(Bio)degradation of ^{14}C-radiolabelled Natural Polymers in Soil in the Presence of Active Coal

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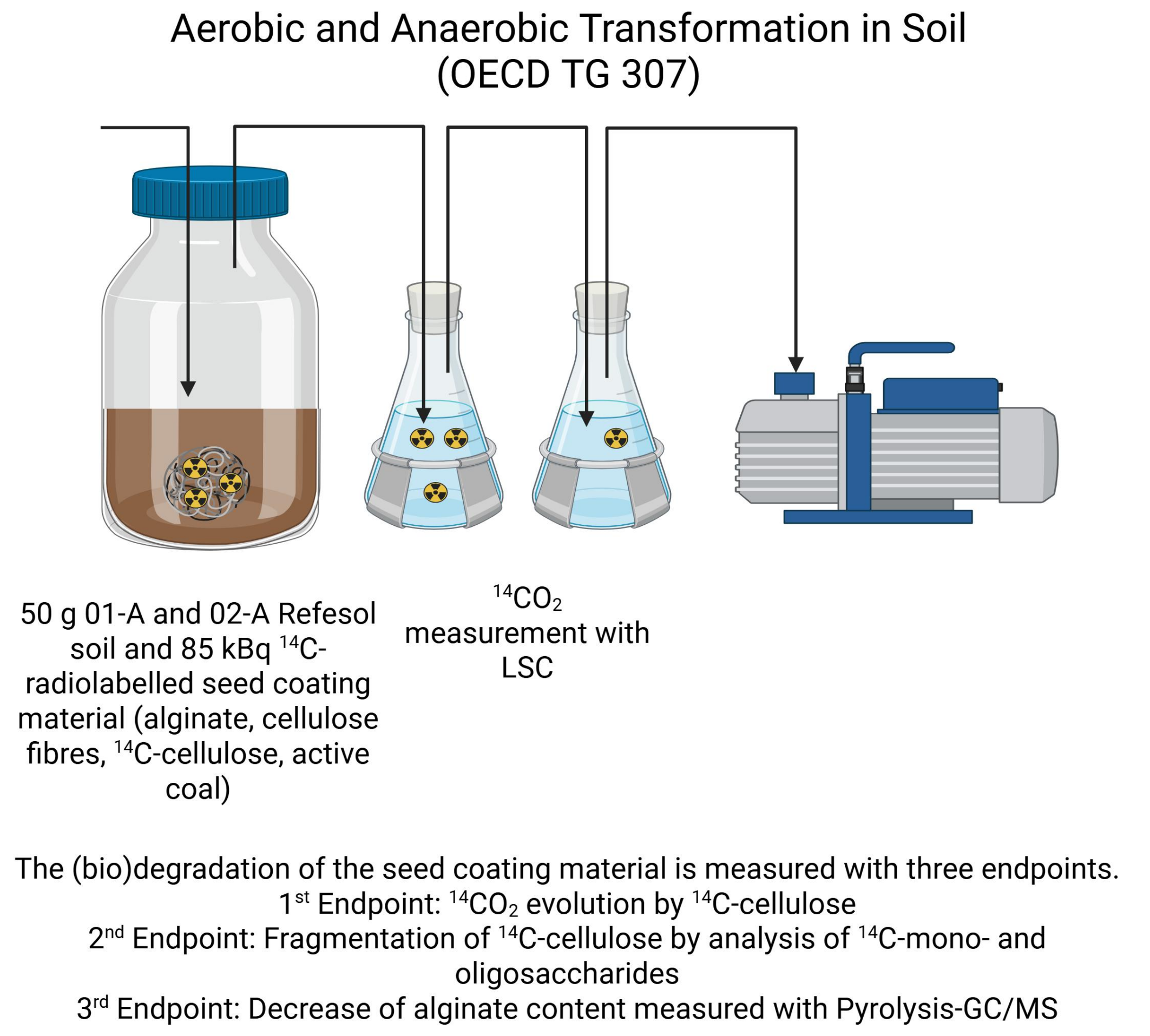
Introduction

The **fate of polymers** has been in the focus of the new restriction (Commission Regulation (EU) 2023/2055) issued by the European Commission [1] on microplastics intentionally added to products. The decisive criterion is the biodegradation of polymers, to be determined according to OECD test guidelines (TG). Since natural polymers are considered degradable by default, they are now considered as replacements for several applications. Our project investigated the degradability of a seed coating consisting of different natural polymers (sodium alginate, cellulose, active coal). Since they failed the **screening test** OECD TG 301 [2] (**<60% mineralization over 28 d)**, a full **simulation test** (OECD TG 307 [3]) was conducted to learn more about the degradation behaviour of this material.

Materials & Methods



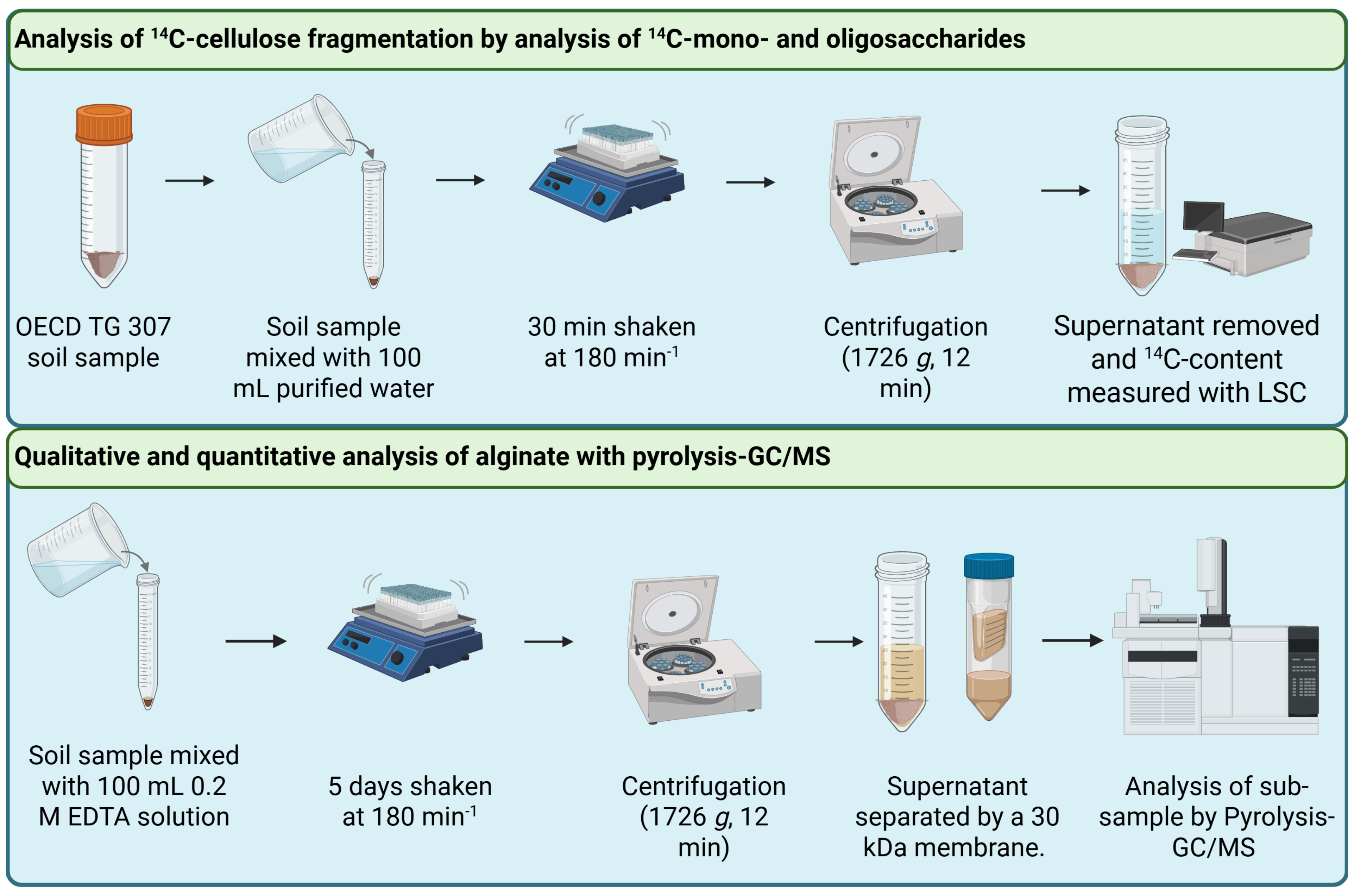
Aerobic and Anaerobic Transformation in Soil (OECD TG 307)

50 g 01-A and 02-A Refesol soil and 85 kBq ^{14}C-radiolabelled seed coating material (alginate, cellulose fibres, ^{14}C-cellulose, active coal)

$^{14}CO_2$ measurement with LSC

The (bio)degradation of the seed coating material is measured with three endpoints.
1st Endpoint: $^{14}CO_2$ evolution by ^{14}C-cellulose
2nd Endpoint: Fragmentation of ^{14}C-cellulose by analysis of ^{14}C-mono- and oligosaccharides
3rd Endpoint: Decrease of alginate content measured with Pyrolysis-GC/MS

Figure 1: Simulation test (OECD TG 307) to determine the degradation of the seed coating.



Analysis of ^{14}C-cellulose fragmentation by analysis of ^{14}C-mono- and oligosaccharides

OECD TG 307 soil sample → Soil sample mixed with 100 mL purified water → 30 min shaken at 180 min^{-1} → Centrifugation (1726 *g*, 12 min) → Supernatant removed and ^{14}C-content measured with LSC

Qualitative and quantitative analysis of alginate with pyrolysis-GC/MS

Soil sample mixed with 100 mL 0.2 M EDTA solution → 5 days shaken at 180 min^{-1} → Centrifugation (1726 *g*, 12 min) → Supernatant separated by a 30 kDa membrane. → Analysis of sub-sample by Pyrolysis-GC/MS

Figure 2: Extraction and analysis of soil samples from simulation test OECD TG 307.

Results & Discussion

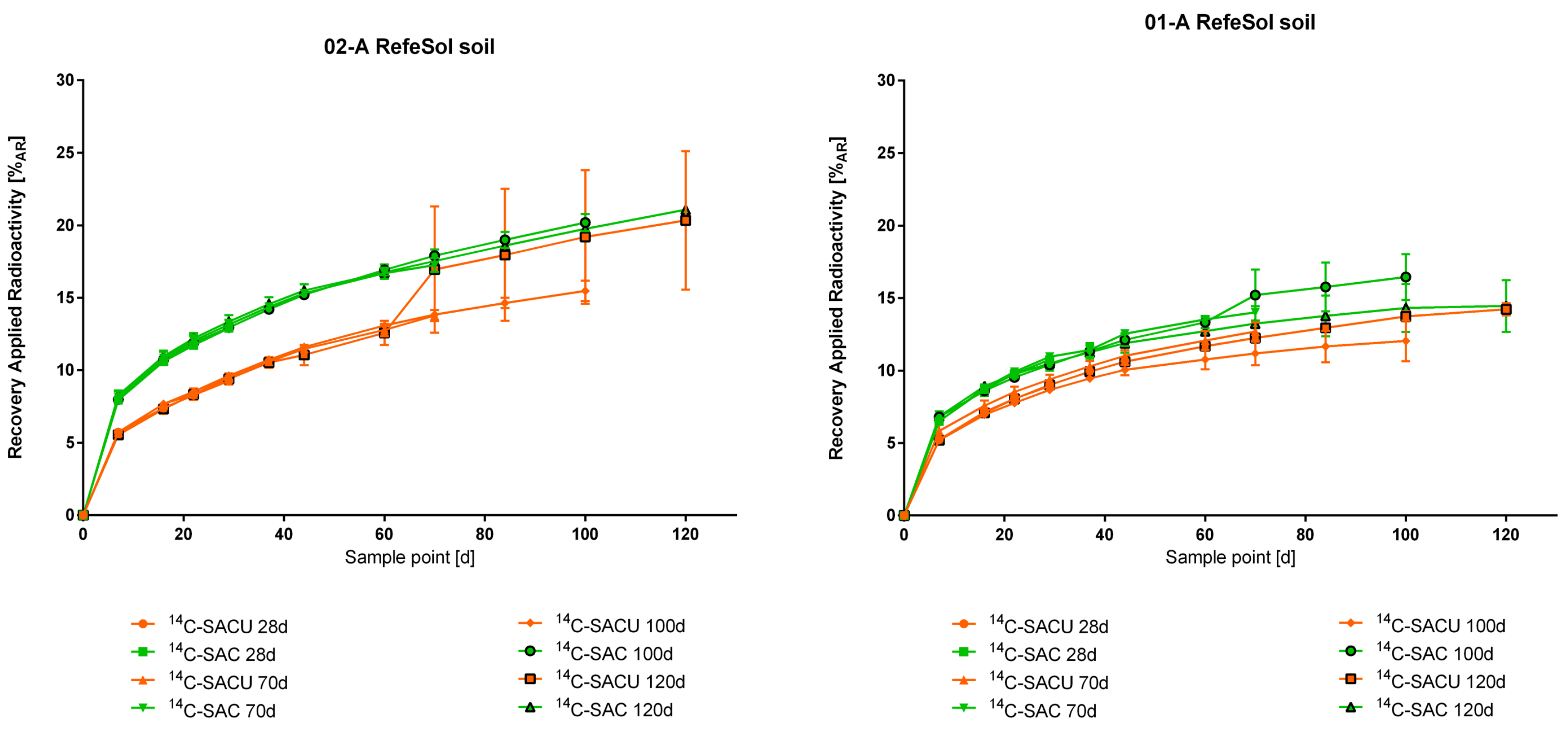


Figure 3: $^{14}CO_2$ evolution for ^{14}C-SACU (seed coating with active coal) and ^{14}C-SAC (seed coating without active coal). Measured in 02-A (left) and 01-A (right) RefeSol soil for 120 d. Two replicates for each sampling day were performed (median ±range).

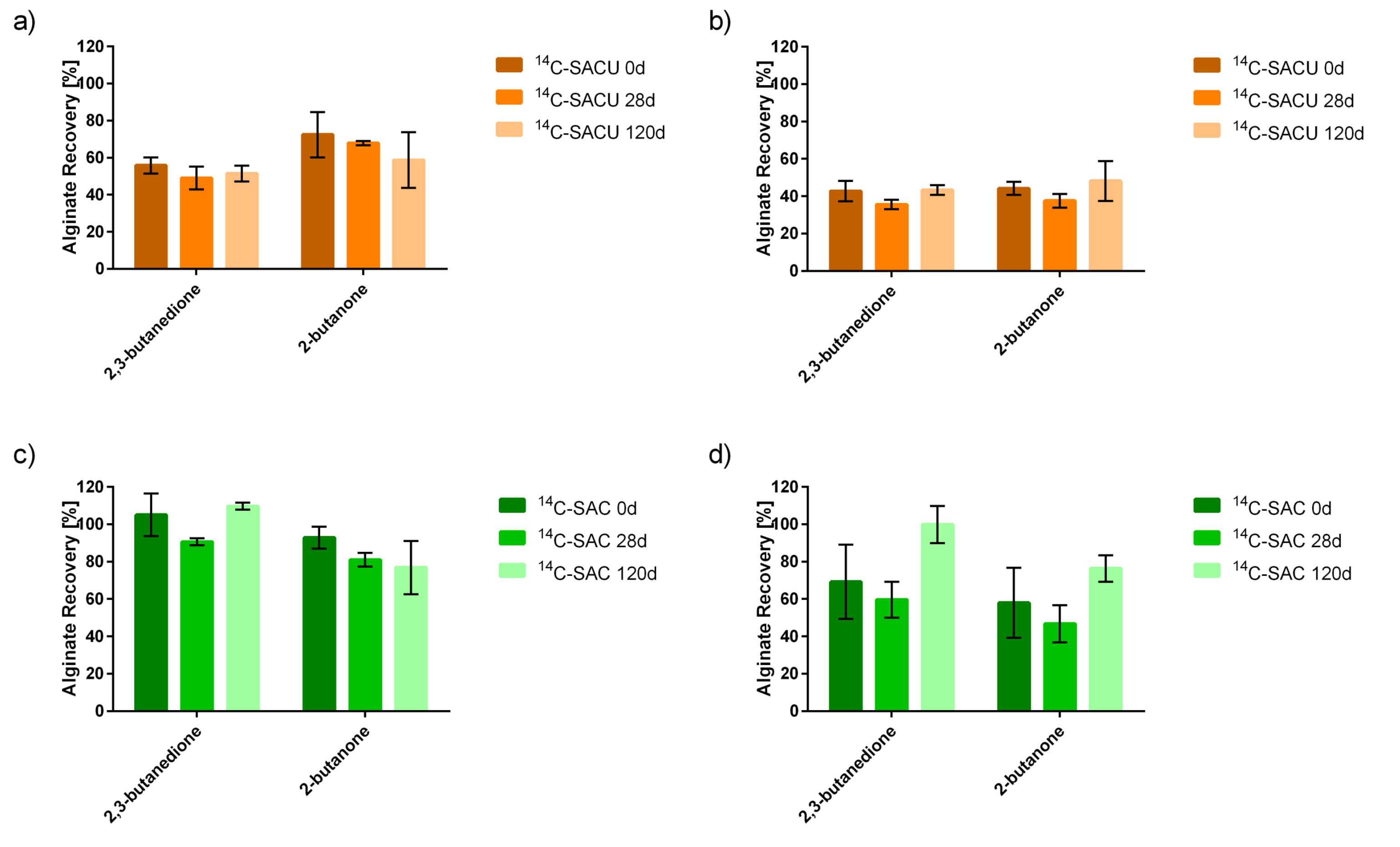


Figure 4: Determination of alginate content in 02-A (a, c) and 01-A (b, d) RefeSol soil for 0, 28 and 120 d incubation time. Measurements were performed in duplicates with pyrolysis-GC/MS. Two replicates for each sampling day were performed (median ±range).

- $^{14}CO_2$ evolution of ^{14}C-cellulose between 14.2 and 21.1%
  - Increased (bio)degradation in 02-A RefeSol soil
  - Increased (bio)degradation for ^{14}C-SAC samples in both soils
- Low (bio)degradation in comparison to studies with microcrystalline cellulose [4]
  - Dependency on environmental compartment
  - Dependency on cellulose structure (amorphous and crystalline ratio)
  - Dependency on soil texture
- Fragmentation of ^{14}C-cellulose also showed a higher decrease of water-soluble mono- and oligosaccharides for 02-A RefeSol soil

- Alginate (bio)degradation analysed with Pyrolysis-GC/MS
  - EDTA solution used to dissolve the alginate cross-linking
  - Decrease in alginate recovery used to calculate (bio)degradation
  - Higher extraction efficiency for ^{14}C-SAC samples
- Alginate degradation between 4.5 and 16.1%
  - Decreased (bio)degradation in comparison to pure alginate (100% (bio)degradation measured with OECD TG 301)
    - Influence of cross-linking and environmental compartment
  - Quantification of (bio)degradation possible, if radiolabelling is not possible
- Limiting factor is the extraction method

The study showed the importance of testing polymers in the **environmentally relevant compartment** and **form**. It also demonstrated the need for **polymer characterization** as **optional additional analysis** to support interpretation of the (bio)degradation pathways.

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Figures created with BioRender and GraphPad Prism 6.

References
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[4] J. Menzies, A. Wilcox, K. Casteel, and K. McDonough, "Water soluble polymer biodegradation evaluation using standard and experimental methods," *Sci. Total Environ.*, vol. 858, no. September 2022, p. 160006, 2023, doi: 10.1016/j.scitotenv.2022.160006.

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