

Biodegradable mulch film monomer metabolization: Optimization via Adaptive Laboratory Evolution

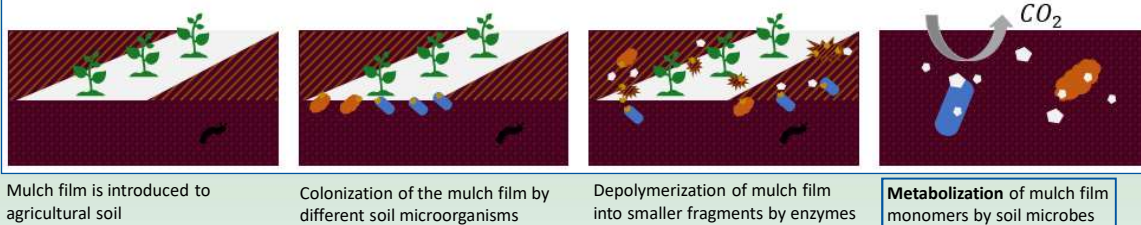
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Background

Biotic degradation of mulch films in soil:



Objective

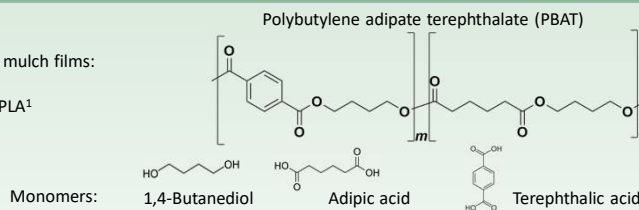
Enhance mineralization performances of biodegradable mulch films to avoid microplastic residues and its imaginably negative effects on soil and soil organisms.

First step: Examine biodegradability of mulch film monomers.

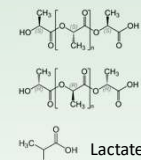
Polymers

Two biodegradable mulch films:

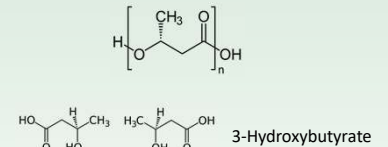
- Oil-based PBAT-PLA¹
- Bio-based PHB



Poly(lactide) (PLA)



Poly(hydroxybutyrate) (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-Butanediol	Adipic acid	Terephthalic acid	Lactate	3-Hydroxybutyrate
<i>Acinetobacter baylyi</i>					
<i>Arthrobacter</i> sp.					
<i>Bacillus licheniformis</i>					
<i>Bacillus subtilis</i>					
<i>Pseudomonas fluorescens</i>					
<i>Pseudomonas putida</i>					
<i>Pseudomonas stutzeri</i>					
<i>Pseudomonas taiwanensis</i>					
<i>Rhodococcus ruber</i>					
nicht getestet	kein Wachstum	Wachstum	Versuch ALE	Successful ALE	

Adaptive Laboratory Evolution (ALE) with promising strains

A) ALE with *P. taiwanensis* on adipic acid (ALE successful with *P. putida* on 1,4-Butanediol in previous work).

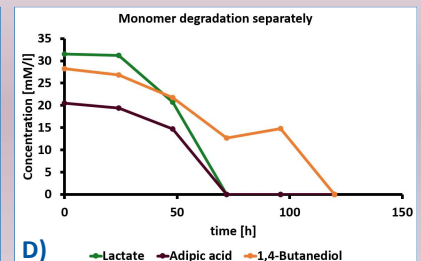
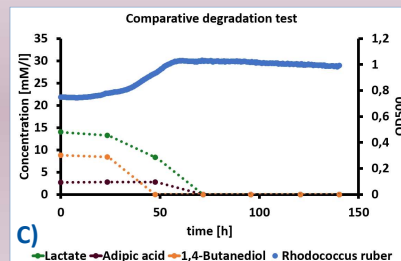
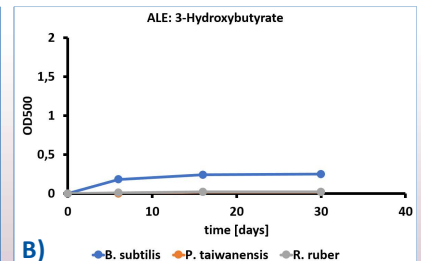
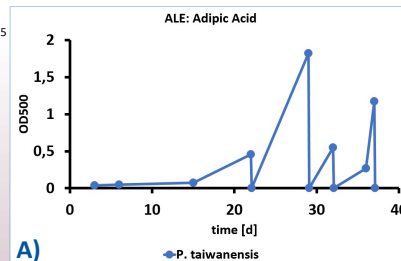
B) ALE with *B. subtilis*, *P. taiwanensis* and *R. ruber* on 3-Hydroxybutyrate.

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.
- This work is part of the iMulch project funded by the European Fond for regional development (EFRE).

References

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