D6.8 Biodegradability of the new materials tested as an end-of-life use

Uusien materiaalien biohajoavuus elinkaaren lopussa

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Tested by Measurlabs

Abstract

Biodegradability of the new materials tested as an end-of-life use

This study was commissioned by the Natural Resources Institute Finland (Luke) as part of the PlastLIFE project. The testing was conducted by Measurlabs, and this report was compiled based on the result reports provided.

Aerobic biodegradation testing was performed according to the ISO 17556 CO_2 Evolution Method (similar to ASTM D5988), while anaerobic biodegradation testing was conducted in accordance with ASTM D5511 under high-solids conditions (20–30% total solids) and static, non-mixed anaerobic conditions. Both test setups were carried out in laboratories accredited according to ISO/IEC 17025.

The ISO 17556 soil test demonstrated that most fibre- and mycelium-based materials were readily biodegradable under aerobic soil conditions. Several materials exceeded 80% biodegradation within 90 days.

In contrast, ASTM D5511 anaerobic digestion testing revealed substantially slower degradation rates. This highlights the importance of end-of-life conditions when assessing biodegradability performance.

Overall, the results indicate that the tested materials are considerably more biodegradable under aerobic soil conditions than under anaerobic landfill-like conditions.

Keywords:

Bio-based materials, biodegradable packaging, circular economy, soil biodegradation, anaerobic biodegradation, plastic-replacing materials, end-of-life performance, sustainable materials, environmental impact

Tiivistelmä

Uusien materiaalien biohajoavuus elinkaaren lopussa

Tämä tutkimus toteutettiin Luonnonvarakeskuksen (Luke) toimeksiannosta osana PlastLIFE-hanketta. Testaukset suoritti Measurlabs, ja tämä raportti on koottu heidän toimittamiensa testitulosten perusteella.

Aerobisen biohajoavuuden testaus suoritettiin ISO 17556 CO_2 Evolution Method -menetelmän mukaisesti (samankaltainen kuin ASTM D5988), kun taas anaerobinen biohajoavuustestaus toteutettiin ASTM D5511 -menetelmän mukaisesti korkeassa kiintoainepitoisuudessa (20–30 % kokonaiskiintoainetta) staattisissa, sekoittamattomissa anaerobisissa olosuhteissa. Molemmat testit toteutettiin ISO/IEC 17025 -akkreditoiduissa laboratorioissa.

ISO 17556 -menetelmällä tehty maaperätesti osoitti, että suurin osa kuitu- ja myseelipohjaisista materiaaleista biohajosi hyvin aerobisissa maaperäolosuhteissa. Useiden materiaalien biohajoavuus ylitti 80 % 90 vuorokauden kuluessa.

Sen sijaan ASTM D5511 -menetelmän mukaisessa anaerobisen mädätyksen testissä hajoamisnopeudet olivat huomattavasti hitaampia. Tämä korostaa loppukäsittelyolosuhteiden merkitystä materiaalien biohajoavuuden arvioinnissa.

Kokonaisuudessaan tulokset osoittavat, että testatut materiaalit biohajoavat merkittävästi paremmin aerobisissa maaperäolosuhteissa kuin anaerobisissa kaatopaikkaa muistuttavissa olosuhteissa.

Asiasanat: biopohjaiset materiaalit, biohajoavat pakkaukset, kiertotalous, maaperässä tapahtuva biohajoaminen, anaerobinen biohajoaminen, muovia korvaavat materiaalit, elinkaaren loppuvaiheen suorituskyky, kestävät materiaalit, ympäristövaikutukset

Contents

1 Biodegradation

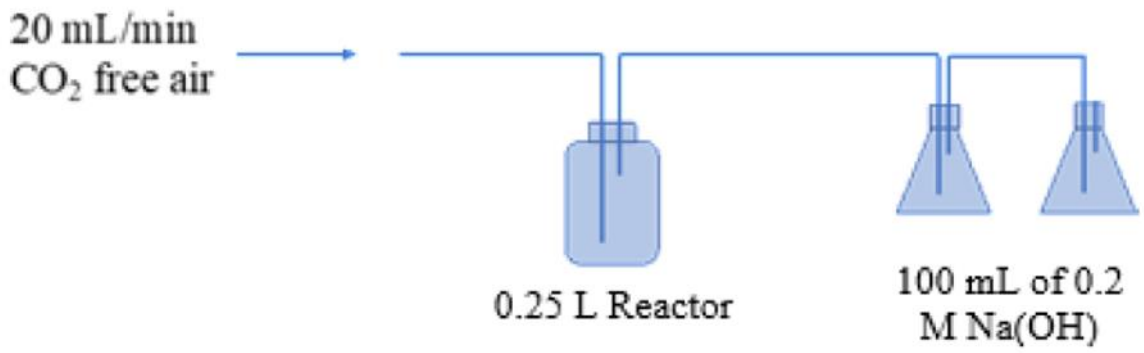
Biodegradation is defined as a natural process in which micro-organisms, such as fungi and bacteria, break down substances. This report provides an overview of the aerobic and anaerobic biodegradation of the novel materials developed to replace plastics. The materials are described in detail in the public deliverable report *D6.6 Report on the technical and mechanical properties of new plastic-replacing materials*.

1.1 ISO 17556 Aerobic biodegradation in soil, 90 days

The ultimate aerobic biodegradability in soil of the samples was determined following the ISO 17556 CO_2 Evolution Method (similar to ASTM D5988). In the CO_2 evolution test, natural soil was dosed with a known amount of test item as the nominal sole source of organic carbon and aerated with CO_2 free air at 10-30 mL/min at room temperature. The biodegradation of the test item was determined by measuring the CO_2 evolution.

The soil was collected from the surface layer (0.2 m) of a garden in the suburb of Cleveland, Ohio, on 6 January 2026, using a thoroughly cleaned container, and transported to the laboratory within 2 hours of collection. The garden had trees and had not previously been used to grow crops or flowers. During transport, the temperature of the sample was not allowed to significantly exceed the temperature to be used in the test. The soil was sieved to obtain particles less than 2 mm in size, and obvious plant material, stones and other inert materials were removed. The soil was preconditioned by aeration before use to reduce background CO_2 formation. No inoculum other than the micro-organisms already present in the soil was added.

The CO_2 evolution test was performed using standard reactor and CO_2 trap systems. Specifically, 0.25 L amber bottles were used as the biodegradation reactors, each containing 100 g of soil. The CO_2-free air was obtained by passing air through three soda lime columns in series to remove any CO_2 originally present in the air. The generated CO_2 was captured in two CO_2 absorption flasks, each containing 100 mL of 0.2 M sodium hydroxide.



Scheme 1. ISO 17556 setup

The reactors were prepared based on the following steps:

(1) Place 100 g of soil at the bottom of each flask.

(2) Add 300 mg of test material or 300 mg of reference material to the soil; this step was omitted for the blank control.

(3) Ensure that the test material is homogeneously mixed with the soil in the case of powder, and spread as widely as possible in the soil in the case of film, to improve contact between the test material and the micro-organisms in the soil.

(4) Press the surface of the test mixture with a spatula to improve the contact between the test material and the microorganisms in the soil.

Two 150 mL flasks, each containing 100 mL of 0.2 M sodium hydroxide solution, were connected in series to each reaction bottle as CO_2 absorption bottles to recover the CO_2 generated during the biodegradation process.

Once the working bottles and CO_2 absorption bottles had been prepared, they were connected in series. CO_2-free air was then pumped through the entire series at a rate of approximately 100 mL/min for each bottle to initiate the reactions. Incubation was maintained at a constant temperature within ± 2 °C in the range of 20–28 °C.

When a constant level of CO_2 release was attained (plateau phase reached) and no further biodegradation was expected, the test was considered to be completed. On the last day of the study, once all CO_2 evolution measurements had been taken, pumping of the CO_2-free air was stopped and the working flasks and CO_2 absorption bottles were disassembled to terminate the tests.

During the first four weeks, the analysis of CO_2 was generally made every three to four days, and then every ten to twenty days until the end of the test. On the days of CO_2 measurement, the CO_2 absorber closest to the test vessel was disconnected and the inorganic carbon (IC) in the absorber was determined. The percentage biodegradation was then calculated from:

$$\%\ degradation = (mg\ IC_{\ from\ test\ flask} - mg\ IC_{\ from\ blank}) / mg\ TOC_{\ added\ in\ the\ reactor} \times 100$$

1.1.1 Results

The soil organic carbon and total carbon contents were 4.62% and 5.77%, respectively. The organic carbon-to-nitrogen ratio was measured as 16.5:1, which was within the recommended range. Water content and soil pH were maintained at approximately 50% and 7, respectively, before use.

The amount of CO_2 formed from the test item, corrected for CO_2 derived from the blank control, was expressed as a percentage of theoretical CO_2 formation ($ThCO_2$). $ThCO_2$ is defined as the amount of CO_2 formed when all carbon is converted to CO_2. The test consisted of a blank control group, a reference group and a test group, each containing three replicates. The blank control was used to measure background CO_2 evolution by the micro-organisms alone and was not dosed with any carbon source. Each replicate of the reference group was dosed with microcrystalline cellulose, a substance known to be biodegradable, at a nominal dosage of 300 mg substance per 100 g of soil. The test item group was also dosed at a nominal dosage of 300 mg item per 100 g of soil for each replicate. The final average biodegradation results are summarised in **Table 1**, and individual aerobic biodegradation percentage results over time are presented in **Figures 1–3**.

Table 1. Aerobic biodegradation of the samples in soil for 90 days

Sample	Biodegradation Average	60% threshold reached
Cellulose Microcrystalline (Reference)	86.1 ± 3.7%	Day 31
Paperboard + Tomato	87.4 ± 1.4%	Day 49.5
Paperboard + Cucumber	114.3 ± 7.5%	Day 34
Mycelia A	86.3 ± 14.6%	Day 38
Mycelia B	54.2 ± 14.7%	Not reached
Mycelia C	83.6 ± 14.3%	Day 28.5
Mycelia D	77.9 ± 6.7%	Day 62
Fish gelatine	33.7 ± 9.0%	Not reached

For the aerobic tests, the cellulose reference sample exceeded 60% degradation by day 31 and reached a final value of 86.1 ± 3.7% by day 90. This indicates that the soil was biologically active and that the system functioned properly.

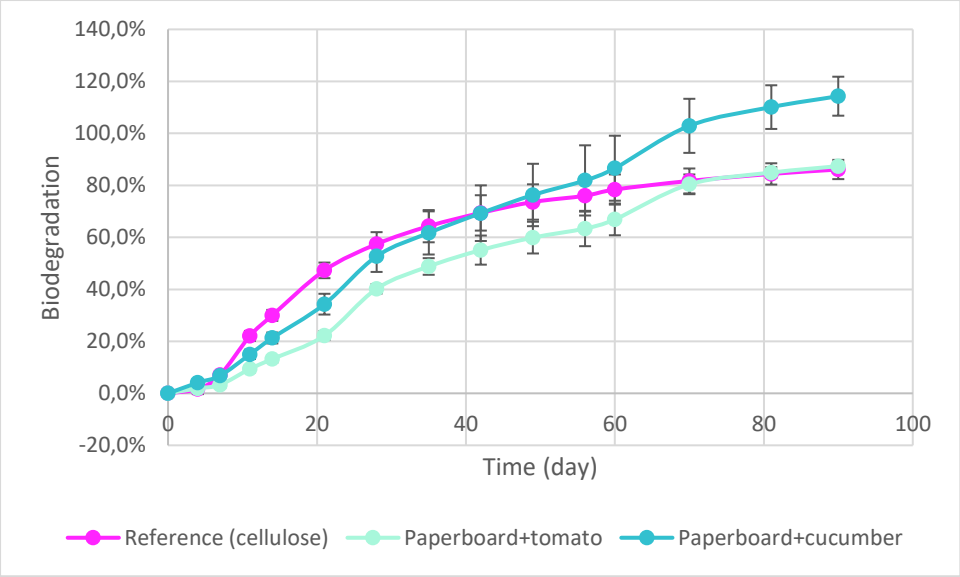


Fig 1. Aerobic biodegradation of cellulose reference and paperboard samples reinforced with tomato and cucumber fibres during a 90-day incubation period.

The aerobic biodegradation profiles differed between the tested paperboard composites and the cellulose reference. The cellulose reference exhibited rapid degradation during the first 20 days, reaching approximately 47% biodegradation, and gradually increased to a final value of 85.3% after 90 days.

Paperboard reinforced with tomato fibres showed a slower degradation rate throughout the experiment. Biodegradation reached approximately 60% by day 55 and continued to increase steadily, reaching a final biodegradation of 87.4% at day 90. In contrast, paperboard reinforced with cucumber fibres exhibited the highest biodegradation among the tested materials. The material reached approximately 60% biodegradation by day 34, surpassed 100% biodegradation around day 70, and achieved a final value of 114.3% at the end of the test period.

Overall, the cucumber fibre-reinforced paperboard degraded more rapidly and extensively than both the tomato fibre-reinforced paperboard and the cellulose reference, whereas the tomato fibre-reinforced paperboard showed a degradation pattern similar to the reference material during the later stages of incubation. Error bars indicate the variability between replicate measurements.

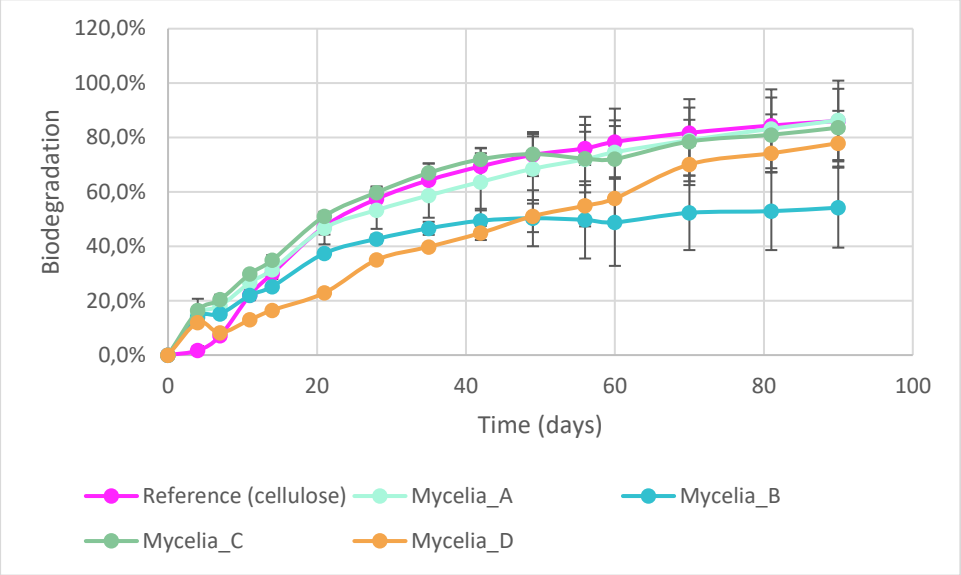


Fig 2. Aerobic biodegradation of cellulose reference and mycelial composite samples during a 90-day incubation period.

The aerobic biodegradation behaviour of the mycelial composite samples varied among the tested materials but was generally comparable to that of the cellulose reference. The cellulose reference exhibited rapid biodegradation during the first 30 days, reaching approximately 60% biodegradation by day 30 and a final value of 86.0% after 90 days.

Mycelia_A and Mycelia_C showed biodegradation profiles closely resembling that of the cellulose reference throughout the incubation period. Both materials reached approximately 60% biodegradation by day 28 and achieved final biodegradation values of 85.0% and 84.0%, respectively, after 90 days. Mycelia_D degraded more slowly during the initial stages of the test, reaching approximately 50% biodegradation by day 49 and a final biodegradation value of 78.0% at the end of the incubation period.

In contrast, Mycelia_B exhibited the lowest biodegradation among the tested composites. The material reached approximately 50% biodegradation by day 49 and showed only limited further degradation thereafter, resulting in a final biodegradation value of approximately 54.0% after 90 days.

Overall, Mycelia_A, Mycelia_C and Mycelia_D exhibited biodegradation levels comparable to the cellulose reference, reaching final biodegradation values of 78–85% after 90 days. In contrast, Mycelia_B showed substantially lower biodegradation, reaching only approximately 54% at the end of the test period.

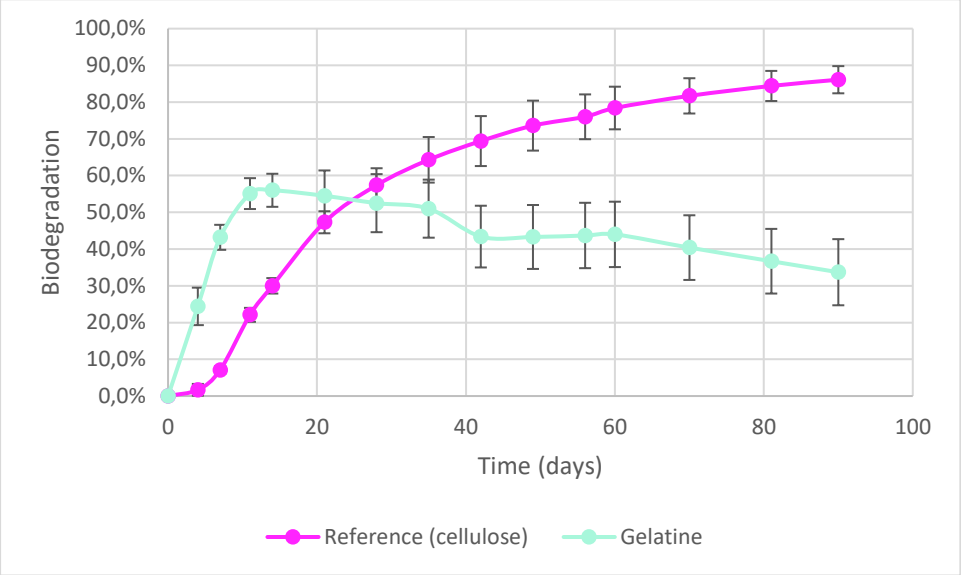


Fig 3. Aerobic biodegradation of cellulose reference and gelatine samples during a 90-day incubation period.

The aerobic biodegradation behaviour of the gelatine sample differed markedly from that of the cellulose reference. The cellulose reference exhibited a steady increase in biodegradation throughout the test period, reaching approximately 69% biodegradation by day 42 and a final value of 86.0% after 90 days.

The gelatine sample showed rapid initial biodegradation, reaching approximately 55% biodegradation within the first 10 days of incubation. Thereafter, the biodegradation curve reached a plateau and gradually declined over the remainder of the test period. By day 90, the measured biodegradation of the gelatine sample had decreased to 33.7%.

The decline in biodegradation observed after the initial rapid degradation phase may be attributed to changes in the accessibility of the remaining degradable material. As the readily degradable fraction was consumed, the remaining gelatine network may have acted as a physical barrier, limiting microbial access to residual biodegradable components and restricting further biological activity. This phenomenon has been reported for materials forming dense polymeric networks, which can hinder microbial colonization and reduce the degradation rate during later stages of incubation.

Overall, although the gelatine sample exhibited rapid initial biodegradation, the material did not maintain the degradation trend observed for the cellulose reference and reached a substantially lower final biodegradation level after 90 days. Error bars indicate the variability between replicate measurements.

1.2 ASTM D5511 Anaerobic biodegradation

The anaerobic biodegradability of the mycelial samples was determined according to ASTM D5511 under high-solids conditions (20–30% total solids) and static, non-mixed anaerobic conditions for 62 days.

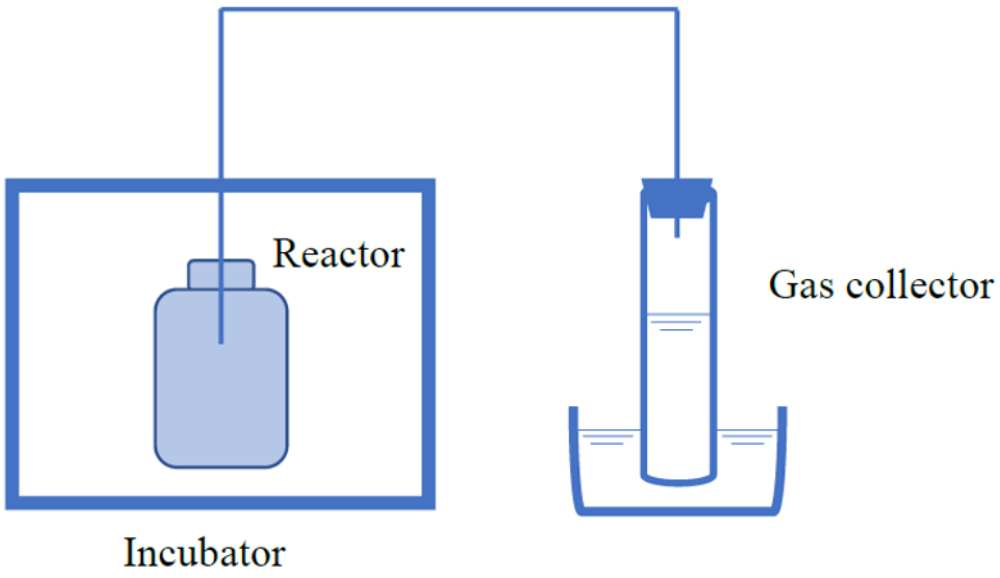
In the test, anaerobically digested sludge was dosed with a known amount of test item as the nominal sole source of organic carbon and incubated at 37 °C. The biodegradation of the test item was

determined by measuring biogas formation, mainly CO_2 and methane. The biogas was collected using inverted plastic columns in water with a pH below 2 to measure gas volume.

The amount of biogas formed from the test item, corrected for biogas derived from the blank control, was converted to carbon weight and expressed as a percentage of the original carbon added. The test consisted of a blank control group, a reference group and a test group, each containing three replicates. The blank control was used to measure background biogas evolution by the micro-organisms alone and was not dosed with any carbon source. Each replicate of the reference group was dosed with microcrystalline cellulose, a substance known to be biodegradable, at a nominal dosage of 15 g reference item per 1,000 g of sludge. The test item group was also dosed at a nominal dosage of 15 g test item per 1,000 g of sludge for each replicate.

The inoculum was derived from a properly operating anaerobic digester using pretreated household waste as the sole substrate. To maximise inoculum activity, post-fermentation preconditioning was not performed. The pH of the inoculum was maintained between 7.5 and 8.5, and the volatile fatty acid (VFA) concentration was kept below 1 g/kg wet weight. The NH_4^+-N concentration was maintained between 0.5 and 2 g/kg wet weight.

The tests were performed using the system shown in **Scheme 2**. Glass bottles of 2 L were used as the biodegradation reactors, each containing 1,000 g of inoculum. The produced biogas was collected using inverted plastic columns in water with a pH below 2 to measure gas volume.



Scheme 2. ASTM D5511 setup

The reactors were prepared based on the following steps:

(1) Place 1,000 g of wet inoculum (20-30% solids) at the bottom of each flask.

(2) Add 15 g test material or reference material to the inoculum (ignore this step for blank control).

(3) Ensure that the test material is homogeneously mixed with the inoculum in the case of powder, and spread as widely as possible in the inoculum in the case of film, to improve contact between the test material and the micro-organisms in the inoculum.

(4) Keep the reactors at 37 °C throughout the testing process.

As illustrated in **Scheme 2**, inverted plastic columns filled with acidified water were used to collect the formed biogas. The pH of the water was kept below 2 using sulphuric acid to minimise CO_2 dissolution in water. Once all the working bottles and gas collectors had been prepared, they were connected as illustrated in **Scheme 2**. Incubation was maintained at a constant temperature of 37 ± 2 °C. The tests were continued until no further biodegradation was observed.

When a constant level of gas production was attained (plateau phase reached) and no further biodegradation was expected, the test was completed. At the end of the testing period, the setup was allowed to cool to room temperature for 8 h, after which the following were determined:

(1) total volume of gas production.
(2) gas composition (CO_2 to methane ratio) at the end of the test.
(3) weight loss of each vessel.
(4) room temperature and atmospheric pressure.

During the first two weeks, gas production was generally measured five times a week and then every three to seven days, or longer, until the end of the test. On the days of gas volume measurement, the volume in the inverted columns was recorded. The increase in volume compared with the original value was taken as the volume of formed biogas.

Given that each mole (12 g) of organic carbon from the test sample can be converted into 1 mole of gaseous CH_4, CO_2, or both, and that one mole of gas occupies 22.4 L at standard temperature and pressure (STP), the measured gas production volume can be converted to the corresponding carbon weight. Temperature and pressure were measured when the conditions differed from standard conditions and were used to convert the gas volumes to STP. Biodegradation was then calculated as follows:

$$\%\ biodegradation = (mean\ Cg,test - meanCg,blank)/Ci,test$$

where Cg indicates the carbon weight in the gas phase, while Ci indicates the carbon weight in the added test compound.

1.2.1 Results

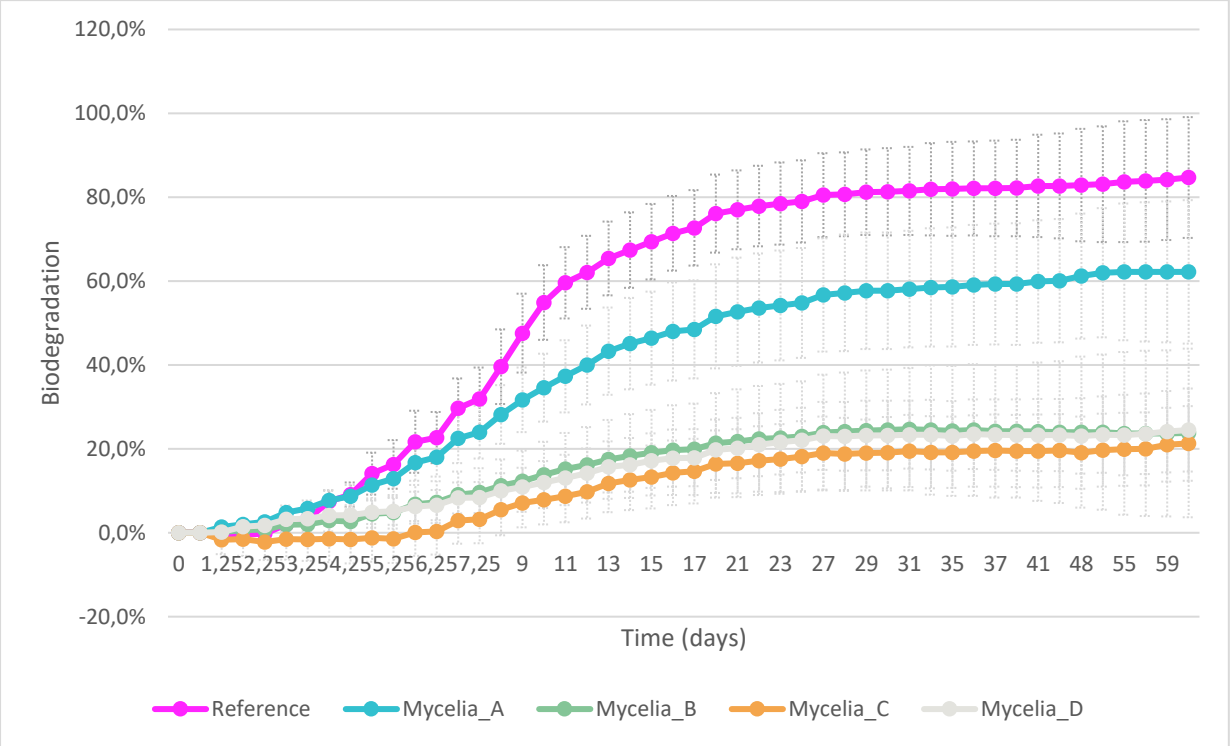


Fig 4. Anaerobic biodegradation of the cellulose reference and mycelial composite samples during a 62-day incubation period.

The anaerobic biodegradation of the mycelial composite samples was lower than that of the cellulose reference throughout the incubation period (**Fig 4**). The cellulose reference exhibited the highest biodegradation, increasing rapidly after day 7 and reaching approximately 85% biodegradation after 62 days.

Among the tested mycelial composites, Mycelia_A showed the highest anaerobic biodegradation. The material reached approximately 35% biodegradation by day 11 and continued to degrade steadily throughout the test, achieving a final biodegradation of approximately 62% after 62 days. In contrast, Mycelia_B, Mycelia_C, and Mycelia_D exhibited considerably lower biodegradation rates. These materials reached final biodegradation values of approximately 24%, 21%, and 24%, respectively, at the end of the incubation period.

The most pronounced increase in biodegradation occurred during the first 10–20 days for all samples, after which the degradation rates gradually declined and the curves approached a plateau. While the cellulose reference continued to show substantial biodegradation throughout the test period, the mycelial composites generally exhibited slower and less extensive degradation under anaerobic conditions.

Overall, Mycelia_A demonstrated the greatest anaerobic biodegradability among the tested mycelial materials, reaching approximately 73% of the biodegradation level of the cellulose reference. The remaining composites showed limited biodegradation, suggesting that their degradation under anaerobic conditions was substantially slower than under aerobic conditions. Error bars indicate the variability between replicate measurements.

2 Conclusions

The tested bio-based materials exhibited diverse biodegradation behaviours depending on their composition and degradation environment.

- Both paperboard composites showed high aerobic biodegradability, with the cucumber fibre-reinforced material demonstrating the highest overall degradation.
- Most mycelial composites achieved aerobic biodegradation levels comparable to the cellulose reference, indicating good biodegradation potential in oxygen-rich environments.
- The gelatine-based material exhibited rapid initial degradation but reached a relatively low final biodegradation level due to a progressive reduction in degradation rate during the incubation period.
- Anaerobic biodegradation was generally lower than aerobic biodegradation for all tested materials.
- Among the mycelial composites, only Mycelia_A exhibited moderate anaerobic biodegradability, while the remaining samples showed limited degradation under anaerobic conditions.

These findings demonstrate that several of the investigated bio-based materials constitute promising alternatives to conventional plastics from an end-of-life perspective, particularly in environments supporting aerobic biodegradation. However, the observed differences between aerobic and anaerobic degradation emphasise the importance of considering disposal conditions when evaluating the environmental performance and circularity of new bio-based materials.

Although no conventional plastic reference material was included in this study, the observed biodegradation levels provide important evidence of the end-of-life performance of the investigated bio-based materials. Future studies would benefit from the inclusion of representative fossil-based and commercially available biodegradable plastic reference materials to enable direct benchmarking. Such comparisons would further support the assessment of environmental performance and application-specific suitability. However, the high biodegradation levels observed for several of the tested materials, particularly under aerobic conditions, indicate considerable potential for reducing the persistence typically associated with conventional plastic products.

References

ISO 17556:2019 Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved.

ASTM D5511-18, Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under High-Solids Anaerobic-Digestion Conditions.