown, the observed changes should be described as material deterioration or fungal-associated changes rather than attributed to the degradation of a specific polymer**[15,16].** The present study examined the effects of a water derived isolate of A. terreus on foam pieces incubated in potato dextrose broth for one and two months. Surface morphology, relative elemental composition, dry-weight changes, FTIR-derived carbonyl index and culture-medium pH were examined. Samples incubated without the fungus were included as time-matched controls. The study aimed to assessed whether fungal incubation was associated with measurable changes in the tested material to assess the value of combining these analytical measurements while investigating potential fungal-mediated deterioration.

2. MATERIALS AND METHODS

2.1. Isolation and identification of the fungal isolate

Fungal isolates were obtained from water samples collected from the Al-Karma Ali River. Isolation was performed using conventional fungal isolation procedures, followed by purification of the resulting colonies. The purified isolate used in the experiment was identified as A. terreus using classical mycological identification methods. The purified isolate was subsequently used in the laboratory treatment of the foam material.

2.2. Preparation of the foam samples

Foam material was cut into small pieces, and a mass of approximately 0.1 g was used for each experimental unit. The samples were prepared for incubation in potato dextrose broth. The chemical composition and specific polymer type of the foam were not established; therefore, the material is referred to as foam throughout this study.

2.3. Preparation of the culture medium and experimental groups

Potato dextrose broth (PDB) was used as the liquid culture medium. A volume of 20 mL was dispensed into each 25 mL glass culture vessel. One foam piece weighing approximately 0.1 g was placed in each vessel assigned to the relevant experimental group. The fungus-associated samples were exposed to the purified A. terreus isolate and incubated at 25°C. The experiment included five groups, with five recorded units in each group: - A: Baseline foam samples examined before incubation. - C+1: Foam samples incubated for one month without fungal inoculation. - A+1: Foam samples incubated with A. terreus for one month. - C+2: Foam samples incubated for two months without fungal inoculation. - A+2: Foam samples incubated with *A. terreus* for two months. The control groups were included to distinguish changes associated with incubation in the culture medium from changes observed in the presence of the fungus.

2.4. Scanning electron microscopy (SEM)

The surface morphology of the foam samples was examined using scanning electron microscopy. Representative micrographs were obtained from the baseline material and the fungus-associated samples collected after one and two months. The images were examined for visible surface features, including irregularities, cavities, cracks, aggregates, and filament-like structures. Recorded feature dimensions were used for descriptive comparisons between the experimental groups. Because the available images did not represent a complete set of independent micrographs for every control unit, morphological comparisons were interpreted in relation to the available imaging records.

2.5. Energy-dispersive X-ray spectroscopy (EDX)

EDX analysis was used to examine the relative elemental composition of the foam-associated material. The reported elemental data included carbon, nitrogen, oxygen, silicon, sulfur, and chlorine. Both weight percentages and atomic percentages were considered when comparing the recorded groups. The results were interpreted as relative measurements of the analyzed areas rather than absolute quantities of elements in the entire samples. EDX data were not used independently to identify the polymer or establish a specific chemical degradation pathway.

2.6. Dry-weight determination

Initial and final dry masses were noted for the foam samples. Percentage weight loss was computed for each recorded unit using the following equation: \[\text{Weight loss (\%)}=\frac{W_i-W_f}{W_i}\times100\]where \(W_i\) represents the initial dry weight and \(W_f\) represents the final dry weight. The calculated values were used to contrast the baseline samples, time-matched controls and fungus associated samples after one and two months. The interpretation of this endpoint viewed the possibility that handling, recovery of small fragments, residual fungal material could affect the final recorded mass.

2.7. FTIR-derived carbonyl index and pH measurement

The FTIR-derived carbonyl index was used to assess changes in the recorded infrared index values across the experimental groups. The index was compared between baseline samples, fungus-free controls, and samples incubated with *A. terreus* for the two incubation periods. The initial and final pH values of the culture medium were also recorded. Variations in pH were examined as an indicator of changes in the culture environment during incubation. The carbonyl index and pH results were explained as complementary measurements and were not considered, alone, definitive evidence of polymer chain cleavage or complete biodegradation.

RESULTS AND DISCUSSION

The new workbook has 25 individually labeled records, each one of them representing a baseline A, no-fungus control after 1 month (C+1), no-fungus control after 2 months (C+2), Aspergillus terreus-associated treatment after 1 month (A+1), and Aspergillus terreus-associated treatment after 2 months (A+2). The laboratory method is to grow 0.1 g of pieces of foam in 20 mL potato dextrose broth at 25 °C. The term foam is not a designation of the formulation of the material and therefore no specific polymer can be named. Initial and final dry weight, four SEM dimensions per record, six element EDX profiles, carbonyl index (FTIR) and initial/final pH are provided. Representative SEM fields and original EDX spectra for A, A+1 and A+2 are in the image archive, but not independent images/spectra for all 25 records and time matched controls. The methodological studies clearly indicate that surface deterioration is not enough to establish biodegradation, but it needs more than one orthogonal endpoint **[17,18].**

3.1. SEM surface morphology and laboratory imaging

Table 1. Available original SEM images and EDX reports for foam-associated samples; the spreadsheet provides additional control records.

Stage	Incubation	Fungal treatment	Original SEM fields	Original EDX spectrum
A	Baseline	Absent	14 images	Available
A+1	One month	Present	14 images	Available
A+2	Two months	Present	13 images	Available
C+1 and C+2	One/two months	Absent	Not supplied	Not supplied

The 41 stored SEM images cover wide and narrow observation fields of the three recorded experimental stages. The individual C+1 and C+2 controls are only available as spreadsheet measurements and only representative images are saved as instrument-derived observations. This separation avoids the possibility of a visual image-to-control comparison which the original archive does not support. The variation in magnifications shown is useful for the study of a heterogeneous surface, but the view is not a random whole-sample survey. Microscopy and weight loss were previously used, as well as a FTIR study on a defined polypropylene substrate, and a methodological study that outlines a screening approach that incorporates imaging, spectroscopic and respiratory tests. These studies show the rationale for images to be supporting evidence rather than proof of polymer mineralization **[18,19].**

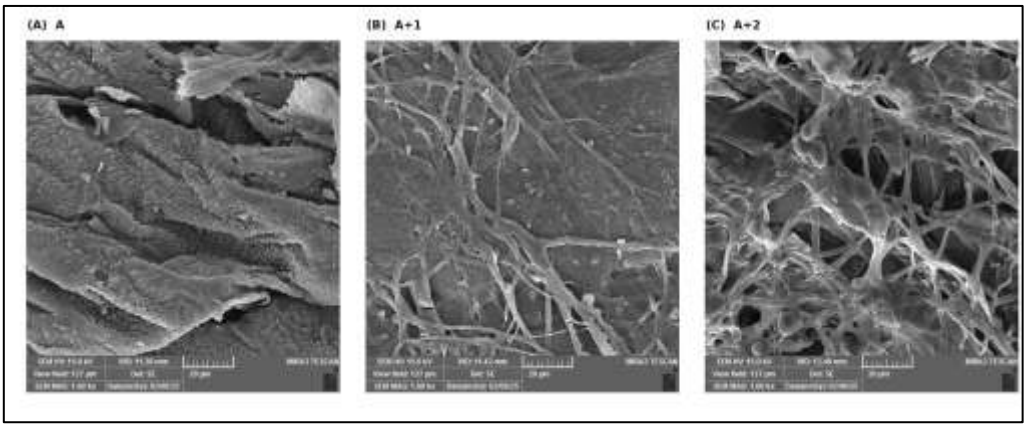


Figure 1. Original lower-magnification SEM fields for (A) baseline A, (B) A+1 and (C) A+2. Magnifications and original scale bars are retained in the micrographs.

In the three archived samples, the surfaces that are associated with the foam are broken up and uneven, as shown in figure 1. The A+2 field has features that are prominent in the form of filaments or bridges, while the A and A+1 fields are porous and particulate in texture. The number of cracks increased by a certain percentage, which is not justified by the images, and the apparent texture is influenced by field selection and by the various effective magnifications. Biological deposition and polymer fragmentation and sample preparation can also yield similar appearances. The chemistry of the present foam is not known and the literature on fungal attachment and surface alteration reported by previous studies provides a plausible biological context, but not confirmation of the chemistry [19,21]

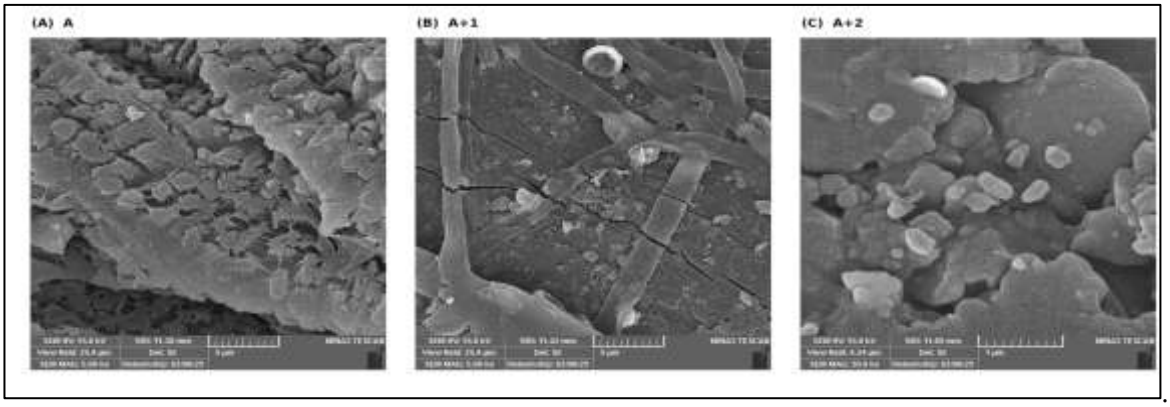


Figure 2. Original intermediate-scale SEM examples of A, A+1 and A+2 displayed as one comparative panel.

At the intermediate observation scale, Figure 2 shows the diversity of local patterns of cavities, aggregates and material continuity. The patterns are similar to the specimen surface being restructured after incubation in the fungal culture and are not able to differentiate between true polymer-chain cleavage and fungal attachment. The conductivity preparation and specimen selection also have an impact on the appearance of a local area. The multi-technique approach is described in methodological studies and reviews of fungal processes, which help to interpret with caution the morphology **[18,20].**

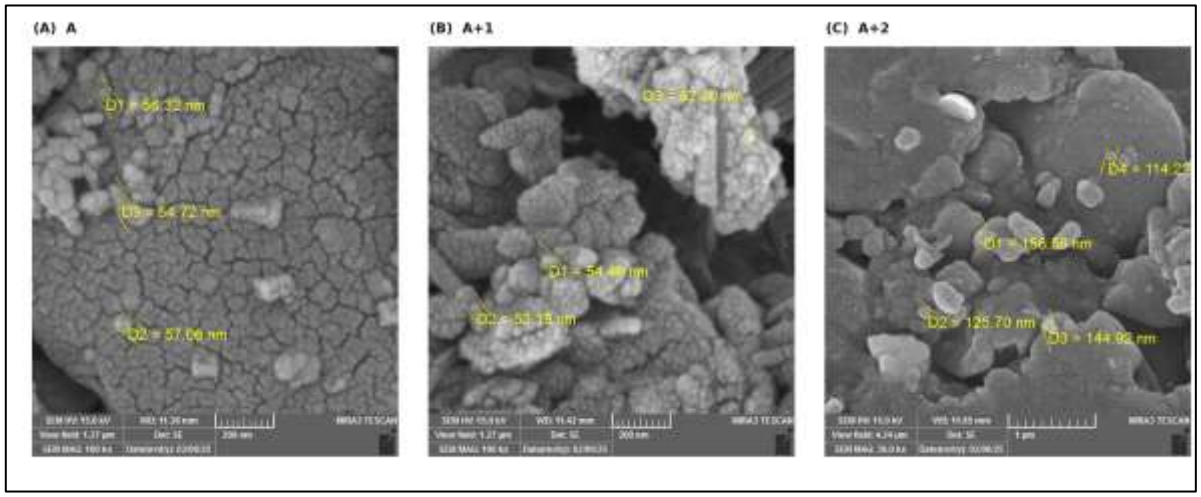


Figure 3. Original laboratory-annotated SEM diameter examples from A, A+1 and A+2; the A+2 annotated image has a different magnification from A and A+1.

The size of the features annotated by the laboratory were about 56 nm in the baseline example, 53 nm after one month and 135 nm after two months. These are selected objects visible in individual archived fields, the identity of the objects is not directly comparable as the A+2 annotated view was taken at a different nominal magnification. Note that the four SEM readings in each row of the new spreadsheet are a separate record-level data set, and are not the same as the small set of annotations shown in Figure 3. Methodological studies caution that changes in the diameter of the features that can be observed with SEM alone are not necessarily indicative of polymer or biochemical degradation **[18,20].**

Group	Records	SEM readings/record	Record-mean SEM size (nm)	Range of record means (nm)
A	5	4	56.27 ± 3.98	51.88–62.65
C+1	5	4	58.16 ± 2.72	54.70–62.26
A+1	5	4	48.73 ± 4.22	42.76–53.91
C+2	5	4	59.36 ± 4.20	52.78–63.33
A+2	5	4	129.38 ± 9.32	117.70–140.88

Table 2. Record-level SEM feature-size summaries (mean ± SD across the five recorded units within each group).

The mean SEM feature size at baseline was 56.27 ± 3.98 nm, which is the record size. At one month, the control mean was 58.16 ± 2.72 nm compared with 48.74 ± 4.22 nm in A+1. At two months, the corresponding means were 59.36 ± 4.20 and 129.38 ± 9.32 nm. So it was not a monotonic size-reduction trend, but rather a reversal, caused by the fungus. The A+1 versus C+1 difference was −9.42 nm (95% CI −14.76 to −4.09), while A+2 versus C+2 was 70.02 nm (95% CI 58.62 to 81.41). The size of the SEM objects is not polymer molecular weights. This discrepancy between the feature trends and the progressive weight-loss is an indication that this is not necessarily a degradation rate, as fungal and abiotic surface changes may occur in different ways **[18,20].**

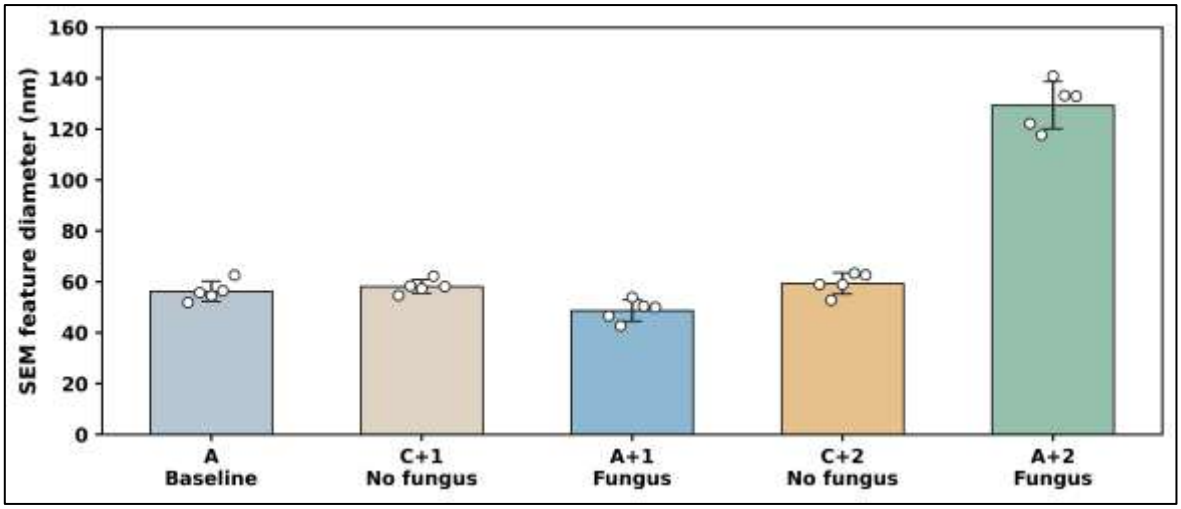


Figure 4. Mean SEM-visible feature diameter ± SD with five record-level observations for each group.

The means of the five spreadsheet records are shown in the scatter and bar graph in Figure 4; 20 independent biological replicates were not performed in each group. A+1 was below its control while A+2 was significantly above C+2 and A+1. The interaction between the fungus and time for feature size was $F(1,16) = 243.47$ ($P < 0.001$) and the recorded five-unit grouping was observed. This interaction exhibits a size contrast, but only when particle identity and field-selection rules are documented, the explanation for this is known. As such, SEM differences are considered complementary morphology and not definitive proof of bulk foam biodegradation **[18,19].**

3.2. EDX composition and original spectra

Table 3. Elemental weight and atomic fractions by EDX (mean ± SD of five spreadsheet entries/group); paired bases describe the same elemental detections.

Group	Basis	C	N	O	Si	S	Cl
A	Weight %	71.55 ± 0.33	9.29 ± 0.11	14.61 ± 0.21	2.57 ± 0.15	1.98 ± 0.11	0.00 ± 0.00
	Atomic %	77.50 ± 0.25	8.63 ± 0.11	11.88 ± 0.18	1.19 ± 0.07	0.80 ± 0.05	0.00 ± 0.00
C+1	Weight %	72.04 ± 0.27	9.10 ± 0.18	14.47 ± 0.32	2.44 ± 0.14	1.95 ± 0.19	0.00 ± 0.00
	Atomic %	77.90 ± 0.19	8.44 ± 0.15	11.75 ± 0.27	1.13 ± 0.07	0.79 ± 0.08	0.00 ± 0.00
A+1	Weight %	70.62 ± 0.27	8.81 ± 0.11	14.30 ± 0.16	2.08 ± 0.15	1.75 ± 0.14	2.43 ± 0.13
	Atomic %	77.36 ± 0.20	8.28 ± 0.11	11.76 ± 0.13	0.97 ± 0.07	0.72 ± 0.06	0.90 ± 0.05
C+2	Weight %	71.49 ± 0.41	9.39 ± 0.08	14.55 ± 0.24	2.37 ± 0.19	2.20 ± 0.11	0.00 ± 0.00
	Atomic %	77.45 ± 0.29	8.72 ± 0.08	11.84 ± 0.21	1.10 ± 0.09	0.89 ± 0.05	0.00 ± 0.00
A+2	Weight %	73.45 ± 0.27	7.10 ± 0.15	11.94 ± 0.22	2.12 ± 0.30	1.84 ± 0.19	3.55 ± 0.07
	Atomic %	80.45 ± 0.21	6.67 ± 0.13	9.82 ± 0.19	0.99 ± 0.14	0.76 ± 0.08	1.32 ± 0.03

The carbon was the main component of all the data. The oxygen content of the fungus-associated groups averaged 11.94 ± 0.22 wt% in A+2 and 14.55 ± 0.24 wt% in C+2. The average chlorine content was 3.55 ± 0.07 wt% in A+2 and zero in both fungus-free time controls. A+1 likewise contained 2.43 ± 0.13 wt% chlorine. The original EDX reports are similar in overall format, but do not contain one original spectrum per spreadsheet record. Note: Non-detection does not imply the absence of the chemical and normalized weight fractions are not a measure of absolute gain or loss of elements in the flask. If the percentages of EDX add up to around 100%, then the percentage of one fraction will decrease and the percentage of the other fraction will increase. The EDX data is carbon-rich and doesn't identify the foam polymer, its functional groups or the mineralization products **[20,21].**

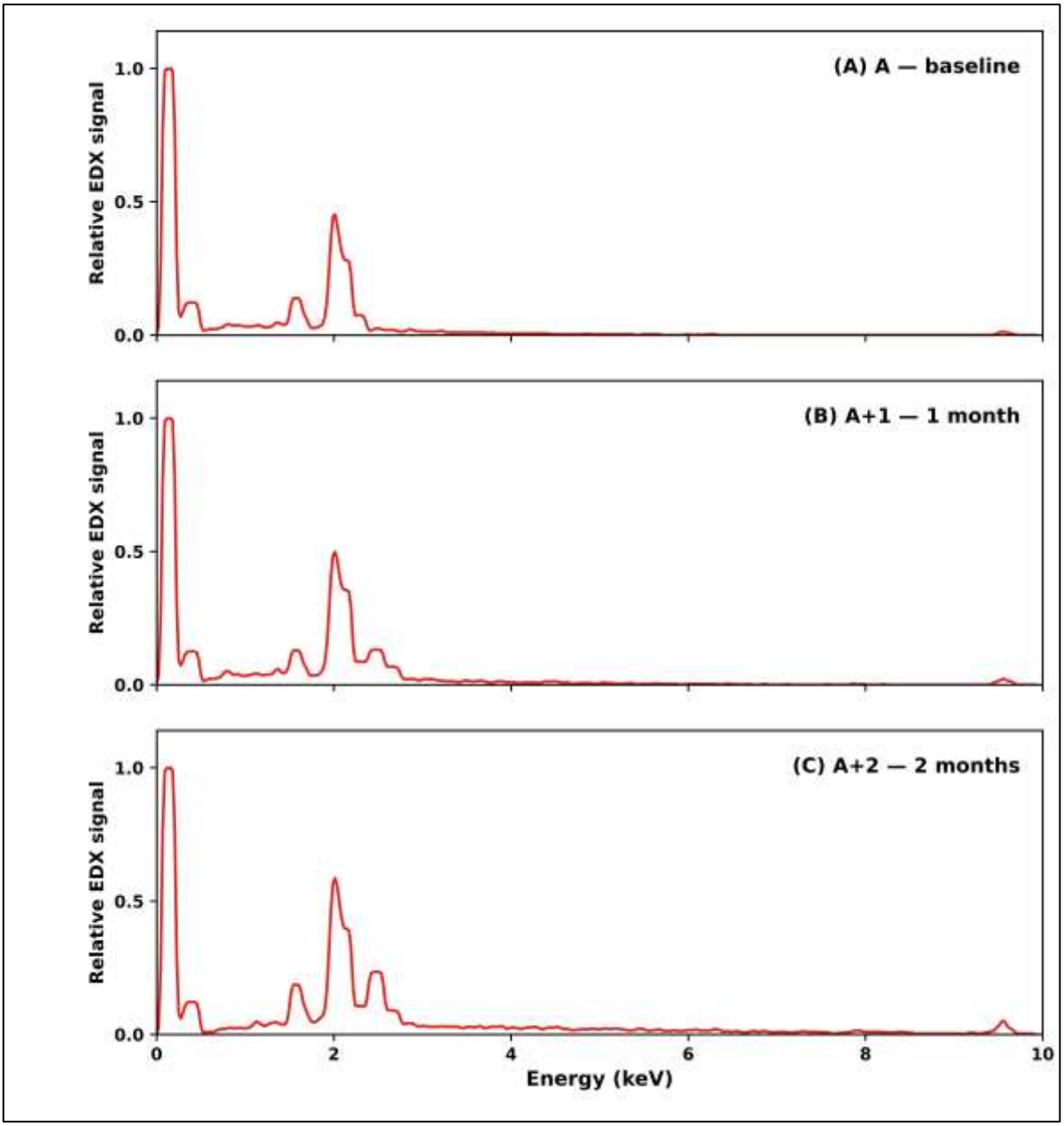


Figure 5. High-resolution redraw of archived EDX spectra for (A) A, (B) A+1 and (C) A+2; signal profiles digitized from the original instrument images and independently normalized within each panel for visual comparison.

The original spectra in Figure 5 are valuable independent archival data that element signals were actually collected for the three imaging stages. The individual spectra were not provided for each new row of the spreadsheet, nor for the time controls (no fungus) so the peaks cannot be used as replicate distributions. Peak heights are not weight percentages and the output of the instrument should be kept if detailed element attribution is desired. This is in line with analytical prudence that calls for a combination of microscopy, spectroscopy and functional assays **[18].**

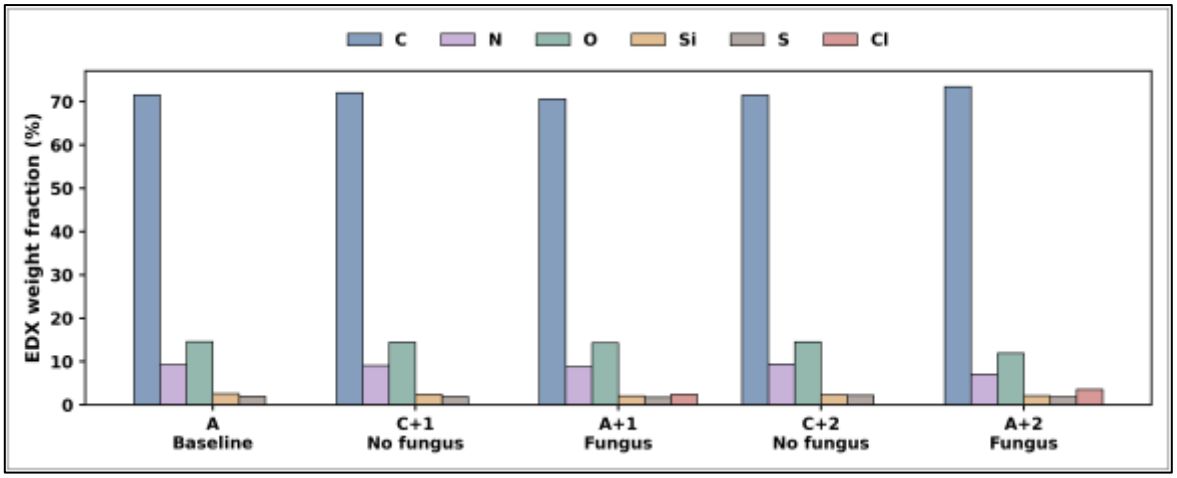


Figure 6. Relative EDX weight percentages for six reported elements in all five groups.

The weight fraction plot indicates that the fungal group had a lower oxygen fraction and that the amount of chlorine was higher in the later fungal group. This does not measure the amount of chlorine that enters or leaves the foam, nor does it prove the oxidation or dechlorination of a particular polymer. Surface selection, fungal biomass and mineral residues and preparation artefacts may still affect local EDX values. The literature on the effects of fungi on plastics can be divided into two categories: evidence of bond-specific change (FTIR/Raman) and changes **[19,20].**

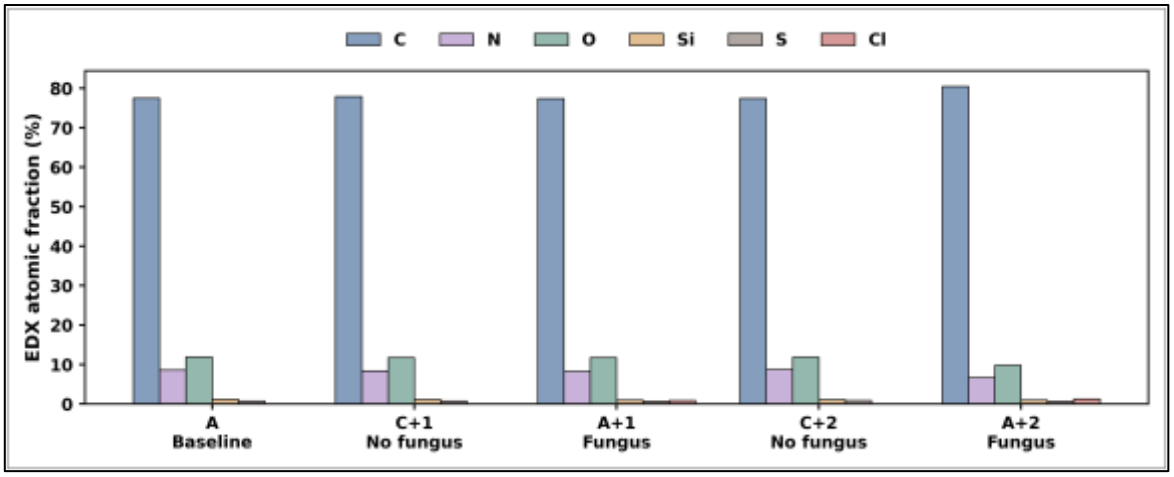


Figure 7. Atomic-percent composition from the same EDX detections, shown separately from the weight-fraction representation.

The atomic-percent plot is the same as the count plot except that the counts are plotted on an atom-number basis. It is not another independent biological assay, but rather an alternative transformation of EDX detections, and apparent agreement between Figures 6 and 7 should not be considered as replicate validation. Specific chemical transformation, compound identification and other methods are required. Comprehensive fungal degradation discussions and multimethod assessments were also found to have the same methodological limitations **[20,21].**

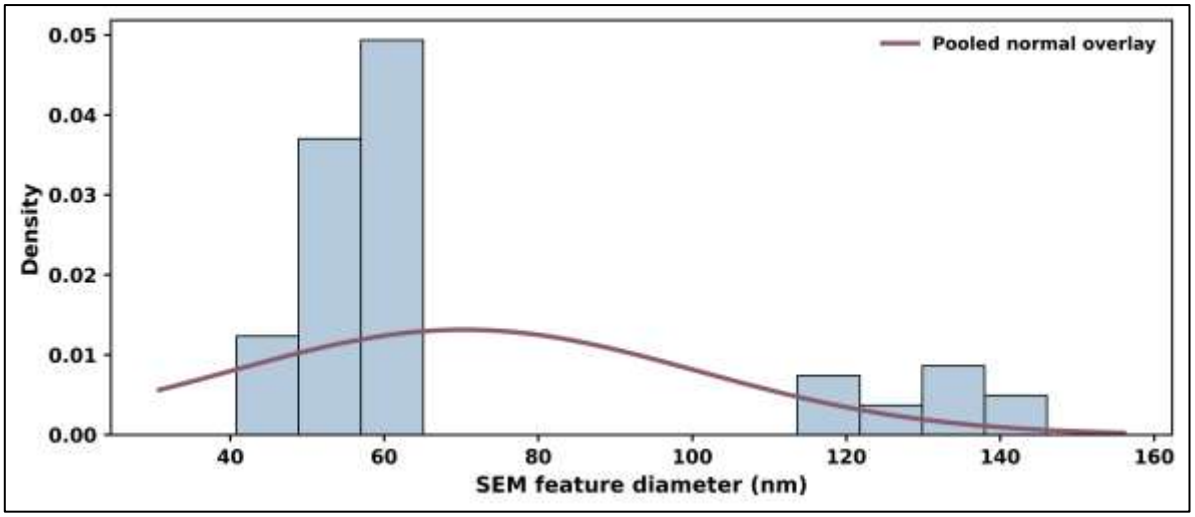


Figure 8. Descriptive histogram of 100 within-record SEM feature dimensions with a pooled normal-density overlay; only 25 record means represent potential independent units.

The histogram is created by combining four feature dimensions from each of the 25 labelled records and by design, groups with very different locations and distributions are combined. Thus, the normal curve is only a visual aid and should not be used to determine the normality of individual treatment groups. These measurements would be considered pseudoreplication if they were combined as n = 100 independent culture replicates. In this report, all inferential statistics have been performed using one average feature diameter per record; the biological independence of the 25 records needs to be verified from the laboratory notebooks. As shown in the previous methodological studies, the quantification of the image is best done if the sampling unit and the limits are well defined **[18].**

3.3. Gravimetric foam mass loss

Table 4. Initial and final foam masses, with gravimetric percentage loss recalculated per record (means ± sample SD).

Group	n	Initial dry mass (g)	Final dry mass (g)	Recorded weight loss (%)
A	5	0.09978 ± 0.00052	0.09978 ± 0.00052	0.00 ± 0.00
C+1	5	0.09950 ± 0.00048	0.09889 ± 0.00039	0.62 ± 0.18
A+1	5	0.09962 ± 0.00046	0.08902 ± 0.00109	10.64 ± 1.30
C+2	5	0.09983 ± 0.00082	0.09825 ± 0.00100	1.58 ± 0.42
A+2	5	0.10003 ± 0.00061	0.07470 ± 0.00262	25.32 ± 2.60

The most direct bulk endpoint is the measured dry foam mass change. The fungus-associated group showed 10.64 ± 1.30% loss after one month versus 0.62 ± 0.18% in the no-fungus control; after two months the means were 25.32 ± 2.60% versus 1.58 ± 0.42%. This resulted in adjusted treatment-associated excesses of 10.02 percentage points at 1 month (95% CI 8.42–11.63), and 23.74 percentage points at 2 months (95% CI 20.53–26.95), both of which were Holm adjusted $P < 0.001$. These group differences are indicative of mass loss due to the treatment and provide more information than a comparison of baseline only because there was mass loss over time without fungus. However, the foam cleaning and drying to constant weight are not recorded and small pieces may be lost during handling, which will impact the measured mass. Gravimetry and complementary imaging and spectroscopy on chemically specified plastics have been used in previous studies, a more solid basis for attributing loss to biodegradation **[19,22].**

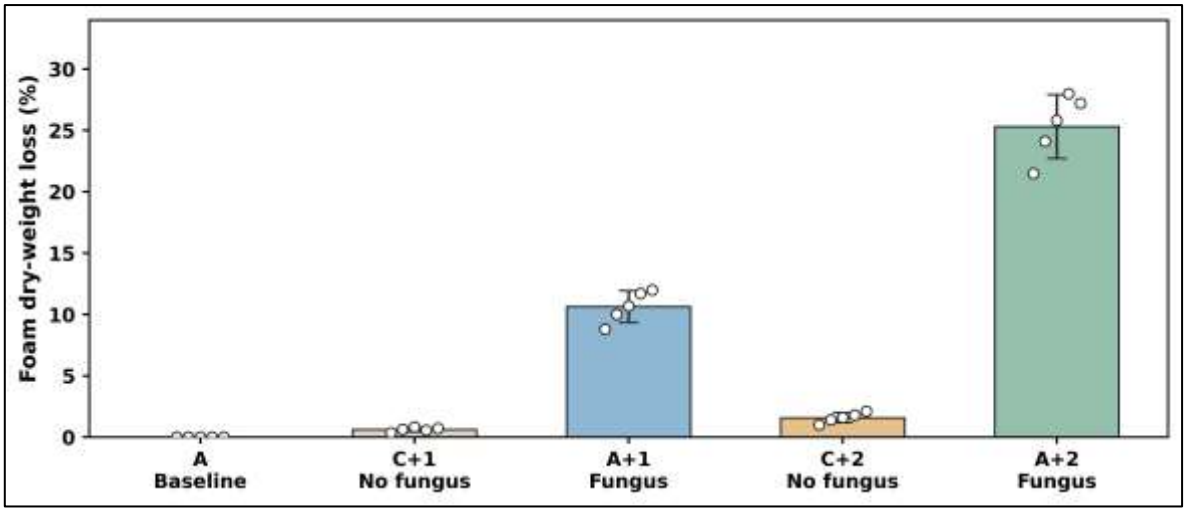


Figure 9. Weight loss (%) across baseline, fungus-free controls and fungal treatments; individual records, means and SD are shown**.**

From the visual trend in figure 9, it is observed that there is a significant difference in the amount of loss after 2 months and 1 month in the fungal treated material. Within the fungal groups, mean loss increased by 14.68 percentage points (95% CI 11.48–17.88; Holm-adjusted $P < 0.001$). The two factor model also showed a significant fungus x incubation interaction for mass loss ($F(1,16) = 108.57$, $P < 0.001$) with the difference between the treatment and control groups increasing with incubation time. The numerical trend does not necessarily imply degradation or mass transfer of the surface, it does not necessarily imply the loss of material is being metabolised, broken up, dissolved or washed away. The difference between gravimetric loss and complete mineralization can be illustrated by the example of the study of polystyrene by isotope tracing **[23].**

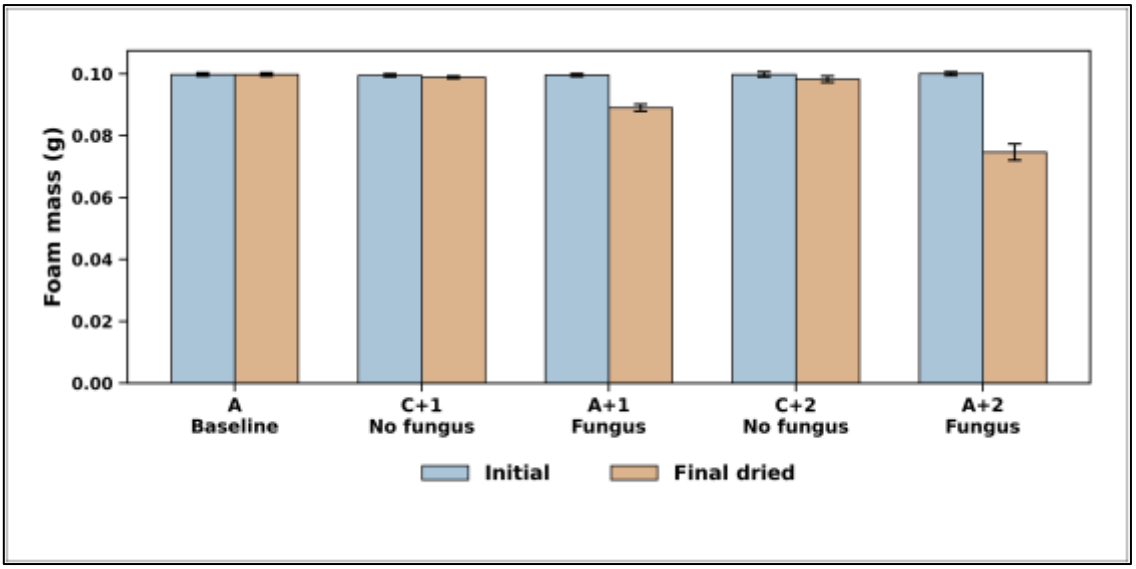


Figure 10. Initial and final recorded dry foam mass (g) by group, mean ± SD.

All groups started with a mass of 0.1 g and the mean mass after the experiment was about 0.0890 g for A+1 and 0.0747 g for A+2. The smaller the initial masses, the less likely that the differences are due to unequal initial masses. However, as biomass, water, medium solids or fragments can be trapped in a foam surface, the recovery, cleaning and drying protocol is important to the direction and amount of the final mass. For instance, gravimetric data obtained in a previous study have been independently verified for polypropylene foam, and the mass-loss data cannot be used as benchmarks for chemically unknown foam **[19].**

3.4. FTIR-derived carbonyl index and medium Ph

Table 5. FTIR carbonyl-index readings, medium pH and C/O ratios (mean ± SD).

Group	FTIR carbonyl index	Initial pH	Final pH	EDX C/O weight ratio
A	0.228 ± 0.011	6.506 ± 0.028	6.340 ± 0.040	4.899 ± 0.087
C+1	0.233 ± 0.009	6.440 ± 0.054	6.236 ± 0.056	4.980 ± 0.127
A+1	0.391 ± 0.023	6.479 ± 0.083	5.880 ± 0.095	4.939 ± 0.061
C+2	0.263 ± 0.016	6.469 ± 0.097	6.154 ± 0.086	4.913 ± 0.102
A+2	0.620 ± 0.016	6.450 ± 0.046	5.471 ± 0.099	6.154 ± 0.139

The FTIR carbonyl index increased from 0.228 ± 0.011 at baseline to 0.391 ± 0.023 in A+1 and 0.620 ± 0.016 in A+2. The matched no-fungus controls were 0.233 ± 0.009 and 0.263 ± 0.016, respectively. Between-group increases were 0.158 after one month (95% CI 0.130–0.186) and 0.357 after two months (95% CI 0.334–0.381). The apparent association of fungal treatment with carbonyl-index values was also correlated with a high interaction with duration ($F(1,16) = 174.04$, $P < 0.001$). However, the raw FTIR absorption spectra, the reference band, the baseline correction and calculation protocol were not provided. The increase should be reported as a change in an index, rather than a confirmed oxidative chemical pathway since the foam polymer is not specified. In previous studies, polymer specific interpretations of FTIR were based on actual band assignment. **[19,21,23].**

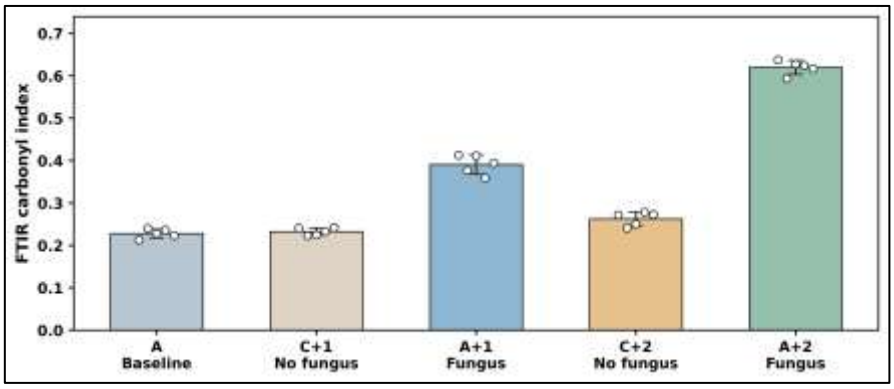


Figure 11. FTIR carbonyl index for the five foam groups, showing five values per group and mean ± SD.

In the fungus-associated groups, the carbonyl-index contrast is raised between 1 and 2 months. It is a potentially useful supplement to the gravimetric result but the index is based on spectroscopy and is not a complete characterization of the peak level. Many of the techniques used in previous studies are included to illustrate the use of an apparent infrared change in combination with a chemically identified substrate and independent observations. The inference is only for numeric index values that are not provided with the raw FTIR spectra here **[18,21].**

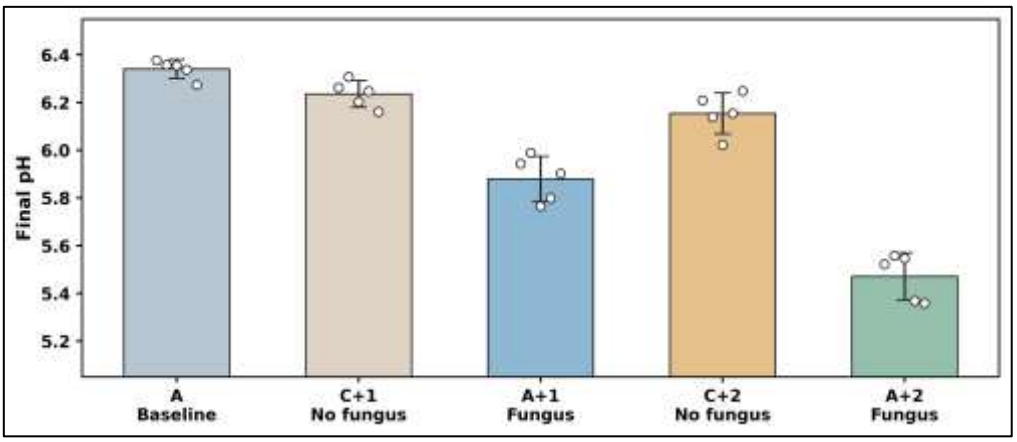


Figure 12. Final medium pH for baseline, one-month and two-month foam groups, with record-level points and mean ± SD**.**

Final pH was 6.340 ± 0.040 at baseline, 6.236 ± 0.056 in C+1 and 5.880 ± 0.095 in A+1; the corresponding two-month values were 6.154 ± 0.086 and 5.471 ± 0.099. The treatment-related pH contrast was −0.357 at one month

(95% CI −0.475 to −0.238) and −0.684 at two months (95% CI −0.819 to −0.548). The fungus-by-time interaction for final pH was significant ($F(1,16) = 18.21$, $P < 0.001$). Acidification is possible during fungal metabolism, even when the desired foam polymer is not utilized as a carbon source, in nutrient-rich PDB. Hence, pH is a good parameter to monitor the alteration of the medium conditions, but is not the only parameter that can indicate the fate of the foam carbon. The pH endpoint is also reported along with the structural and mass loss endpoints as in the previous studies **[23].**

3.5. Statistical comparisons of treatment and duration

Table 6. Two-factor ANOVA of timed groups only (fungus present/absent × one/two months; n = 5 records per cell).

Outcome	Fungus F(1,16)	Time F(1,16)	Interaction F(1,16)
Weight loss (%)	658.03; P < 0.001	141.27; P < 0.001	108.57; P < 0.001
SEM diameter (nm)	141.66; P < 0.001	258.48; P < 0.001	243.47; P < 0.001
FTIR carbonyl index	1162.73; P < 0.001	294.53; P < 0.001	174.04; P < 0.001
Final pH	184.30; P < 0.001	41.00; P < 0.001	18.21; P < 0.001

The factorial analysis separates out differences and duration of the fungus and their interaction. The differences in fungus–control were not consistent over the two periods for all four outcomes, and were significant at $P < 0.001$ for recorded dry-mass loss, SEM feature size, carbonyl index and final pH. The time-zero A group was not used as a fifth cell in this 2 × 2 factorial model. These tests are for detecting associations in the data at the row level, and are not tests of enzymatic polymer cleavage; the five records per group are treated as independent experimental units. Since several endpoints are based on the same specimens, the results cannot be considered as independent mechanistic confirmations as the EDX fractions are compositional. A major drawback of the multimethod analysis **[18,22].**

Table 7. Planned independent-sample Welch contrasts with 95% confidence intervals and Holm adjustment within each outcome. pp = percentage points.

Outcome	Comparison	Mean difference (95% CI)	Holm-adjusted P
Weight loss	A+1 − C+1	10.02 (8.42, 11.63) pp	P < 0.001
	A+2 − C+2	23.74 (20.53, 26.95) pp	P < 0.001
	A+2 − A+1	14.68 (11.48, 17.88) pp	P < 0.001
SEM diameter	A+1 − C+1	-9.42 (-14.76, -4.09) nm	P = 0.004
	A+2 − C+2	70.02 (58.62, 81.41) nm	P < 0.001
	A+2 − A+1	80.64 (69.24, 92.05) nm	P < 0.001
Carbonyl index	A+1 − C+1	0.158 (0.130, 0.186)	P < 0.001
	A+2 − C+2	0.357 (0.334, 0.381)	P < 0.001
	A+2 − A+1	0.229 (0.200, 0.259)	P < 0.001
Final pH	A+1 − C+1	-0.357 (-0.475, -0.238)	P < 0.001
	A+2 − C+2	-0.684 (-0.819, -0.548)	P < 0.001
	A+2 − A+1	-0.409 (-0.550, -0.267)	P < 0.001

Planned contrasts give an indication of the precision of the differences observed as well as an indication of the practical size of the differences. The most significant treatment related effect was seen after 2 months (23.74 percentage points compared to C+2). The direction of the changes in the size of the SEM features was not the same as the direction of the changes in the gravimetric loss: A+1 had smaller features than C+1, and A+2 had much larger features than C+2. This difference is not due to a calculation error but to the difference between the image features and the dry-weight recovery process. Corrected P values should be used for statistical comparison of these specific endpoints recorded and not as evidence of a specific degradation mechanism **[20,22].**

CONCLUSION

The 25-record foam dataset shows that the groups with Aspergillus terreus had substantial loss of dry weight after one month (10.64% vs. 0.62%) and after two months (25.32% vs. 1.58%) compared to the fungus-free time-matched controls. Treatment by incubation ($P < 0.001$) had a significant effect on the recorded weight loss. There were also some differences between the groups in the measurements of SEM features, carbonyl index (FTIR) and final pH and relative EDX elemental composition. The representative original SEM images and EDX traces are given for the evolution of the specimen's appearance and composition during incubation. The original FTIR spectra were not available for all records, however, and the mass-recovery protocol could not be verified from the

spectra provided. Thus, the results indicate that there is a significant difference in the gravimetric loss achieved under the conditions used and the exact biochemical pathway and mineralization of foam carbon is not supported. For more robust biodegradation claims, material identification, documented cleaning/drying steps, and corroborative spectroscopy and degradation-product or carbon-balance measurements would be needed.

STATISTICAL ANALYSIS

There are 25 records in the workbook, divided into 5 groups, with 5 records in each group. The percentage weight loss per record was re-calculated as 100 × (initial dry mass – final dry mass)/initial dry mass. The four SEM dimensions in each record were summarized and one mean calculated per record, group-level variation was calculated from the five record means, not the 100 image measurements, which were not biologically independent. Data are shown as mean ± sample SD. For the timed groups (fungus present/absent × one/two months), a balanced fixed-effects 2 × 2 factorial ANOVA was performed, and three comparisons within each outcome were assessed by independent-sample Welch tests, Welch 95% confidence intervals and Holm adjusted P values. The significance level was 0.05 (two-sided). The results of the pH, gravimetric, FTIR-index and SEM tests are based on the assumption that each row in the workbooks represents an independent sample, which needs to be confirmed from the source records. There are no original spectra for all records and the weight/atomic fractions are compositionally closed, so they have been used descriptively. The 100 dimension SEM histogram is a descriptive one. The statistical graphics are consistent with those found in IBM SPSS Statistics 32 and GraphPad Prism 11, but are not meant to imply that these programs were used to calculate the statistics.

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