# Comparative Soil Burial and Microbial Degradation of Starch-Based Bioplastics

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***Abstract***

*Starch-based films are renewable materials with potential for short-lived packaging and agricultural applications, but soil-burial mass loss depends on formulation, film geometry, plasticizer leaching, soil properties, environmental conditions, and microbial activity. To compare the four-week soil-burial mass-loss proxy of locally extracted wheat- and rice-starch films prepared under the reported nominal formulation, and to describe presumptive culturable bacterial morphotypes recovered from the surrounding soil. Films were prepared from 2 gram (g) starch, 100 mL distilled water, 5 mL glycerol, and 2 mL vinegar or acetic acid, then buried approximately 5 centimeter (cm) deep in garden topsoil for four weeks. The source record reported three samples per starch type, but did not document whether they were independent biological batches or technical subsamples. Endpoint mass-loss percentages were reconstructed from the displayed replicate masses. Welch and exact permutation tests were calculated, with the exact permutation test designated as the primary inference because n = 3 per group. Wheat films showed a mean endpoint mass-loss proxy of 75.98 % (SD = 2.09 %) and rice films 50.95 % (SD = 8.61 %), a mean difference of 25.02 percentage points. The reconstructed Welch test gave t (2.24) = 4.89, p = 0.031, whereas the exact two-sided permutation test gave p = .10. Wheat films showed greater endpoint mass loss under the reported preparation and burial conditions, but the experiment does not isolate starch source as the causal determinant.*

***Keywords:*** Starch Bioplastic, Wheat Starch, Rice Starch, Soil Burial, Mass-Loss Proxy, Plasticizer, Microbial Morphotypes, Experimental Design

## Introduction

The terms petroleum derived plastics, bio-based, biodegradable and compostable are often used interchangeably, though they represent different properties, and petroleum derived plastics last in terrestrial and aquatic environments. However, factors such as the structure of the polymer, additives, geometry, receiving environment and end-of-life

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conditions can all affect environmental performance, making the bio-based material not always biodegradable in natural soil (Lambert & Wagner, 2017; Fòlino et al., 2020).

Starch is a good polysaccharide for film formation and the relatively simple solution casting method is employed for this. The behavior of the film is known to be influenced by factors such as starch source, amylose–amylopectin ratio, gelatinization, crystallinity, water absorption capacity, plasticizer concentration and thickness of the film (Marichelvam et al., 2019; Surendren et al., 2022; Vaz Matheus et al., 2023). The structure, film strength, and loss of weight could also be changed due to the use of some additives and processing conditions, glycerol can also improve the water sensitivity and flexibility of films (Shafqat et al., 2020, 2021).

Soil-burial tests provide good screening tests; however, percentage mass loss is not synonymous with ultimate biodegradation. It is important to note that the sample may not be entirely converted into polymer CO2, water, biomass and inorganic products, meaning that mass can be lost from the sample through dissolution, fragmentation or leaching of soluble components (Pires et al., 2022; Polman et al., 2020; Zambrano et al., 2020). ISO 17556:2019 and ASTM D5988 are based on the measurement of oxygen demand, or the production of evolved carbon dioxide, for ultimate aerobic biodegradation evaluations.

There is also a variability in the soil environment. Microbial activity and polymer transformation is affected by moisture, pH, temperature, texture, organic matter, nutrient status and oxygen availability. The degradation rate and the structure of the microbial community can change with different soil materials, soil environment, temperature and time (Guliyev et al., 2022; Romano et al., 2024; Samuelson et al., 2021).

There are many studies that focused on one starch source, one commercial starch–polymer blend and one composite film, and this is a challenge for comparison but this pilot study attempts to fill the gap with a very specific and methodological approach. This study is a comparison of mass data for the wheat-starch and rice-starch films produced locally in the same garden-soil experiment done nominally for four weeks, and mass-loss proxy information, with the addition of some microbial information that is presumed. Film geometry, purity of the material, the environment and the process of replications were not completely documented, so the work is exploratory.

The goals were to compare endpoint mass-loss assays for wheat-starch and rice-starch films; to document the challenges involved with culturing and reporting data that affects interpretation relating to the buried

soil film; and to report on culturable bacterial morphotypes recovered from the wheat-starch and rice-starch film without assigning a species identity or degradative function.

## Methodology

### *Materials and Starch Extraction*

The wheat and rice were bought from local market in Lundkhwar, Pakistan. Wheat grains were washed and soaked for three days while rice grains were washed and soaked overnight. A slurry was made with each material and the slurry was filtered, settled, decanted and dried to recover the sedimented starch fraction.

The recipe documented for each starch source was 2.00 gram (g) of extracted starch, 100 milliliters (mL) of distilled water, 5.0 mL of glycerol and 2.0 mL of vinegar or acetic acid. No information was provided regarding the grade or purity of glycerol or acetic acid, the density or concentration of either and no information was provided about the supplier. The term vinegar was used inconsistently with the term acetic acid. These should be checked from the laboratory records before submitting and should not be assumed from the size of the volume. Nutrient agar: 3.5 g per 125 mL of water (or 28 g/L).

### *Film Preparation and Replicate Definition*

The starches were all heated with magnetic stirring for 30 minutes at about 80 °C and then cooled to room temperature and dried in the air for three days on flat trays or aluminum foil. The record did not state the casting area, wet-film volume per unit area, final thickness, density, residual moisture, or batch-to-batch variability.

The source record describes three replicate samples per starch type. Each reported replicate consisted of one film sample with its own initial mass and final mass. However, the available documentation does not establish whether the three samples were generated from three independent starch-extraction and film-preparation batches, which would support biological or batch-level replication, or were cut from one preparation batch, which would make them technical subsamples. Accordingly, this manuscript refers to them as reported sample-level replicates and does not claim confirmed biological independence.

A confirmatory design should prepare at least three independent batches per starch source, cut multiple coupons from each batch to identical dimensions, and use the batch as the experimental unit or model batch as a random effect. Initial dry mass, area, thickness, and mass per area should be standardized before burial for both starch sources. The

present dataset did not meet these requirements, and the unequal starting masses cannot be corrected post hoc.

### *Soil Burial and Environmental Conditions*

Prepared films were buried approximately 5 centimeter (cm) deep in garden topsoil collected from a house-lawn area in Lundkhwar and monitored for four weeks. The initial mass for wheat films and rice films was reported as 1.8 g and 0.6 g, respectively. As a qualitative visual test, the conventional plastic sample was buried with a starting mass of 2.0 g, but without reporting the final mass. No data were collected or reported for temperature, rainfall, relative humidity, irrigation, soil moisture, pH, texture, organic matter, carbon-to-nitrogen ratio, oxygen status, and background microbial biomass. So, it is not possible to be certain of the environmental conditions during the burial interval. For repeat studies, samples must be placed in a mosaic soil under either field conditions (with temperature and moisture logged) or a controlled chamber (with temperature and moisture logged).

### *Weighing and Degradation Proxy*

Weight loss was determined by subtracting final weight from initial weight. Degradation (%) was estimated as follows: Degradation (%) = [(Initial weight - Final weight) / Initial weight] × 100. The term mass-loss proxy is used here because carbon mineralization, oxygen consumption, leaching from the residual moisture, and from the soluble components were not measured separately.

The mass source table reports masses to one decimal gram. There were no records of balance model, readability, calibration status, calibration standard, tare procedure, drying-to-constant-mass procedure or number of repeat weighing's. Accordingly, the precision and accuracy with which the reported measurements were taken cannot be independently verified. A confirmatory protocol should include a calibrated balance with documented readability, weigh samples after a specified drying and equilibration time, include duplicate readings and report the balance calibration check.

### *Microbial Isolation and Presumptive Characterization*

About 1g of soil was sampled from the area around the films after four weeks, serially diluted in sterile distilled water, and then plated on nutrient agar and incubated for about 24 to 28 hours. Representative colonies were sub cultured. The observations were made based on the following: colony morphology, cell morphology, gram reaction. The observations reported as presumptive bacterial morphotypes. There was

no biochemical identification, MALDI-TOF analysis, measurement of abundance, enzyme assay, or isolate-level degradation test reported.

### *Statistical Analysis*

The endpoint percentages of the replicates were calculated from the initial and final masses shown. Means and sample standard deviations were kept. The Welch two-sample t-test was used as a secondary parametric description since the variances of the observed groups were unequal and pooling of groups of equal variances would be hard to justify. The Welch test is not considered the principal test since the normality and independence can't be determined for n = 3 per group.

The most important inference was the two-sided exact permutation test. It listed all 20 distinct assignments for the 6 listed endpoint values in two sets of three, thus not depending on a large sample approximation. This test can serve as a way to conduct a small-sample robustness analysis in a manner that is transparent, but it still requires exchangeability of the observations. As a sensitivity analysis, an exact Mann-Whitney U test was performed. All statistics are necessarily reconstructed and exploratory, as it is not possible to provide the original raw weighing records (Table 1).

***Table 1: Replicate-level mass and endpoint mass-loss proxy data.***

| Material | Replicate | Initial mass (g) | Final mass (g) | Mass loss (g) | Mass-loss proxy (%) |
|---|---|---|---|---|---|
| Wheat starch | 1 | 1.8 | 0.4 | 1.4 | 77.78 |
| Wheat starch | 2 | 1.7 | 0.4 | 1.3 | 76.47 |
| Wheat starch | 3 | 1.9 | 0.5 | 1.4 | 73.68 |
| Rice starch | 1 | 0.6 | 0.3 | 0.3 | 50.00 |
| Rice starch | 2 | 0.5 | 0.2 | 0.3 | 60.00 |
| Rice starch | 3 | 0.7 | 0.4 | 0.3 | 42.86 |

* Initial masses were recorded to the nearest 0.1 g. Unequal initial masses, together with unreported film thickness, coupon dimensions, and surface area, prevent attribution of the observed degradation differences solely to starch source.

## Results

### *Film Appearance Before Burial*

Both starch sources produced visible films. The wheat-starch film was described as relatively rigid and harder to handle, whereas the rice-starch film was described as smoother, thinner, and more flexible. These observations were qualitative and were not accompanied by tensile strength, elongation, thickness, moisture, contact-angle, or microscopy measurements.

### *Endpoint Mass-Loss Proxy*

After four weeks of burial, wheat films showed endpoint mass-loss proxies ranging from 73.68 % to 77.78 %, with a mean of 75.98 %

(SD = 2.09 %). Rice films ranged from 42.86 % to 60.00 %, with a mean of 50.95 % (SD = 8.61 %). The mean difference was 25.02 percentage points. The direction of the difference was consistent across the three displayed replicates in each group (Figures 1 and 2).

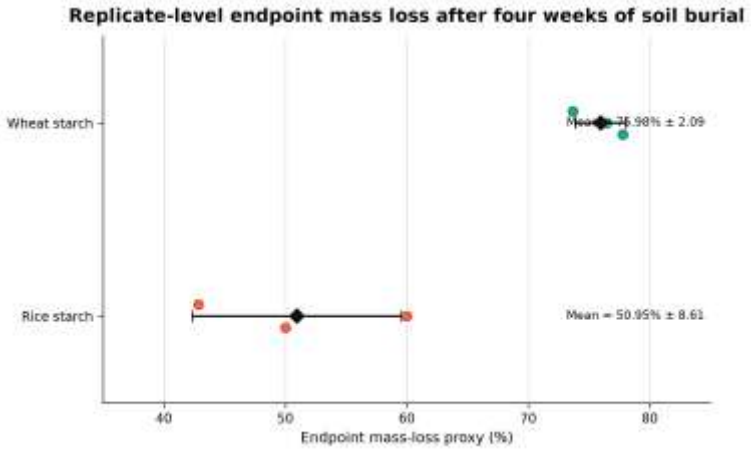



***Figure 1: Individual endpoint mass-loss proxies after four weeks of soil burial. Diamonds show group means and horizontal whiskers show +/- 1 sample SD; n = 3 per starch source. The primary exact permutation test gave p = .10, and the secondary Welch test gave p.***

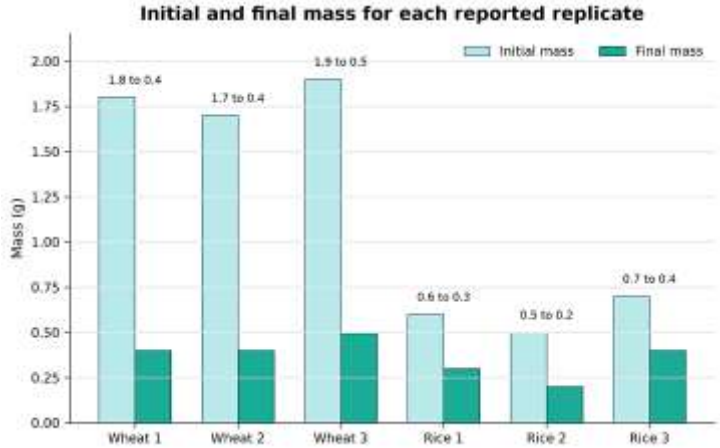



***Figure 2: The mass of each replicate before and after the test. The unequal initial masses can be seen and constitute a significant design constraint.***

### *Statistical Comparison*

The Welch test was reconstructed and t (2.24) = 4.89, p = 0.031, with a 95 % confidence interval for the mean difference of 5.09 to 44.95

percentage points. Both the exact two-sided permutation test and the exact two-sided Mann-Whitney test had p = 0.10. The permutation test was declared as the main inference and not significant at 0.05, therefore, the result should be interpreted as suggestive, but not statistically supported (Table 2). The traditional plastic control was assessed visually and did not exhibit any visible change. Its end weight was not stated so it was not used in the quantitative comparison.

***Table 2: The displayed endpoint values are used to provide an exploratory statistical comparison.***

| Analysis | Estimate | p value | Interpretation |
|---|---|---|---|
| Mean difference, wheat minus rice | 25.02 percentage points; 95 % CI 5.09 to 44.95 | n/a | Higher endpoint mass loss for wheat; uncertainty is wide |
| Welch two-sample t-test | t(2.24) = 4.89 | 0.031 | Secondary parametric description; assumptions cannot be checked with n = 3 |
| Exact permutation test | 20 unique label assignments | 0.10 | Primary inference; no large-sample approximation; not confirmatory |
| Exact Mann-Whitney U | U = 9.0 | 0.10 | Nonparametric sensitivity analysis; not definitive |

### *Presumptive Microbial Observations*

Copying of the buried films was accomplished by recovering culturable colonies from soil around the films. The rod-shaped and coccoid morphotypes were suggested by gram-stained microscopy, showing both Gram-positive and Gram-negative reactions. Although recovered culturable bacterial morphotypes in the burial soil, these observations are not proof of the degradation of the film, or enrichment with either type of starch, or species identification (Wicaksono et al., 2022; Rüthi et al., 2020).



***Figure 3: Representative post-burial appearance of films made from wheat- or rice-starch study records. The original photo was only available at low resolution.***

The supplied post-burial appearance and Gram-stained images are shown in (Figures 3 and 4). The photographs are supportive context rather than quantitative outcomes.

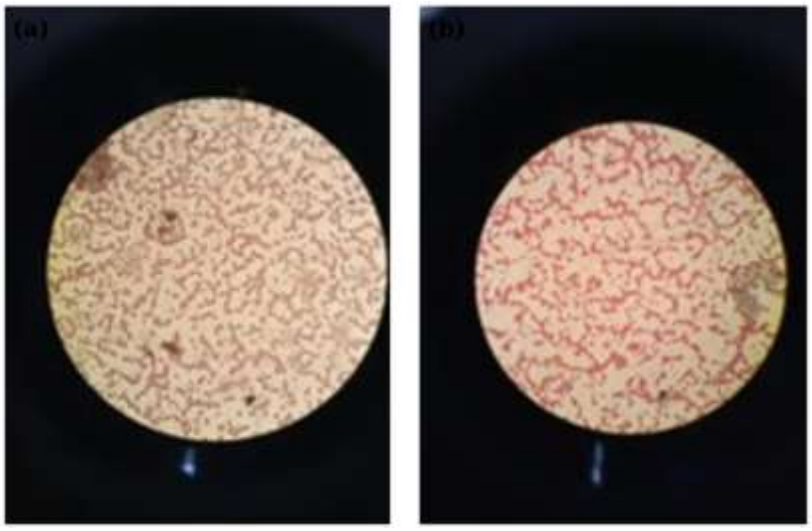


***Figure 4: Soil was collected around the buried films, and representative isolates were found to be stained with Gram. Presumptive characterization is only achieved with morphology and Gram reaction; it does not indicate species identity or degradative function.***

## Discussion

The reported formulation and 4-week garden-soil burial conditions indicated that the wheat-starch films incurred a higher endpoint mass loss proxy than did the rice-starch films. The primary inference was from the exact permutation test which gave $p = 0.10$ and the secondary Welch test, $p = 0.031$. In this pilot experiment, three samples were collected from each group, and the results of the replicates are not necessarily independent, so the best possible conclusion is that the wheat formulation demonstrated a suggestive higher endpoint mass-loss proxy, not that wheat starch is necessarily more biodegradable than wheat flour.

The observed values fall within the wide range of the starch-containing materials reported, but cannot be directly compared without matching formulation, thickness, soil, temperature, duration and endpoint. Wicaksono et al. (2022) found that the weight of the commercial cassava-starch bioplastic reduced by 74 % after 120 days in compost soil. Malekzadeh et al. (2024) observed that the starch-nanocellulose films lost 35 % – 67 % of their weight after 30 days of soil exposure, while Ruggero

et al. (2021) found that a commercial material based on starch lost 4 5% +/- 4.7 % after 60 days of simulated composting. Chaisuwan et al. (2023) applied a 20-day soil-burial test on cassava-starch films and applied mass loss coupled with FTIR, microscopy and microbial counts.

The value of wheat in the present study was 75.98 % after about 28 days which might seem high as compared to certain of the published studies, and the value of rice was 50.95 %. Several potential explanations exist: the glycerol, acid content was not completely specified in the present films, the amount of glycerol and acid in the films was not measured, the thickness, area, density, and residual moisture were not measured, the burial soil was not characterized, and there was no attempt to measure carbon mineralization, just mass loss. Studies published also involve different soil types or different compost products, either neat starch film or a composite or commercial product, and different methods of incubation (e.g., garden soil, agricultural soil, compost, or controlled incubation) (Francioni et al., 2021; Følino et al., 2020; Polman et al., 2020).

This comparison serves a methodological purpose and does not rank materials in terms of endpoint mass loss being indicative of rapid disintegration of starch-rich components, or of whether the remaining material was mineralized. In composite studies, starch-rich phases can be lost before more resistant phases (Convertino et al. 2024; Zambrano et al. 2020), and/or residues or fragments can persist in soil.

Masses used for wheat films and rice films started with were 1.8 g and 0.6 g, respectively. The direct effect of the starting mass on the numerator is reduced by percentage normalization, but not the confounding due to area, thickness, density, exposed surface, surface-area-to-volume ratio or plasticizer leaching. The qualitative description of the wheat films being more rigid and the rice films being thinner and more flexible is consistent with possible differences in network structure, but cannot answer the question of the mechanism without thickness and material characterization.

The dimensions of the dry coupon, target mass per area, thickness determined at several locations, extent of drying, glycerol-to-starch ratio, concentration of acid, and moisture content at the end of drying should be the same for both sources of starch in a confirmatory study. It should also make independent preparation batches as well as randomize coupons onto burial containers. These controls are more significant than to repeat the same statistical tests on this limited data set (Surendren et al., 2022; Shafqat et al., 2021).

Only garden topsoil was mentioned as the type of burial soil. Ecological interpretation is restricted by unmeasured pH, moisture, temperature, texture, microbial biomass, carbon-to-nitrogen ratio, oxygen

status, and background microbial biomass. Degradation can be significantly affected by soil location and local environmental factors, and temperature can affect material loss and microbial community structure (Guliyev et al., 2022; Romano et al., 2024; Samuelson et al., 2021). Generalization to other soil systems, seasons, climates and disposal systems is not to be expected.

Since both Gram-positive and Gram-negative morphotypes were recovered it is concluded that culturable bacteria were present, but were unable to be detected by a Gram stain because they did not degrade the films. The microbial community associated with soil or plastic also can evolve over time and may contain microbes, which are not necessarily degrading the polymer, but rather reacting to other soluble components (Rüthi et al., 2020; Wicaksono et al., 2022). A more robust study should compare viable counts, enzyme assays, 16S rRNA amplicon sequencing, in addition to tests of degradation of isolates, with abiotic and non-inoculated controls.

The mass loss endpoint is a beneficial screening endpoint and should not be reported as evidence of ultimate biodegradation. Aerobic biodegradation is defined according to international standards, by evolution of carbon dioxide or oxygen demand under certain soil conditions (International Organization for Standardization, 2019; ASTM International, 2025). The best terms for this current study are endpoint mass loss, disintegration proxy or mass-loss proxy. If biodegradation is claimed in the future, mineralization should be measured or a standard endpoint should be used which has been validated (Pires et al., 2022; Ahsan et al., 2023).

### *Strengths, Limitations, and Reporting Upgrades*

The study has three merits: it is based on readily available agricultural feedstocks; it reports masses at replication level and provides a quantitative mass-loss proxy along with a conservative microbial observation. It has a few drawbacks such as unequal initial masses, unmeasured film geometry, lack of detailed materials specification, unknown balance performance, uncharacterized soil and weather conditions, uncertain replicate independence, conventional rather than molecular microbial identification, and presumptive identification. Limitations should be identified and then tackled using a design for confirmatory testing (Table. 3).

## Conclusion and Future Work

After 4 weeks of incubation in the soil used in the test, wheat-starch films achieved higher endpoint mass-loss proxies than rice-starch

films. The main inference was the exact permutation test, which was not confirmatory at the 0.05 level. The difference is hard to attribute to the starch source alone, due to the fact that the masses were not equal, the film geometry was not measured, the material specifications were not complete, the weighing performance was not known, the soil and environmental conditions were not known and the starch source may have contained plasticizer that leached out during the manufacturing process.

***Table 3: Minimum reporting framework for a confirmatory wheat-versus-rice film experiment***

| Domain | Current record | Required upgrade |
|---|---|---|
| Replication | Three reported samples per starch source; biological independence not verifiable | At least three independent preparation batches per source; randomize coupons and define the experimental unit |
| Film geometry | Initial masses differed; area and thickness not reported | Use identical dimensions, area, thickness, density, dry mass per area, and residual-moisture criteria |
| Materials | Glycerol and vinegar/acetic acid volumes reported; purity and concentration absent | Record supplier, grade, purity, density, acid concentration, lot, and formulation by mass and volume |
| Soil and weather | Garden topsoil; pH, moisture, temperature, rainfall, humidity, and irrigation not documented | Homogenize and characterize soil; log temperature and moisture; report outdoor weather or use a controlled chamber |
| Weighing | Values reported to 0.1 g; balance model and calibration not reported | Use a documented calibrated balance; report readability, calibration check, drying protocol, and repeated readings |
| Endpoint | Four-week mass-loss proxy | Add time-series sampling plus CO2 evolution or oxygen demand under ISO 17556 or ASTM D5988-compatible conditions |
| Controls | Conventional plastic assessed visually only | Add quantitative conventional plastic, positive biodegradable reference, blank soil, and abiotic or no-soil controls |
| Microbiology | Colony morphology and Gram staining | Add CFU counts, enzyme assays, biochemical or molecular identification, and isolate-level degradation tests |

## *Practical Applications*

The use of wheat-starch and rice-starch films as short-lived materials for dry-goods packaging, produce liners, seedling or nursery films, and the development of agricultural mulches as prototypes can be screened. Starch films are desirable due to the potential of using abundant feedstock and are produced by solution casting, but there are various properties that must be evaluated prior to being put into practice, including water sensitivity, mechanical properties, barrier properties, food contact safety and end of life performance (Vaz Matheus et al., 2023; Marichelvam et al., 2019; Gamage et al., 2022). These are four-week mass loss data that are only for feasibility screening, and do not imply product readiness or environmental safety.

### *Future Research Directions*

Future work should employ independent preparation batches of samples, identical coupon samples, measured thickness and mass per area, fully specified reagent grade and concentration, calibrated weighing, characterized and monitored soil, moisture and temperature controls, multiple time points of burial, and quantitative positive and negative controls. Studies should be performed with CO2 evolution and/or oxygen demand, FTIR or other chemical characterization, microscopy, mechanical testing, 16S rRNA sequencing, or metagenomic sequencing. One should evaluate if there is any difference between wheat and rice starch under matched conditions before a prespecified power analysis is performed, to determine the sample size.

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