

Biodegradable mulch film monomer metabolization: Optimization via Adaptive Laboratory Evolution

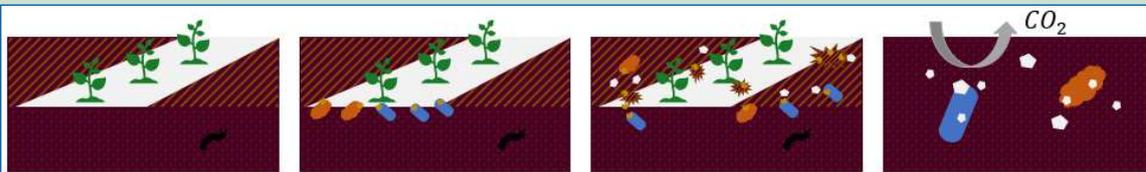
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Background

Biotic degradation of mulch films in soil:



Mulch film is introduced to agricultural soil

Colonization of the mulch film by different soil microorganisms

Depolymerization of mulch film into smaller fragments by enzymes

Metabolization of mulch film monomers by soil microbes

Objective

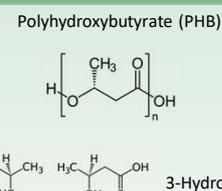
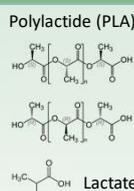
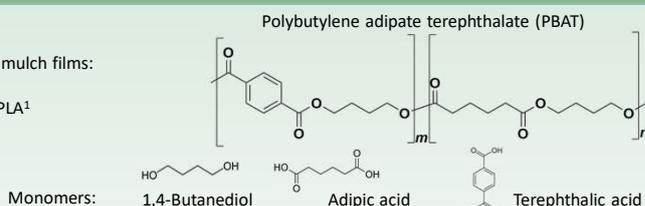
Enhance mineralization performances of biodegradable mulch films to avoid microplastic residues and its imaginably negative effects on soil and soil organisms.

First step: Examine biodegradability of mulch film monomers.

Polymers

Two biodegradable mulch films:

- Oil-based PBAT-PLA¹
- Bio-based PHB



Methods

Literature study



Screening growth of potential monomer metabolizing, available microbes on monomers in EnzyScreen growth profiler²



Adaptive Laboratory Evolution³ with most promising strains



Comparative monomer degradation test with *Rhodococcus ruber* in EnzyScreen growth profiler and System Duetz shaker; Analytics of monomer dissipation with High Pressure Liquid Chromatography

Results

Screening tests for monomer metabolization with potential degrading soil microbes^{1,4,5}

	1,4-Butandiol	Adipic acid	Terephthalic acid	Lactate	3-Hydroxybutyrate
<i>Acinetobacter baylyi</i>	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum
<i>Arthrobacter</i> sp.	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum
<i>Bacillus licheniformis</i>	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum
<i>Bacillus subtilis</i>	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum
<i>Pseudomonas fluorescens</i>	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum
<i>Pseudomonas putida</i>	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum
<i>Pseudomonas stutzeri</i>	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum
<i>Pseudomonas taiwanensis</i>	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum
<i>Rhodococcus ruber</i>	Wachstum	Wachstum	Wachstum	Wachstum	Wachstum

Legend: nicht getestet (white), kein Wachstum (red), Wachstum (green), Versuch ALE (yellow), Successful ALE (blue)

Adaptive Laboratory Evolution (ALE) with promising strains

A) ALE with *P. taiwanensis* on adipic acid

(ALE successful with *P. putida* on 1,4-Butandiol in previous work).

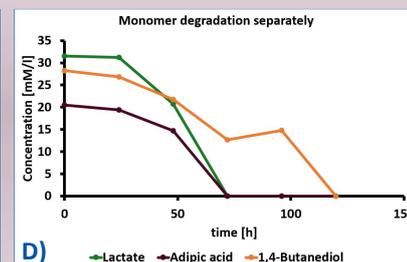
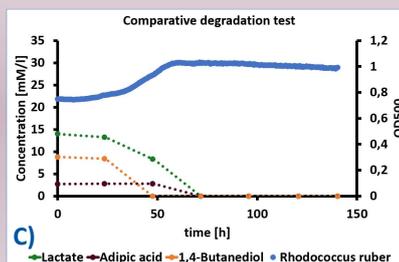
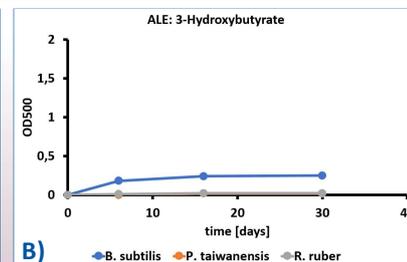
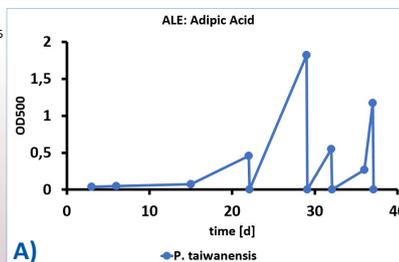
B) ALE with *B. subtilis*, *P. taiwanensis* and *R. ruber* on 3-Hydroxybutyrate.

Almost all tested microorganisms were able to grow on 3-Hydroxybutyrate in the screening tests, but only to a small extent which did not increase during ALE.

Comparative degradation test with monomer-metabolizing *Rhodococcus ruber* and PBAT-PLA monomers

C) Monomer dissipation and microbial growth during one week degradation test with monomer mixture according to [1] (terephthalic acid dissipation will be analyzed).

D) Monomer dissipation test with *Rhodococcus ruber* incubated with single monomers separately under same conditions as C) (terephthalic acid dissipation will be analyzed).



Outlook

- This work was a first step into examination of the biodegradation process of biodegradable mulch films based on screening for potential key species in literature.
- In the next step, soil samples from different agricultural fields will be incubated with mulch films to isolate and identify polymer-degrading soil microbes or consortia.
- Further, the degradation performance under different conditions shall be examined.
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References

- Morro A, Catalina F, Sanchez-León E, Abruscì C. 2019. Photodegradation and Biodegradation under thermophile conditions of mulching films based on Poly(Butylene Adipate-co-Terephthalate) and its blend with Poly(Lactic Acid). *Journal of Polymers and the Environment* 27:352-363.
- Ruedi L, Herrmann R, O'Connor K, Büchs J, Witholt B. 2000. Methods for intense aeration, growth, storage, and replication of bacterial strains in microtiter plates. *Applied and Environmental Microbiology* 66:2641-2646.
- Dragostis M, Mattanovich D. 2013. Adaptive Laboratory Evolution – principles and applications for biotechnology. *Microbial Cell Factories* 12:1-64.
- Engelhardt G, Wallnöfer P, Rast HG. 1976. Metabolism of o-Phthalic acid by different gram-negative and gram-positive soil bacteria. *Archives of Microbiology* 109:109-114.
- Mergaert J, Webb A, Anderson C, Wouters A, Swings J. 1993. Microbial degradation of Poly(3-Hydroxybutyrate) and Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) in soils. *Applied and Environmental Microbiology* 59:3233-3238.