



Kristina Bitter

Nach der Anwendung: Kann man Mulchfolien stofflich nutzen?

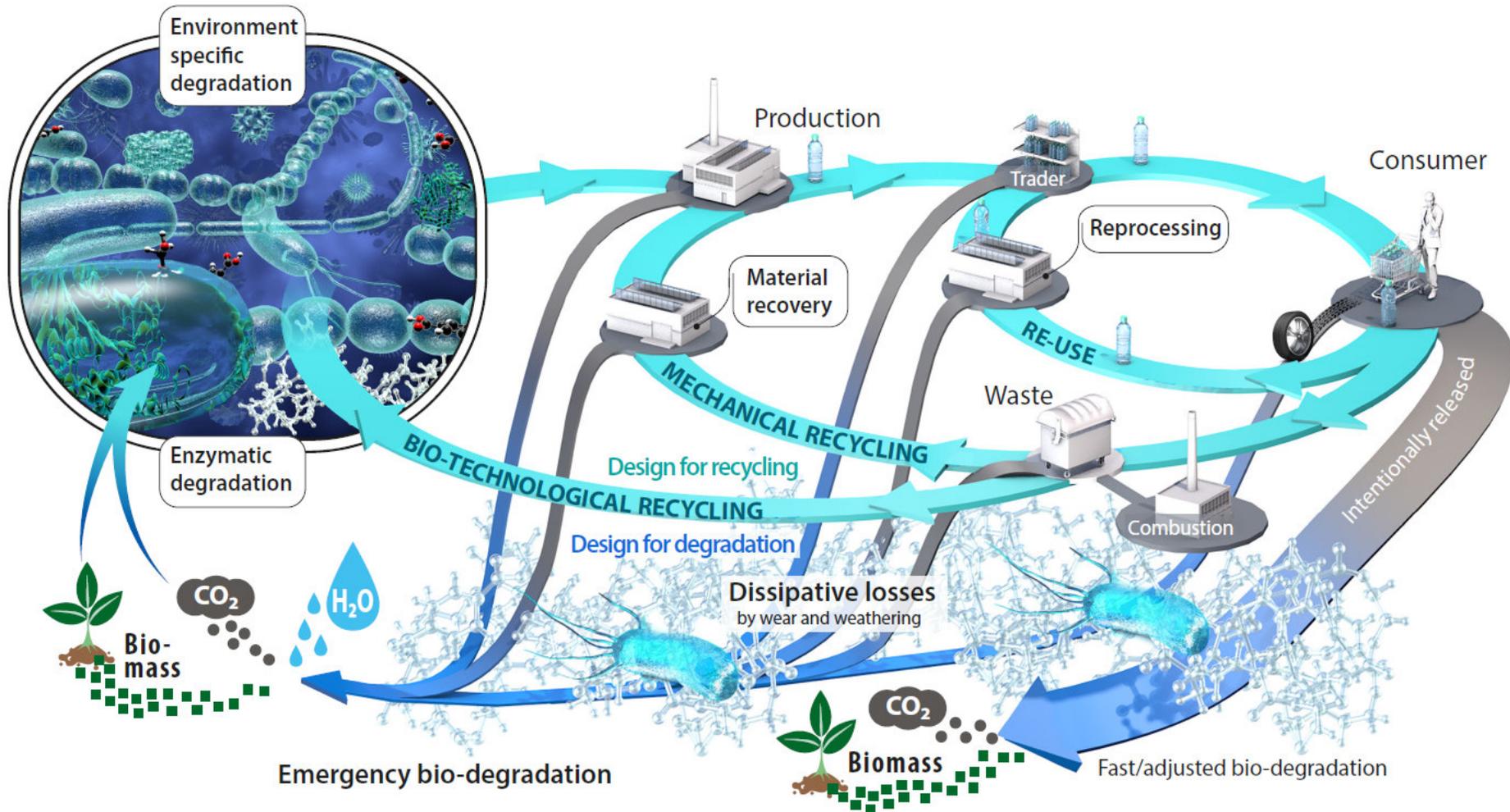
Lars M. Blank, Kristina Bitter

Institut für Angewandte Mikrobiologie
RWTH Aachen University

Super dünne PE Mulchfolien als Herausforderung



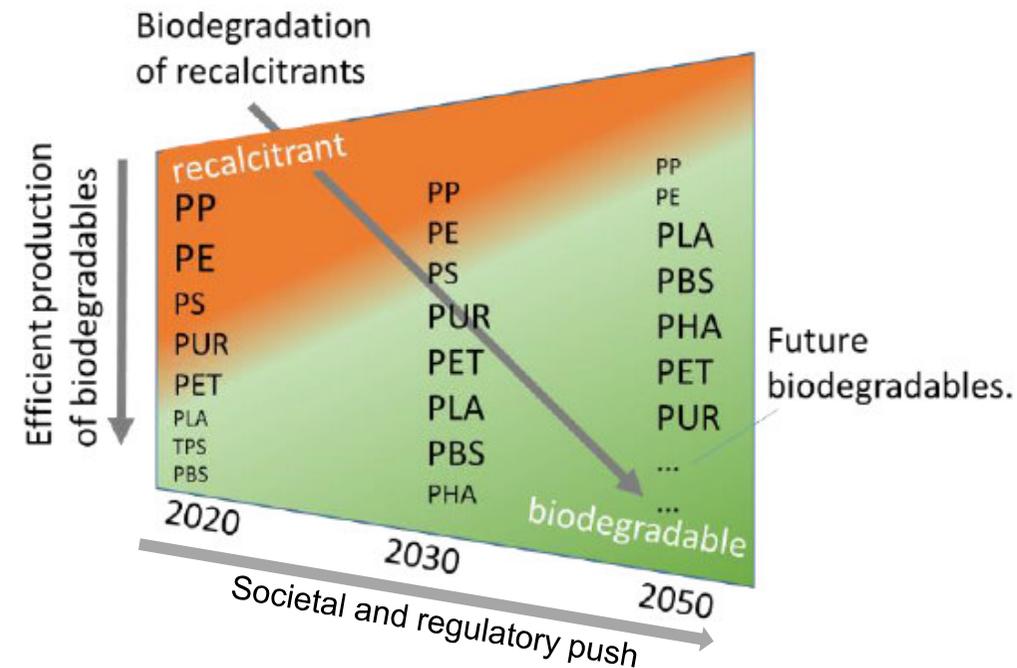
Vision einer nachhaltigen Plastikwirtschaft



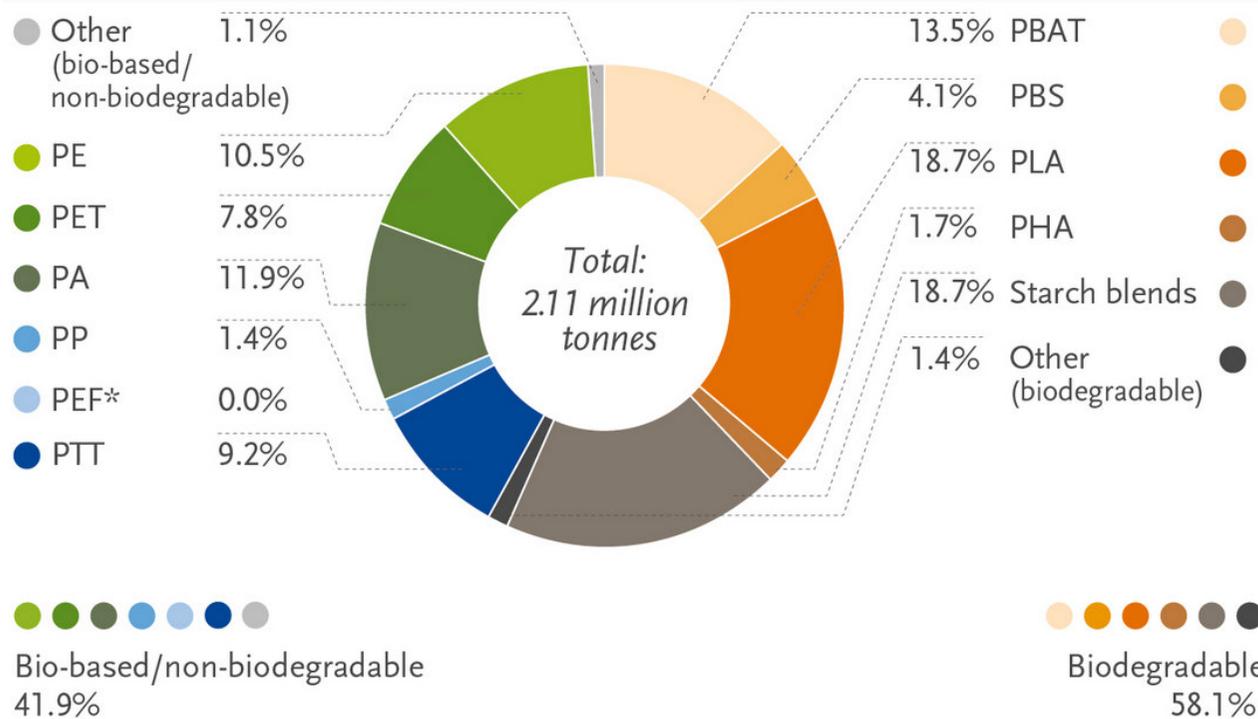
Was muss getan werden?

1. Von fossilen zu nachhaltigen C-Quellen
 - 🦠 Biomasse, CO₂, Plastikabfall, ...
2. Mischplastik Recycling
 - 🦠 Biokatalyse
 - 🦠 Chemokatalyse
3. Polymere, die im Notfall sich zersetzen
„emergency bio-degradation“

=> Bioplastik



Bioplastik: Jetzt geht es los!



*PEF is currently in development and predicted to be available in commercial scale in 2023.

Source: European Bioplastics, nova-Institute (2020)

Pressemitteilungen:

2020:

- PHA in the 5 kT scale (Japan, USA)
- Total/Corbion 125 kT PLA, France

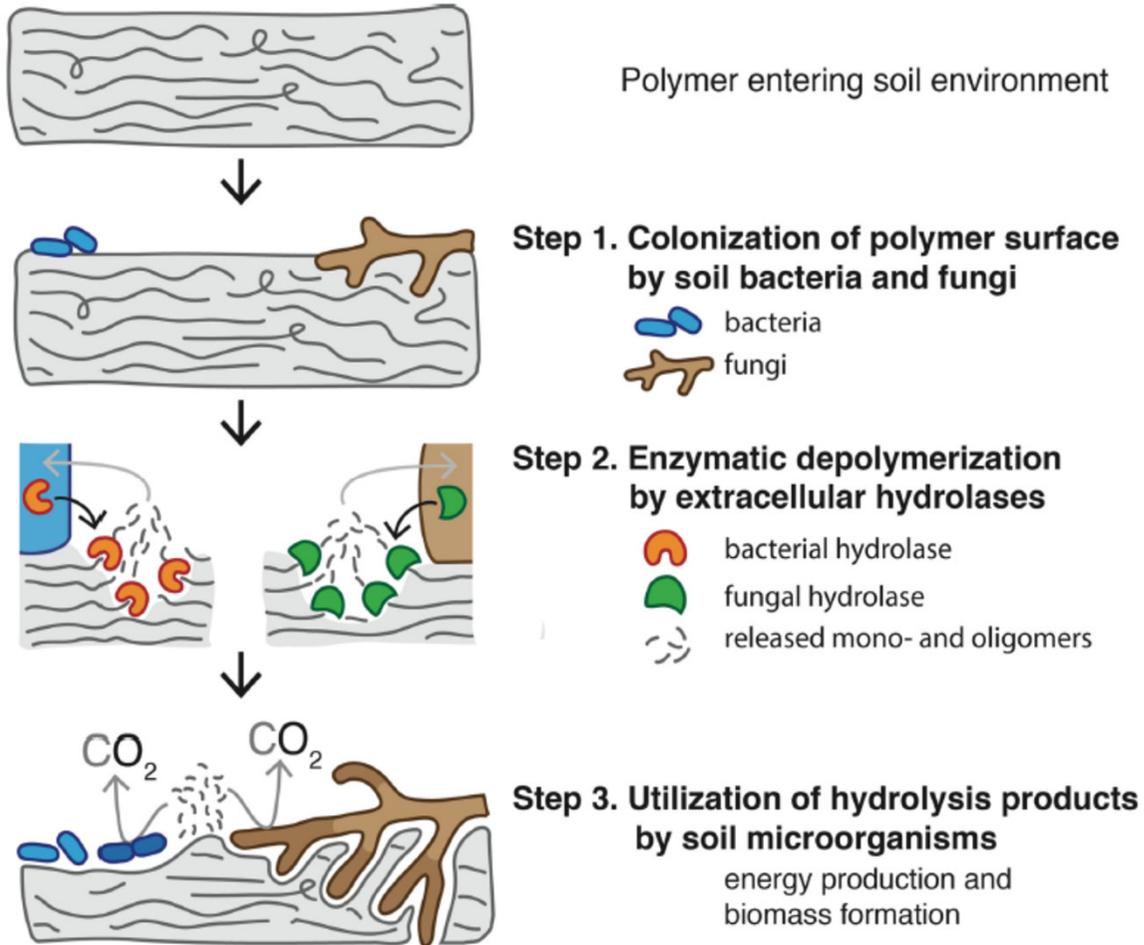
2021:

- Zhejiang Youcheng 200 kT PLA, China
- Shanxi Huayang, 500 kT PBAT, China
- Dawn, 120 kT PBAT, China
- Hengli Kanghui, 900 kT PBS, China
- BluePHA, 20 kT PHA, China

...

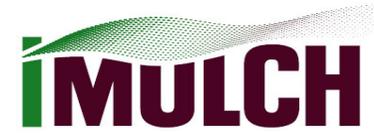
➔ Mehr als 15 MT in 2021!!

Polymerabbau im Boden



[Sander M, Environ. Sci. Technol. 2019, 53, 2304-2315]

Unterschiedliche Mulchfolien - Polymertypen



PHB Mulchfolien



	Öl basiert	Bio basiert
Nicht biologisch abbaubar	PE	PE
Biologisch abbaubar	PBAT-PLA	PHB PBAT-PLA
	Öl basiert-Bio basiert	Bio basiert Öl basiert-Bio basiert



PHA Produktion mit *Halomonas bluephagensis* in 200 m³ Fermenter

NGIB:

Simple facilities

Not sterile

Easy downstream

Easy scaleup

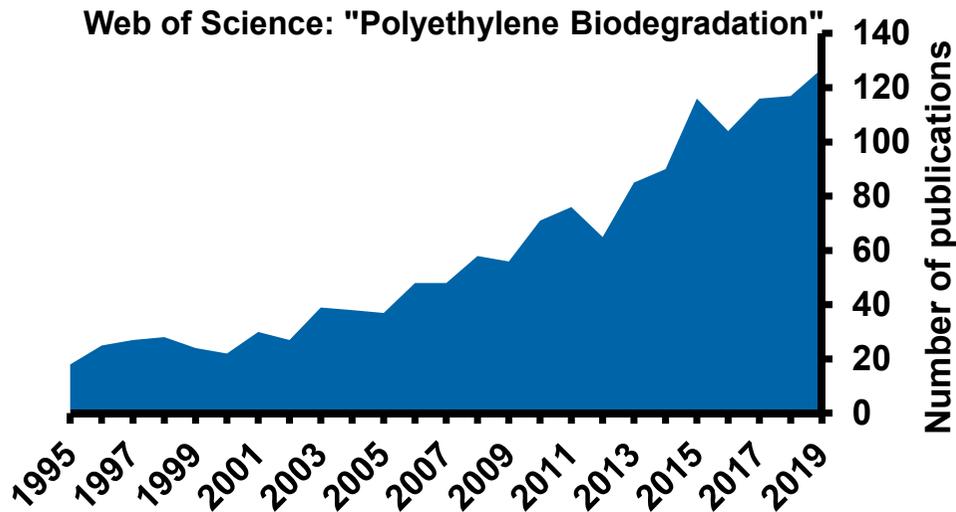
A 200 cubic meters
airlift fermentor in

A small village in GD
Province in China

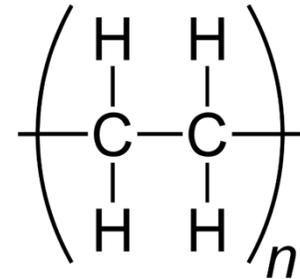
**Carbon source:
glucose and γ -
butyrolactone**



Konventionelle Mulchfolie : Polyethylen (PE)



Oligo- / Monomers = Alkanes,
similar paraffin (mixture azycllic alkanes)



- Nicht biologisch abbaubar
- Potentielle Enzyme: Cutinase, Laccase, Lignin peroxidase, Mangan peroxidase, ...
- Abbauraten:
3% in 80 days (Delacuvellerie et al, 2019)

Polyolefine sind NICHT biologisch abbaubar



IOP Publishing

Environ. Res. Lett. 17 (2022) 043002

<https://doi.org/10.1088/1748-9326/ac59a7>

ENVIRONMENTAL RESEARCH LETTERS



TOPICAL REVIEW

Microbial abilities to degrade global environmental plastic polymer waste are overstated

OPEN ACCESS

RECEIVED

4 January 2022

REVISED

19 February 2022

ACCEPTED FOR PUBLICATION

1 March 2022

PUBLISHED

15 March 2022

G Lear^{1,*} , S D M Maday¹, V Gambarini¹ , G Northcott², R Abbel³ , J M Kingsbury⁴ , L Weaver⁴,
J A Wallbank¹  and O Pantos⁴ 

¹ School of Biological Sciences, University of Auckland, 3a Symonds Street, Auckland 1010, New Zealand

² Northcott Consulting Ltd, 20 River Oaks Place, Hamilton 3200, New Zealand

³ Scion, Whakarewarewa, Rotorua 3010, New Zealand

⁴ Institute of Environmental Science and Research, 27 Creyke Rd, Ilam, Christchurch 8041, New Zealand

* Author to whom any correspondence should be addressed.

E-mail: g.lear@auckland.ac.nz

Keywords: bacteria, fungi, polystyrene, biodegradation, nylon, polycarbonate, polyurethane

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Polyethylen (PE) Nutzung über Pyrolyse



- Idee: 1) Depolymerisierung von PE mittels Pyrolyse
RWTH-TEER Institut: Pyrolyse von PE-Mulchfolien
2) Mikrobielle Verwertung von Pyrolyse-Kondensat

Aktueller Stand:

- Wachstum von Mikroben auf Pyrolyseöl durch CO₂-Entwicklung (GC) gezeigt



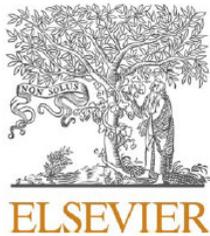
Irene Cardaio



PE stofflich nutzen: thermo/chemische Vorbehandlung



Waste Management 135 (2021) 60–69



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Waste Management

journal homepage: www.elsevier.com/locate/wasman



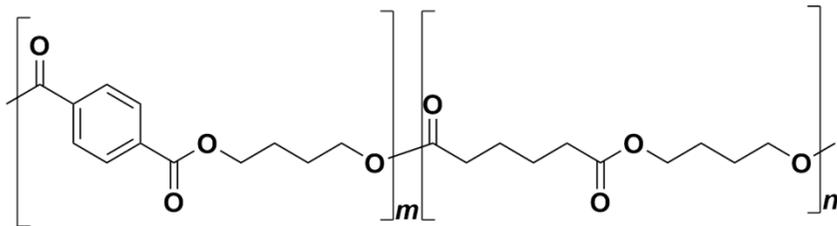
Robust process for high yield conversion of non-degradable polyethylene to a biodegradable plastic using a chemo-biotechnological approach[☆]

Maciej W. Guzik^{a,b}, Tomasz Nitkiewicz^c, Magdalena Wojnarowska^d, Mariusz Soltysik^e, Shane T. Kenny^a, Ramesh P. Babu^{f,g}, Marshall Best^h, Kevin E. O'Connor^{a,*}

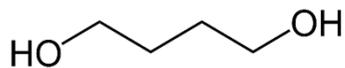
^a School of Biomolecular and Biomedical Sciences, BiOrbic Bioeconomy SFI Research Centre, UCD O'Brien Centre for Science, University College Dublin, Belfield, Dublin 4, Ireland



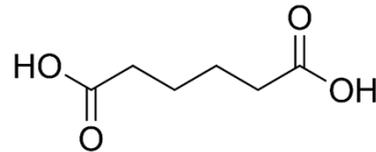
Bioabbaubare Polymere: PBAT-PLA (Ecovio[®], Bioflex[®])



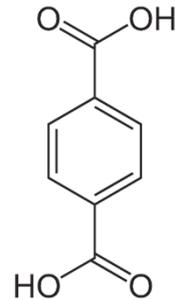
Polybutylene adipate terephthalate (PBAT)



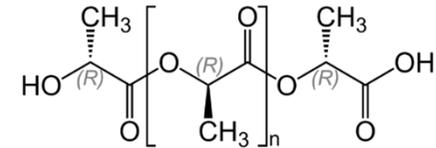
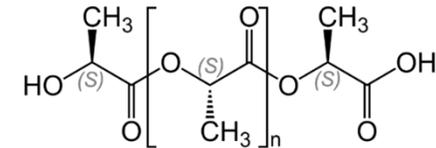
1,4-Butanediol
50.0 mol%



Adipic acid
27.8 mol%

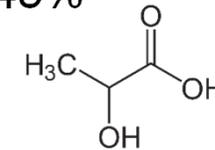


Terephthalic acid
22.2 mol%



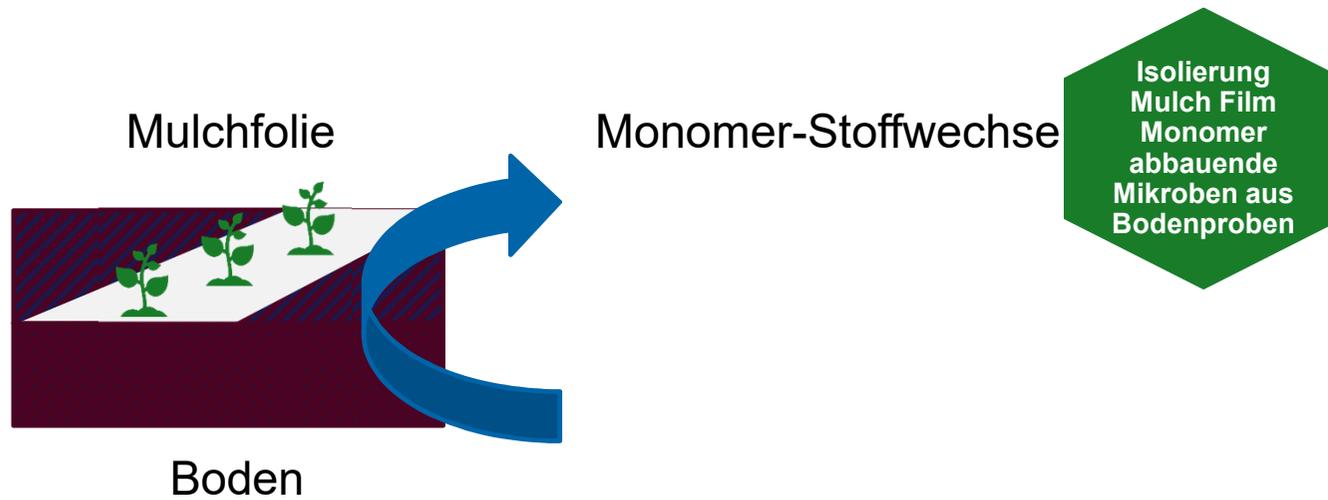
Poly(lactide) (PLA)

45%

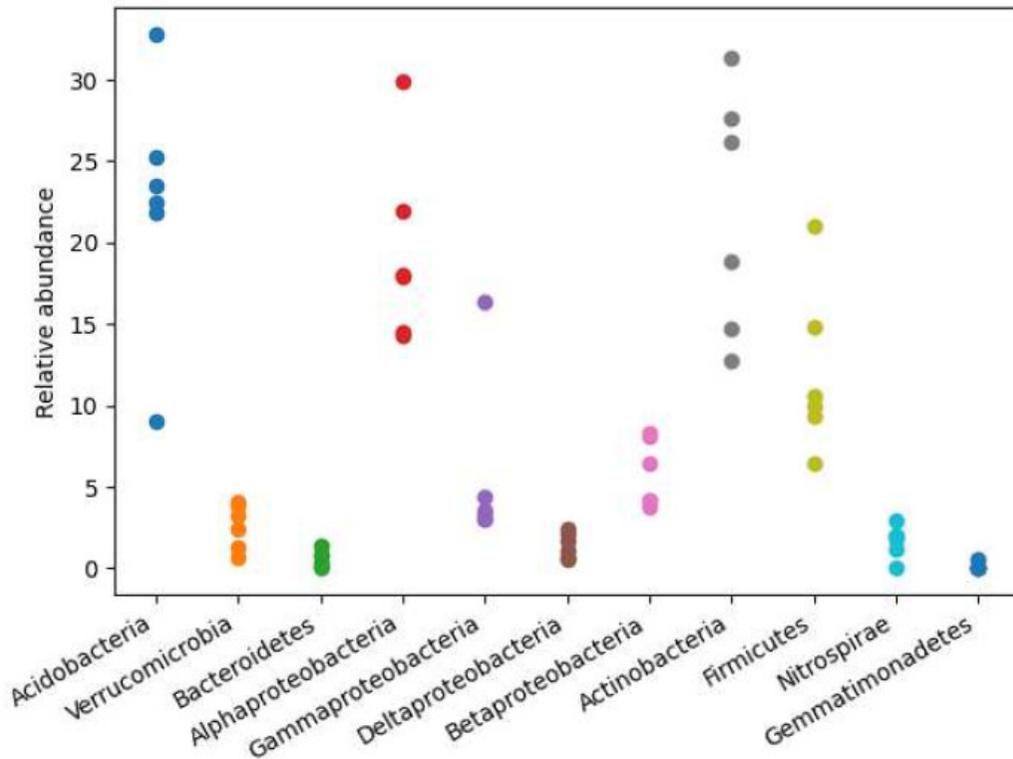


Lactate

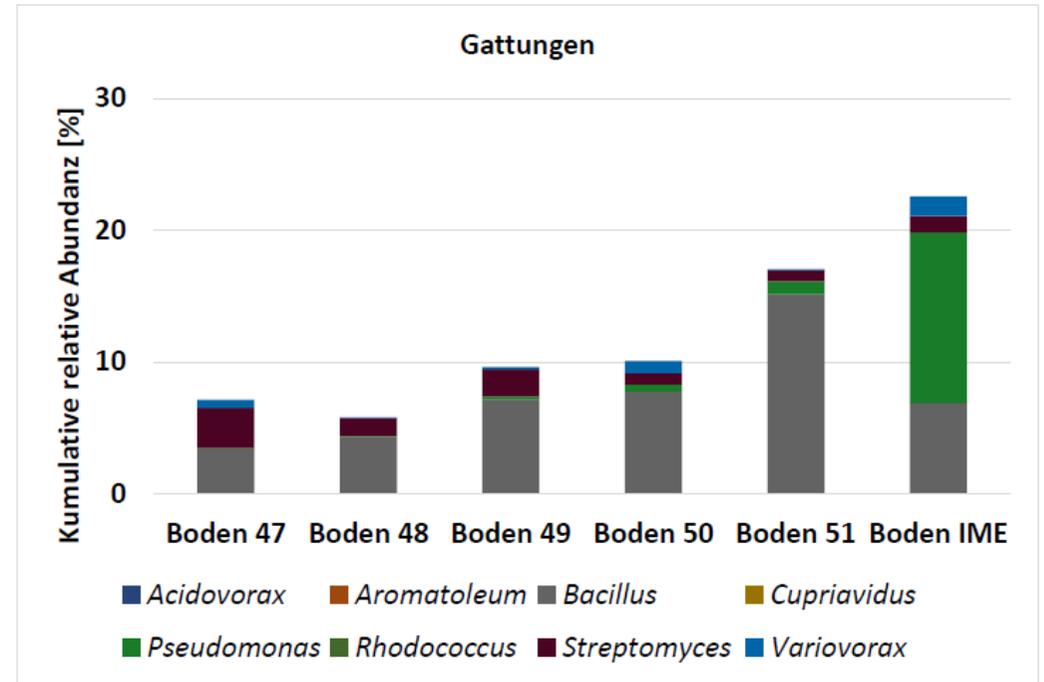
Unser Beitrag zum Projekt iMULCH



Welche Mikrobe ist da? Das Bodenmikrobiom

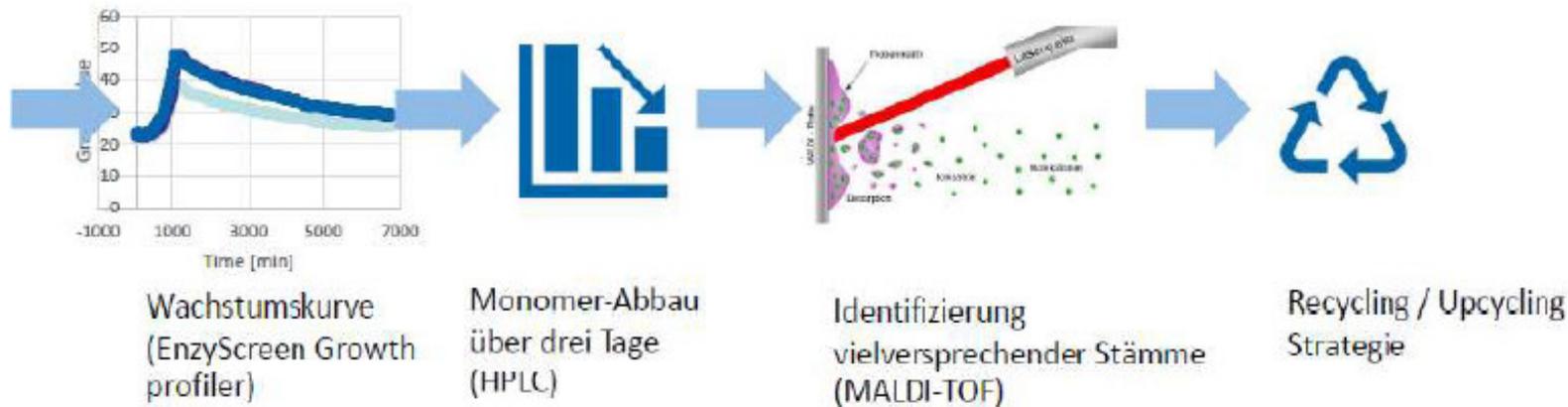


Relative Abundanz der Phyla in den Bodenproben



Kumulative relative Abundanz der Gattungen der isolierten und identifizierten Bakterien in Bodenproben

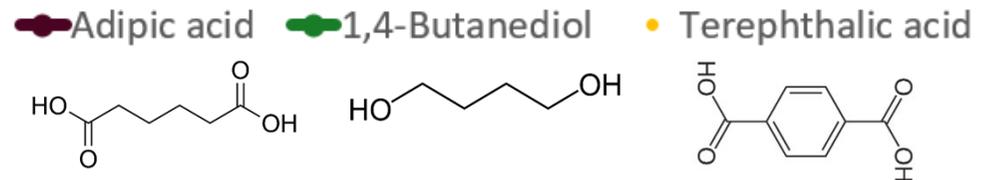
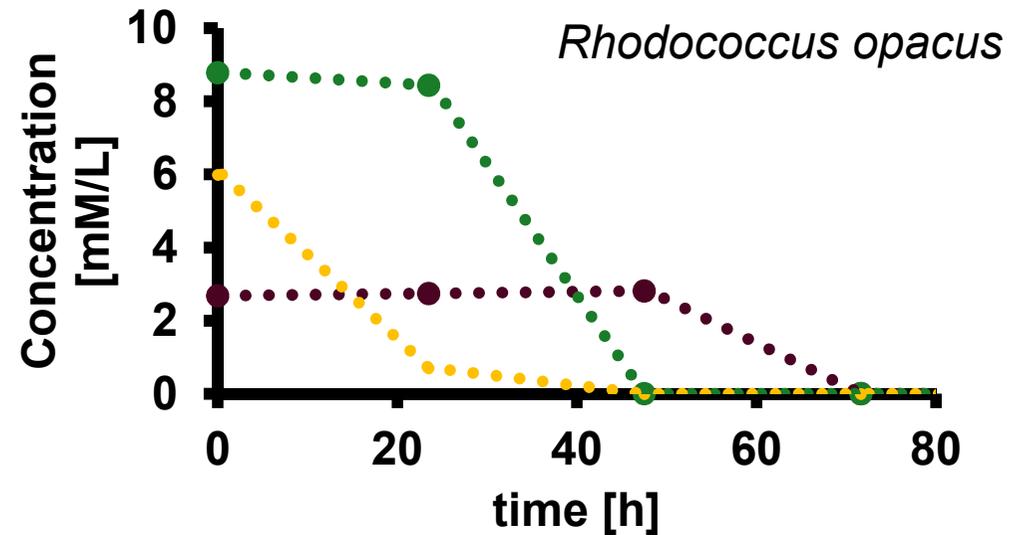
Isolierung und Identifizierung vielversprechender Bakterien



Ergebnisse: PBAT Monomer Abbau



	1,4-Butanediol	Adipic acid	Terephthalic acid
Ecovio1.1	Green	Green	Red
Ecovio 1.3	Red	Green	Light Blue
Ecovio 1.5	Red	Green	Light Blue
Ecovio 1.6	Green	Green	Red
Ecovio 1.8	Green	Green	Red
Ecovio 1.10.2	Green	Green	Red
Ecovio 2.4	Red	Green	Light Blue
Ecovio 2.7.2	Red	Green	Light Blue
Bioflex 1.3	Red	Green	Light Blue
Bioflex 1.4	Green	Green	Red
Bioflex 1.5.2	Red	Green	Light Blue
Bioflex 1.9	Green	Green	Red
Bioflex 2.6	Green	Green	Red
<i>P. putida</i>	Green	Red	Light Blue
<i>R. opacus</i>	Green	Green	Green



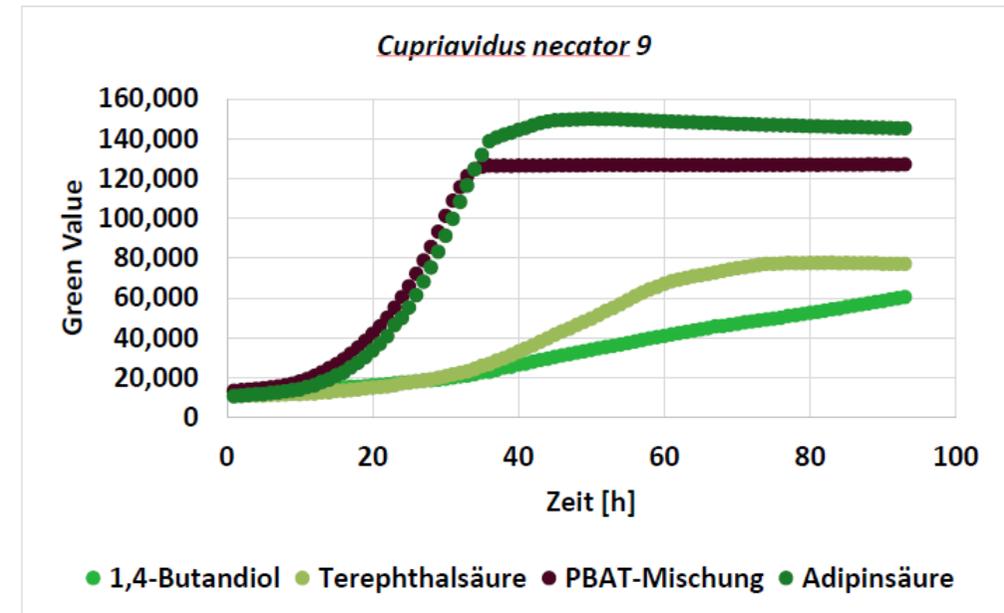
- Umwelt: vermutlich aufgeteilter Prozess
- Biotechnologische Anwendung, Upcycling: Stamm, welcher alle Monomere verstoffwechselt

Charakterisierung der bakteriellen Isolate

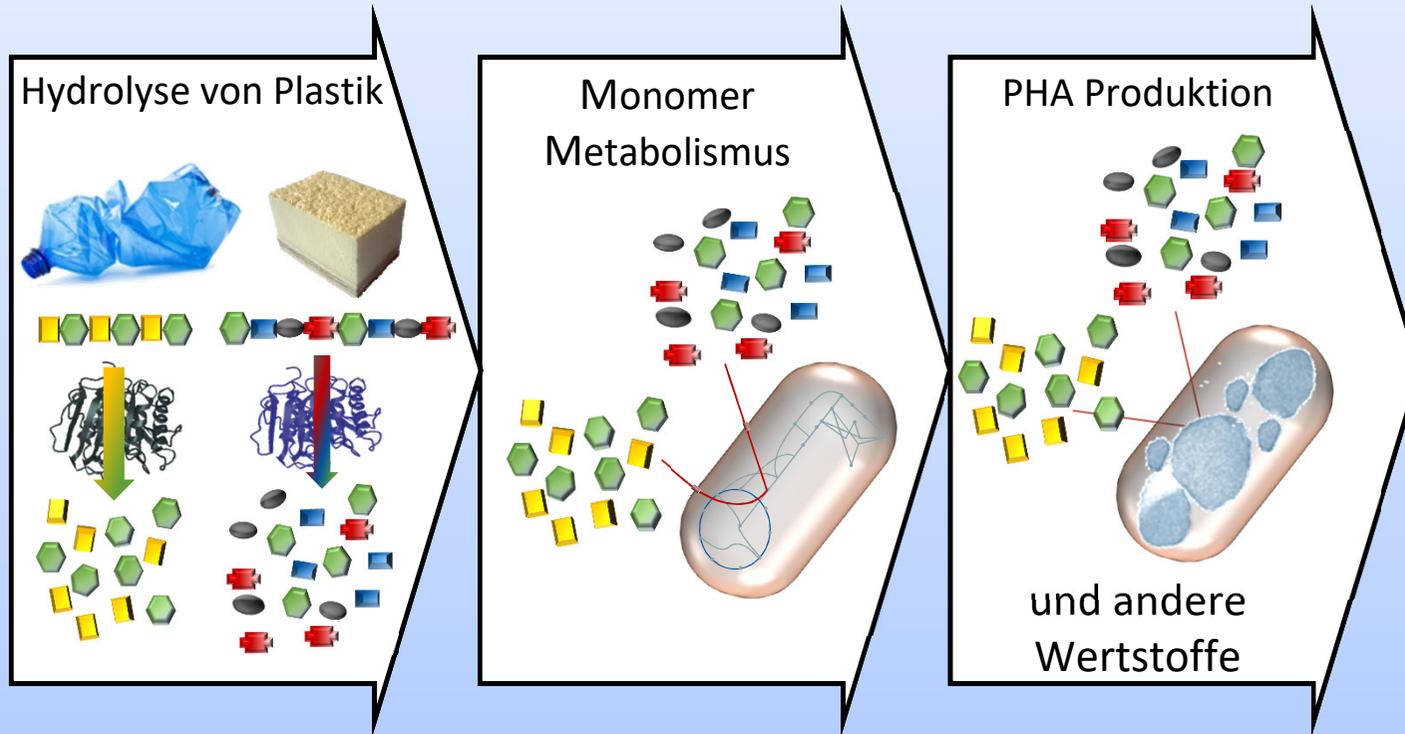
Art	1,4-BDO	AA	TA	Wachstumsrate μ [/h]	OD600_max
<i>Aromateum tolulyticus</i>	rot	grün	grün	0.28	1.5
<i>Bacillus simplex</i>	grün	rot	rot	0.2	1.5
<i>Cupriavidus necator 1</i>	gelb	grün	grün	0.37	1.5
<i>Cupriavidus necator 3</i>	gelb	grün	grün	0.1	1.5
<i>Cupriavidus necator 7</i>	gelb	grün	grün	0.3	1.5
<i>Cupriavidus necator 9</i>	gelb	grün	grün	0.31	1.5
<i>Pseudomonas chlororaphis</i>	grün	rot	gelb	0.29	1.5
<i>Rhodococcus opacus</i>	rot	grün	grün	0.25	1
<i>Variovorax paradoxus 2</i>	rot	grün	grün	0.05	1.5
<i>Variovorax paradoxus 3</i>	grün	rot	gelb	0.07	1

*Kompletter Abbau der einzelnen Monomere im Überstand ist grün, teilweiser Abbau gelb, kein Abbau rot gekennzeichnet.

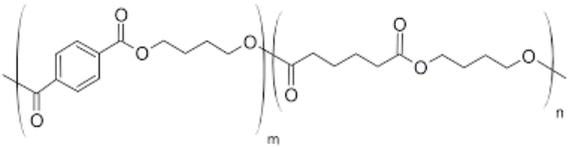
*OD600_max zeigt, ob ein Schwellenwert von 1 oder 1,5 erreicht wurde



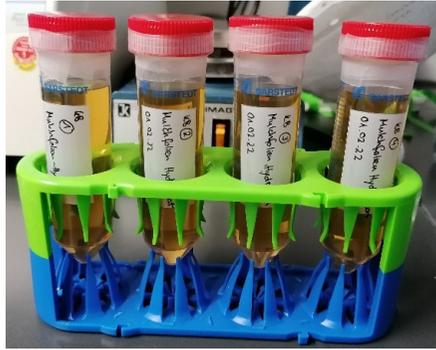
*Isolat *C. necator 9* das größte Wachstum auf Adipinsäure, abgeschwächtes Wachstum auf Terephthalsäure sowie nur geringes Wachstum auf 1,4-Butandiol im angelegten Testzeitraum



Upcycling von Mulchfolie

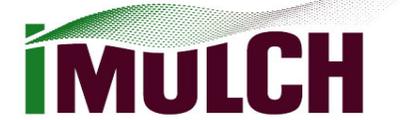
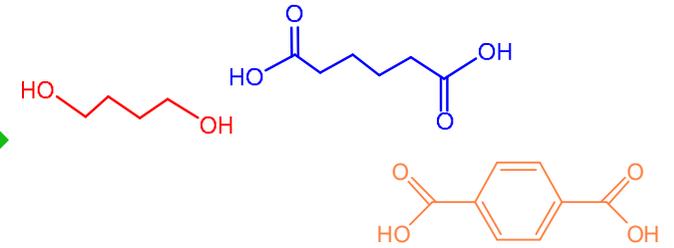


PBAT



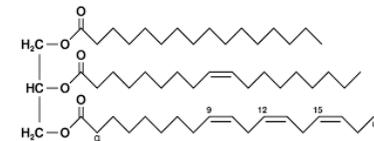
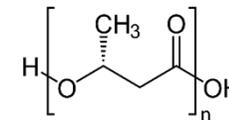
Enzymatische Depolymerisierung

UNIVERSITÄT GREIFSWALD
Wissen lockt. Seit 1456



Cupriavidus necator

Rhodococcus opacus



Polyhydroxybutyrat (PHB)

Triacylglycerol (TAG)



Mulchfolie



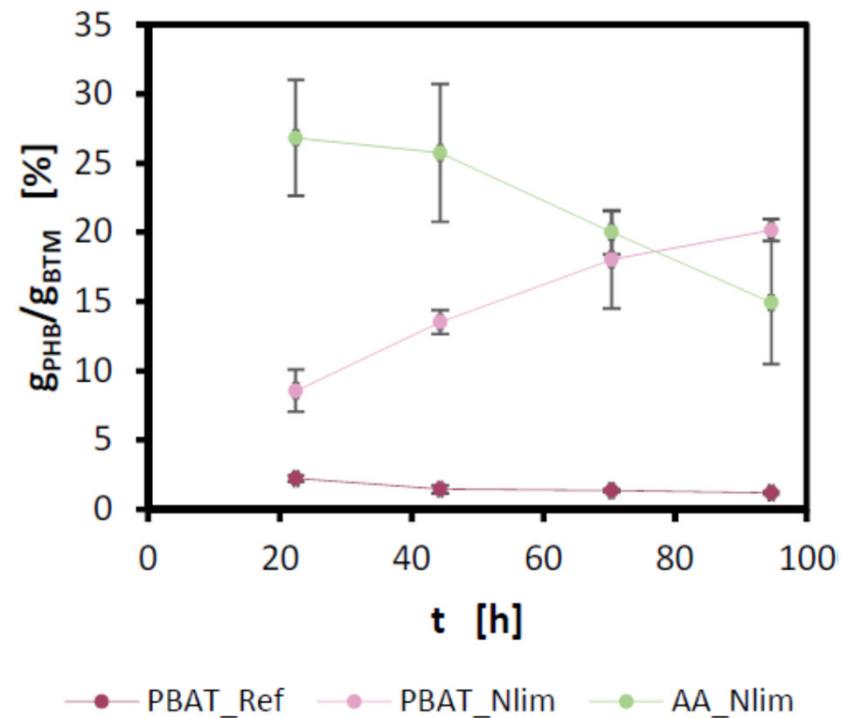
Selina
Granderath
-Miegel



PBAT Upcycling: Bioplastik (PHB) Produktion mit *C. necator*

Upcycling mit *C. necator*: Kultivierung bei Stickstoff-Limitierung

→ > 25% Zelltrockenmasse PHB

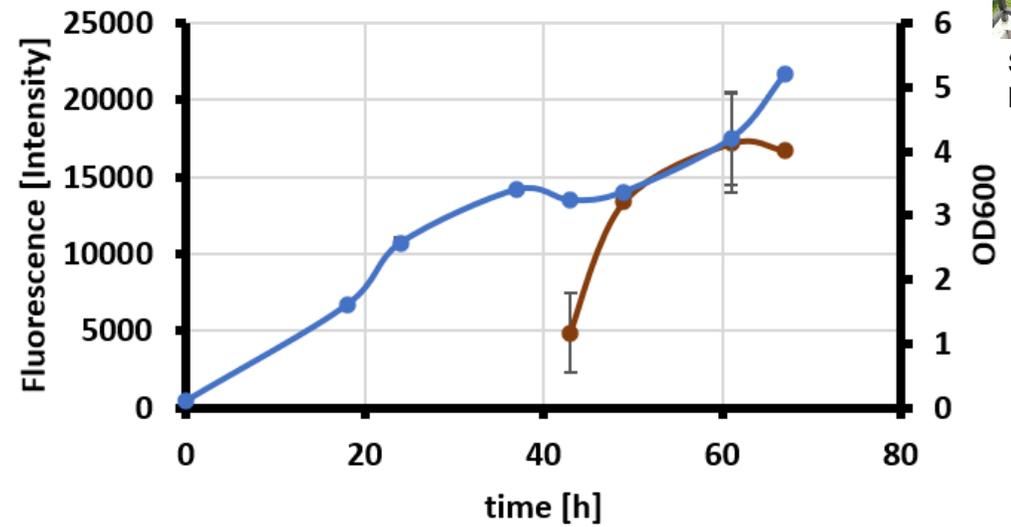
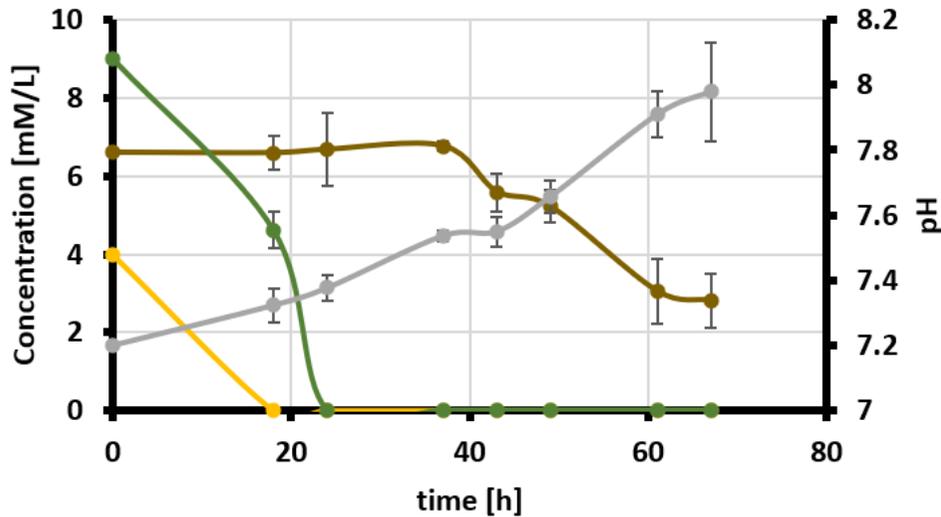


PBAT Upcycling: Triacylglycerol (TAG) mit *Rhodococcus opacus*

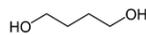
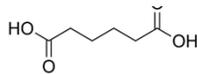
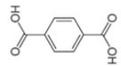


Sofia Bratchenko

Shake flask, V=50 ml; 30°C, 200 rpm, 67 h, initial OD 0.1; C-source: PBAT-monomers, 20 mM; C/N ratio: 12/1



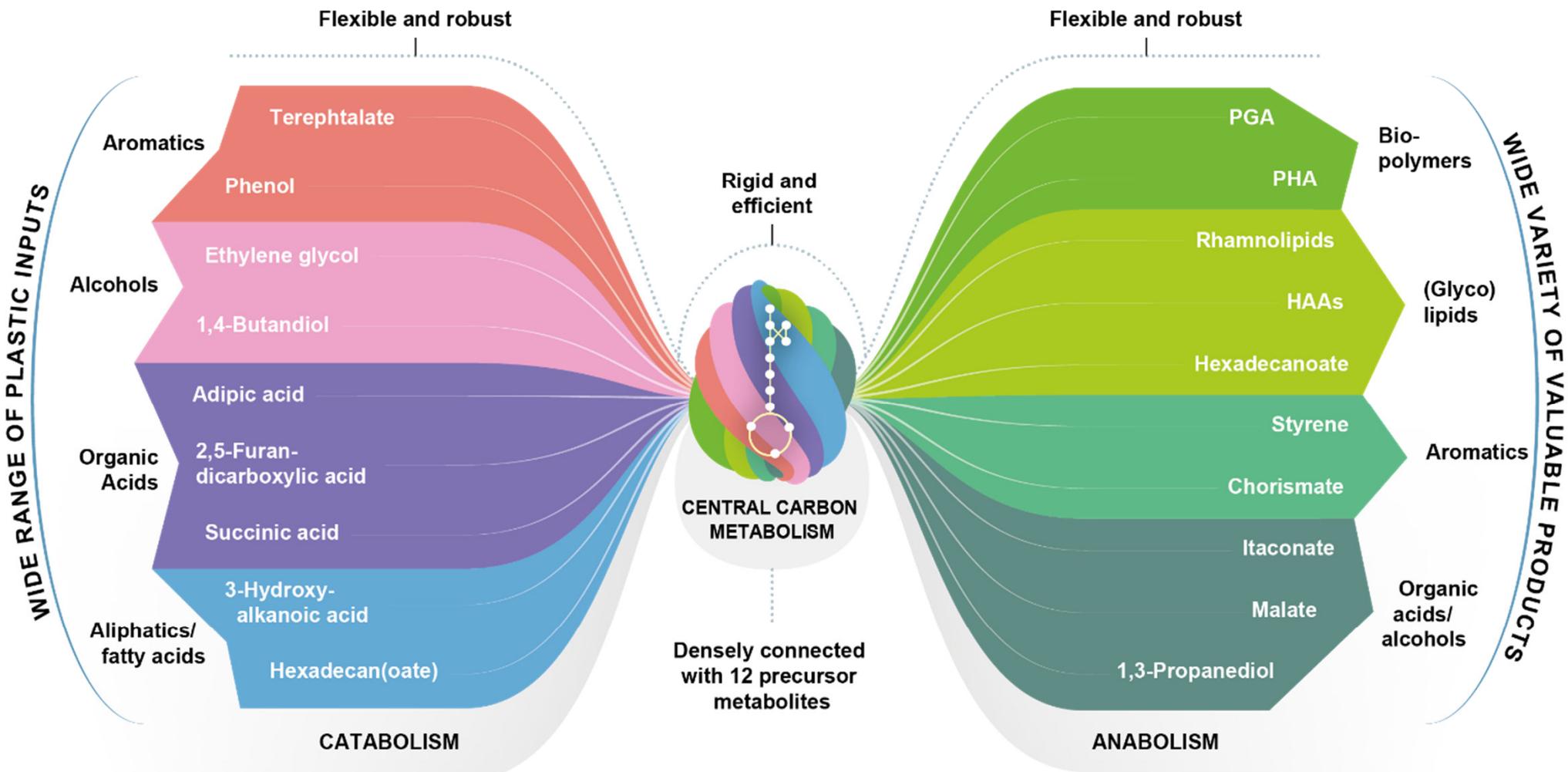
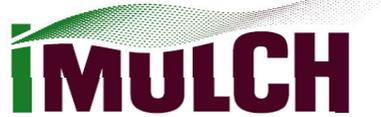
● Terephthalic acid
 ● Adipic acid
 ● 1,4-Butanediol
 ● pH



● TAG
 ● OD



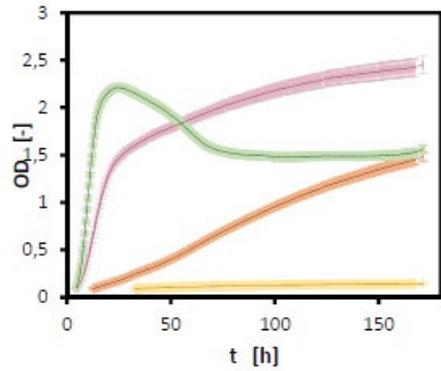
Ziel – die “bow-tie” Struktur des mikrobiellen Metabolismus nutzen



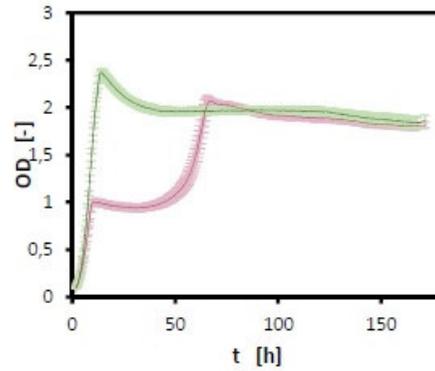
Wierckx et al., Plastic waste as a novel substrate for industrial biotechnology, *Microbial Biotechnology*, 2015, 8: 900–903
 Blank et al., Biotechnological upcycling of plastic waste and other non-conventional feedstocks in a circular economy. *Curr Opin Biotechnol.* 2019;62:212-219
 Wei et al., Possibilities and limitations of biotechnological plastic degradation and recycling. *Nature Catalysis*, 2020, 3 (11), 867-871

**Und dann noch diese immer wieder gestellt Frage:
Können wir mithelfen die Umwelt “aufzuräumen”?**

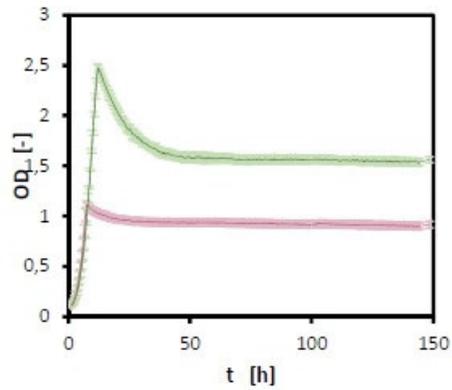
Wachstum auf PBAT-PLA Monomeren muss gesichert sein



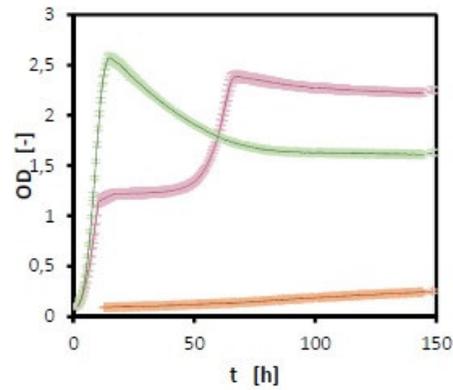
C. necator K1



C. necator K3



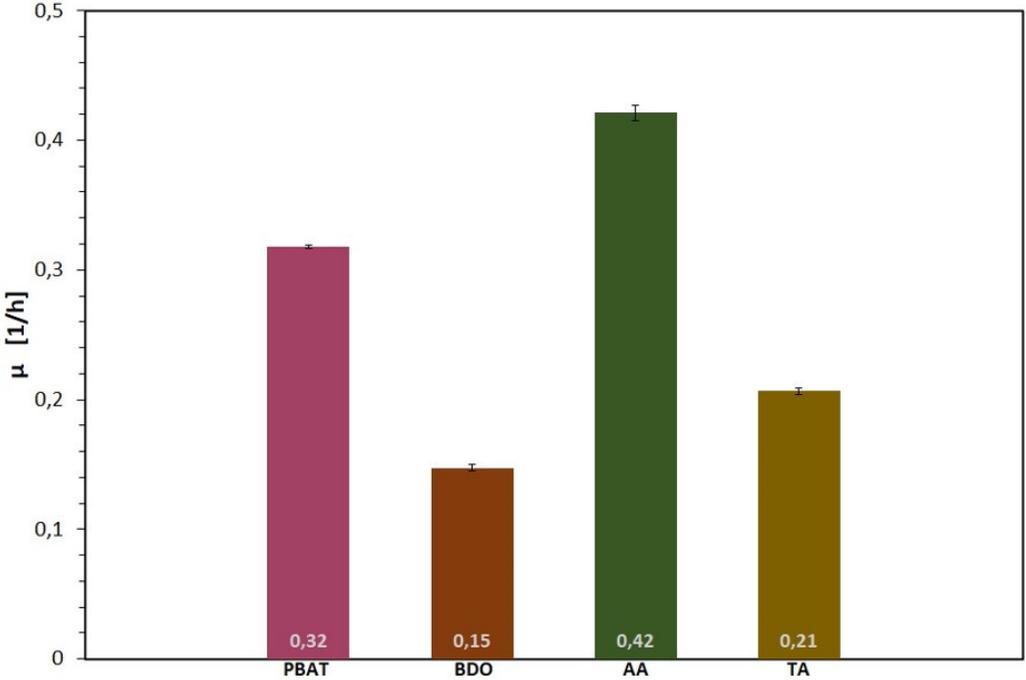
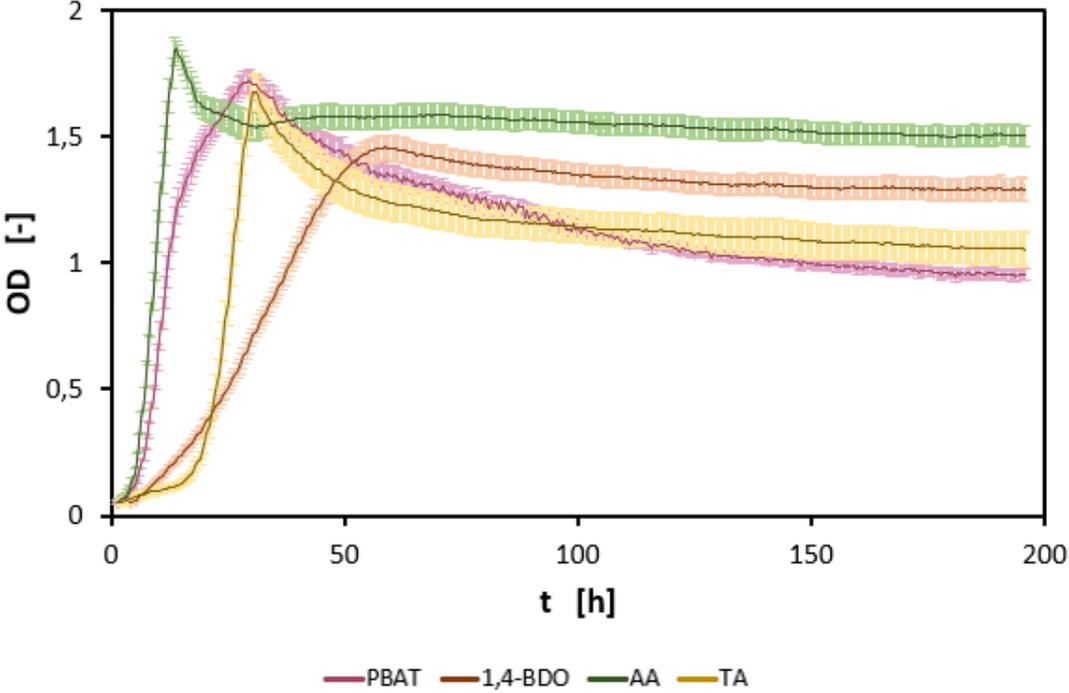
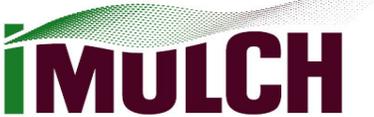
C. necator K7



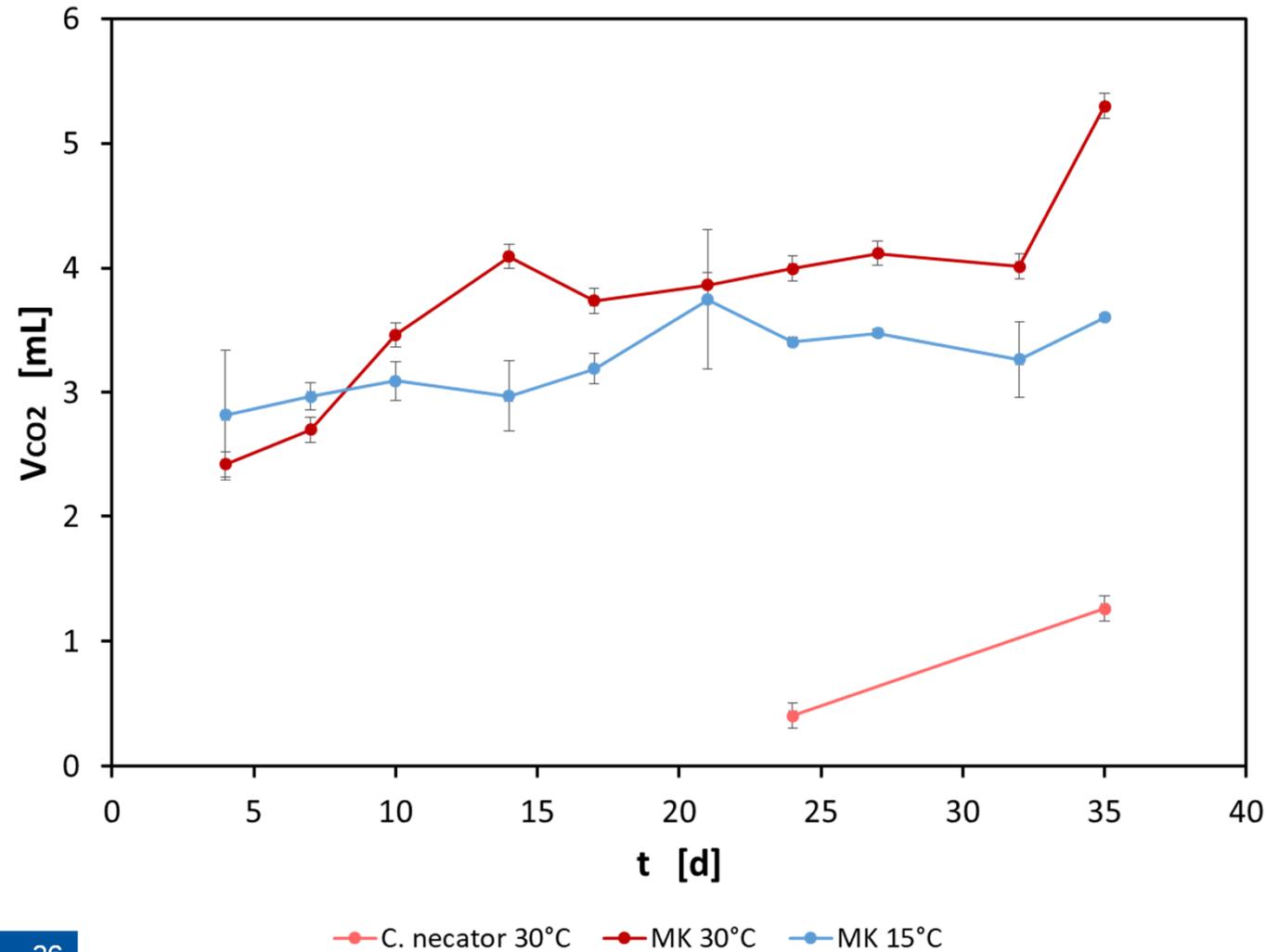
C. necator K9



Wachstum auf PBAT-PLA Monomermischungen muss klappen



Gibt es eine Hydrolyseaktivität? Abbau von Folienpartikeln



Medium: MSM mit 10 g/L PBAT-Folienpartikeln

Kultivierung:

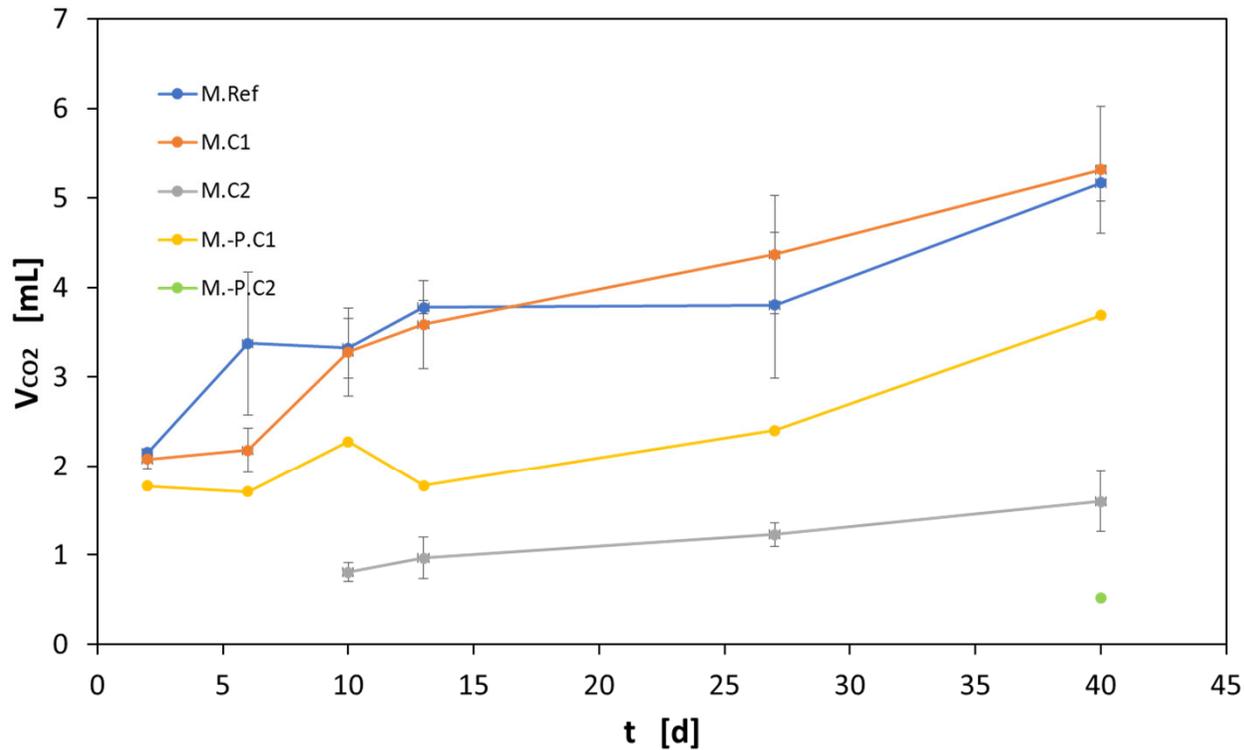
- 30°C; 200 rpm
- 15°C; 100 rpm

Quantifizierung des CO₂-Volumens:

- Multiple Gas Analyzer (SRI Instruments)
- Molsieve 13x; TCD; He

➔ PBAT-PLA wird abgebaut

Abbau PBAT-PLA-Folienpartikel + Folicur



Mischkultur *C. necator* +
P. polychromogenes

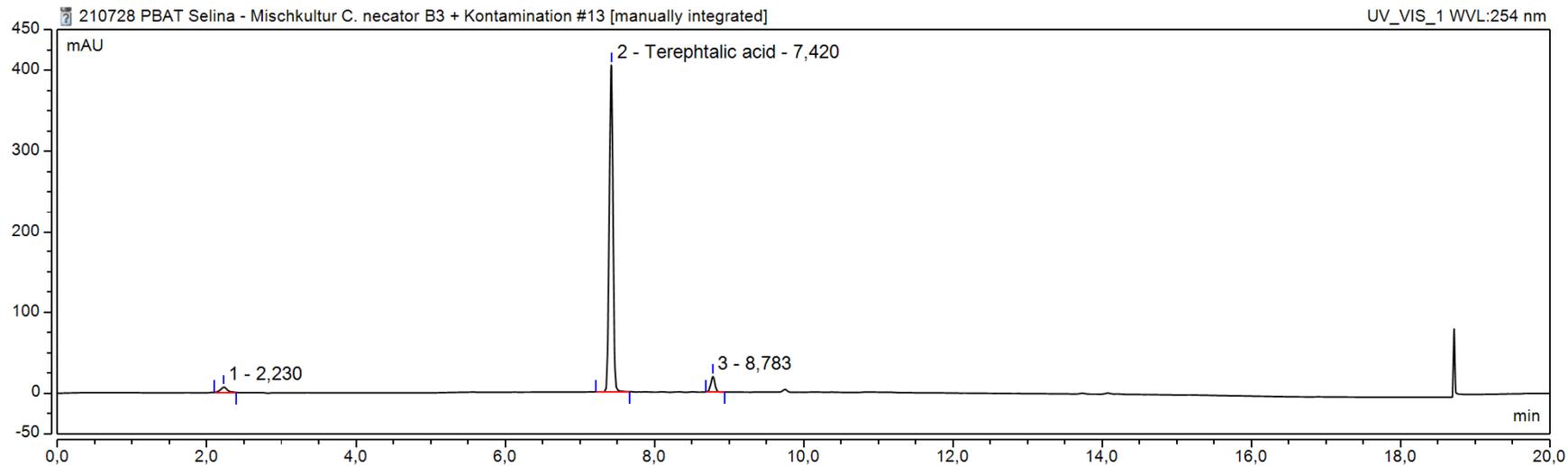
- M.Ref: Mischkultur mit Folienpartikeln, ohne Pestizid
- M.C1: Mischkultur mit Folienpartikeln, [Tebuconazol]=1,875 µg/mL
- M.C2: Mischkultur mit Folienpartikeln, [Tebuconazol]=608 µg/mL
- M.-P: Mischkultur ohne Folienpartikel mit Folicur (C1/C2)

- ➔ Tebuconazol scheint bei hohen Konzentrationen zu inhibieren
- ➔ Tebuconazol wird verstoffwechselt?!

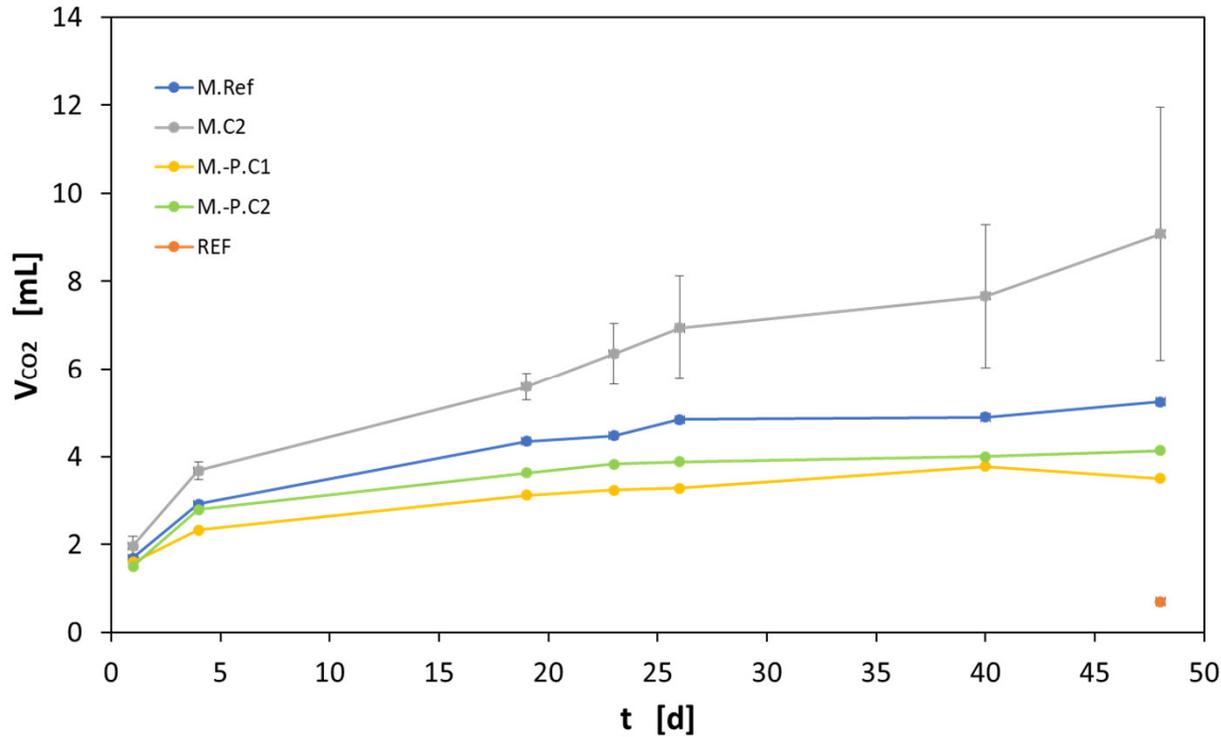
Monomeranalytik: Partikelabbau + Folicur



- Keine Adipinsäure oder 1,4-Butandiol
- Terehptalsäure (ca. 1 mM) in Proben mit 608 µg/mL Tebuconazol



Abbau PBAT-PLA-Folienpartikel + Biscaya



Mischkultur *C. necator* +
P. polychromogenes

- M.Ref: Mischkultur mit Folienpartikeln, ohne Pestizid
- M.C2: Mischkultur mit Folienpartikeln, [Thiacloprid]=360 µg/mL
- M.-P: Mischkultur ohne Folienpartikel mit Folicur (C1/C2)
- REF: MSM mit Folienpartikeln, ohne MOs

→ Thiacloprid inhibiert NICHT
→ Thiacloprid wird verstoffwechselt?!

Beiträge zu einer biobasierten zirkulären Plastikwirtschaft



- Mischplastikmonomer Verstoffwechslung nachgewiesen
- Wertstoffsynthese mit Mikroben gezeigt
- Können wir mit Mikroben helfen „aufzuräumen“?!



PHA resin, Bioplastech Ltd, Dublin

=> Vom Plastikmüll zum Plastikwertstoff

Danke für Ihre Aufmerksamkeit!

By Gabe Martin



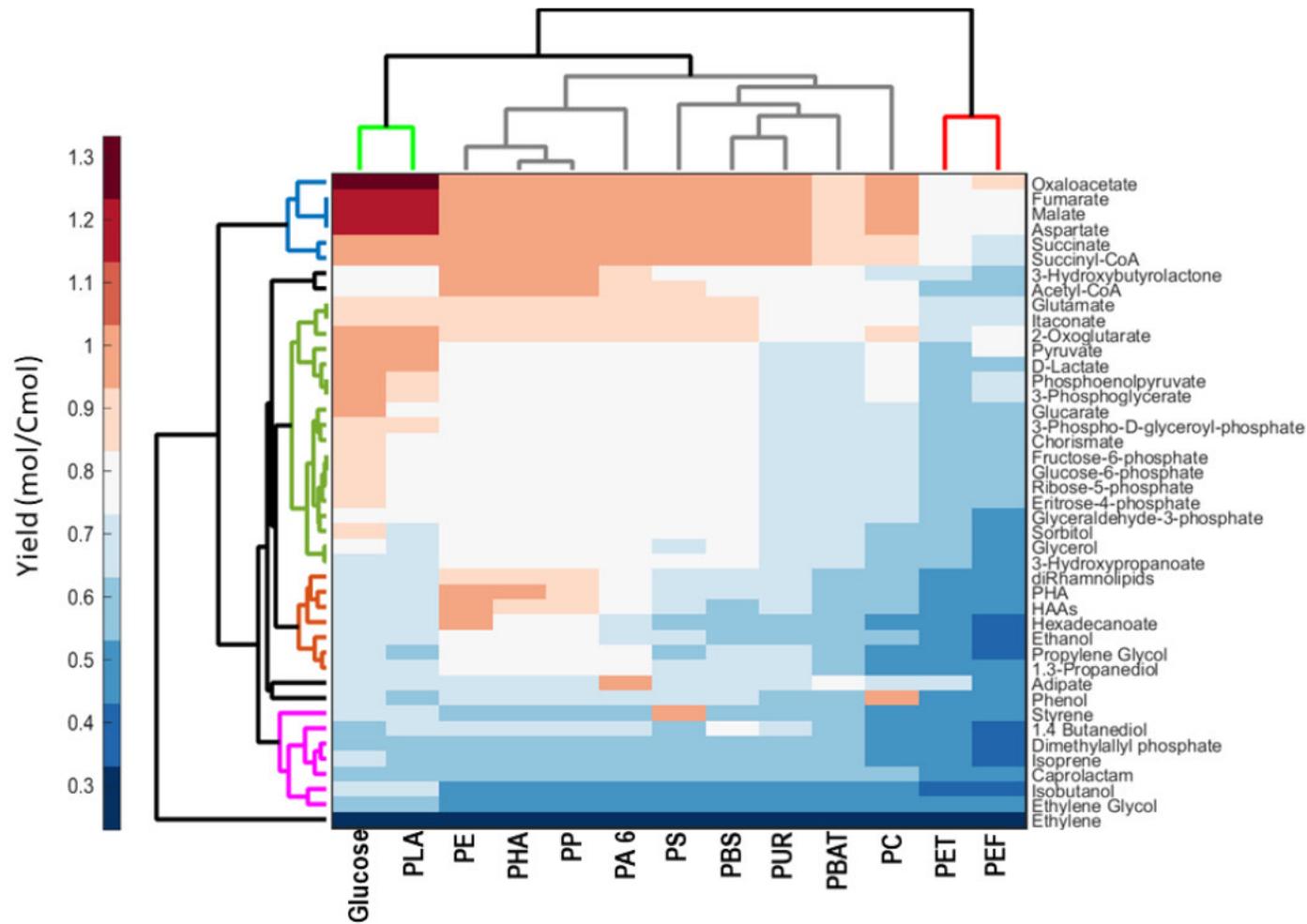
<http://www.cts.com/~borderln/>

Fragen bitte!

Now the question:

Which plastic as substrate for which product?

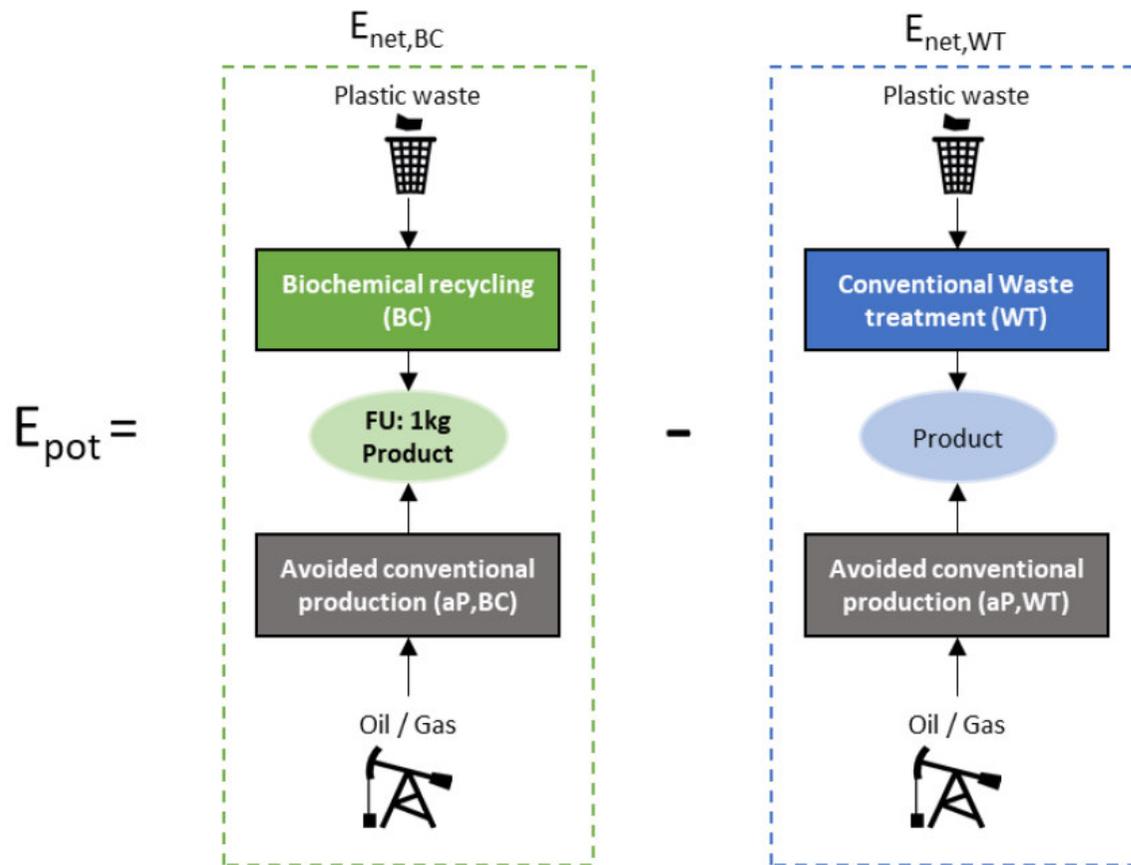
Product yield on plastic substrate – an evaluation



- Genome scale metabolic model for estimating theoretical yields
- Substrate/product pairs result in differences in yield

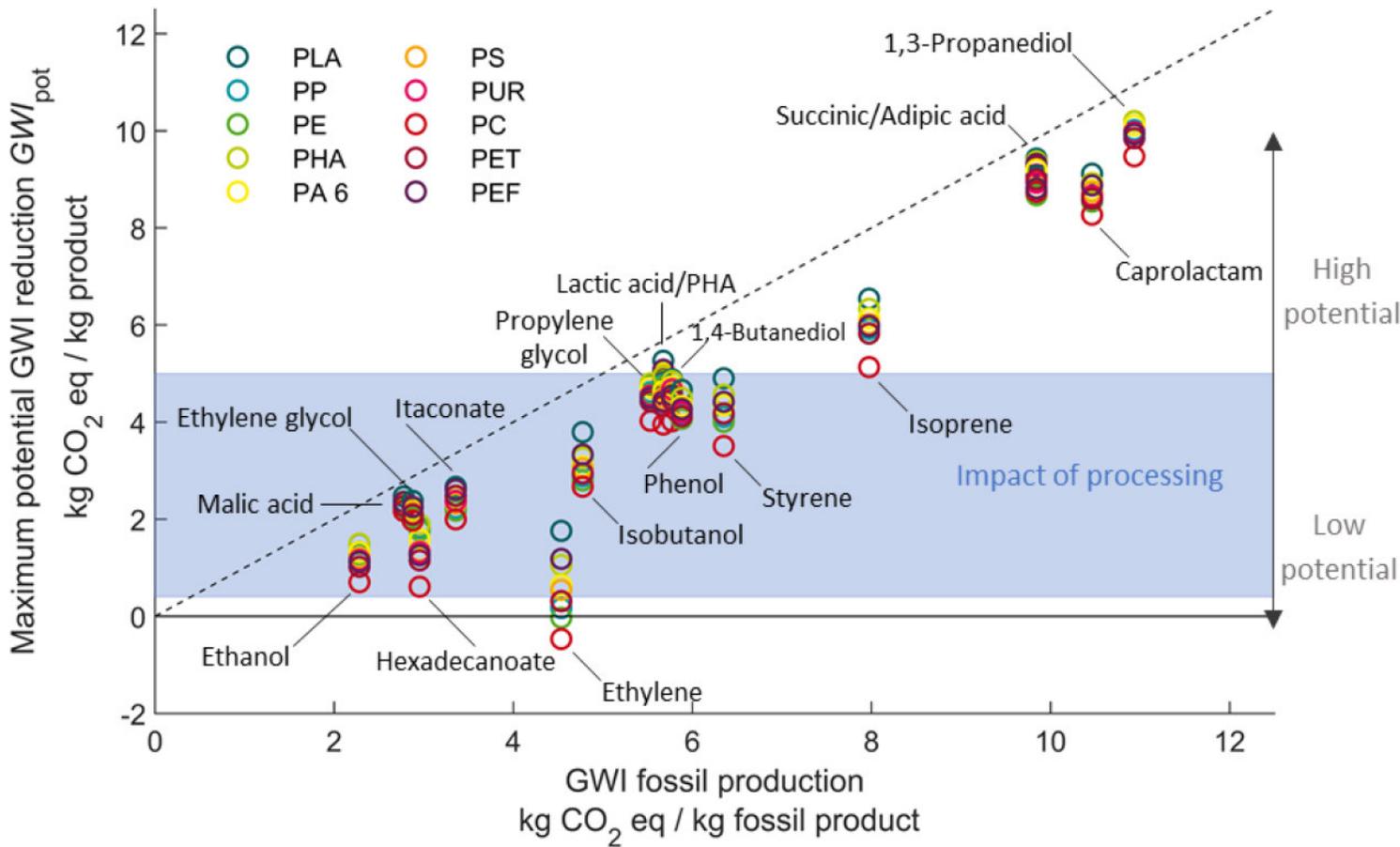
Tiso, Winter, Wei, Hee, de Witt, Wierckx, Quicker, Bornscheuer, Bardow, Nogales, Blank. the metabolic potential of plastics as biotechnological carbon sources – Review and targets for the future. Metab Eng.,

System boundaries – estimating maximal impact reduction



E_{pot} : environmental impact reduction potential

Not all products are good targets!



- Biochemical yield less important, chemical route decides on GWI reduction
- Some products geared for synthesis from mixed monomers

GWI: global warming impact

Tiso, Winter, Wei, Hee, de Witt, Wierckx, Quicker, Bornscheuer, Bardow, Nogales, Blank. the metabolic potential of plastics as biotechnological carbon sources – Review and targets for the future. Metab Eng., Online ahead of print

Stay tuned: visit the webpage – NEWS, BLOG, and more



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▶ We use bacteria for MIXed plastics biodegradation and UPcycling!

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Will This UN Treaty Really Change The (Plastic) World?

03 March 2022 | By Leon Kirschgens & Jaqueline Plaster

A historic week is behind us. The UN Environment Assembly has agreed to a treaty elaboration that could effectively fight the plastic problem. An overview and report of an assembly in which we too have placed a lot of hope.

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16 February 2022

Mr. Tiso, Are Plastic Enzymes and Bacteria Dangerous When Released...



10 February 2022

How the Triple S Approach Can Help a Growing Number of Sustainability...



04 February 2022 | By Leon Kirschgens & Jaqueline Plaster

"The Boundary Has Long Been Exceeded"

PBAT Upcycling: Neutrale Lipide (TAG) Produktion mit *Rhodococcus opacus*

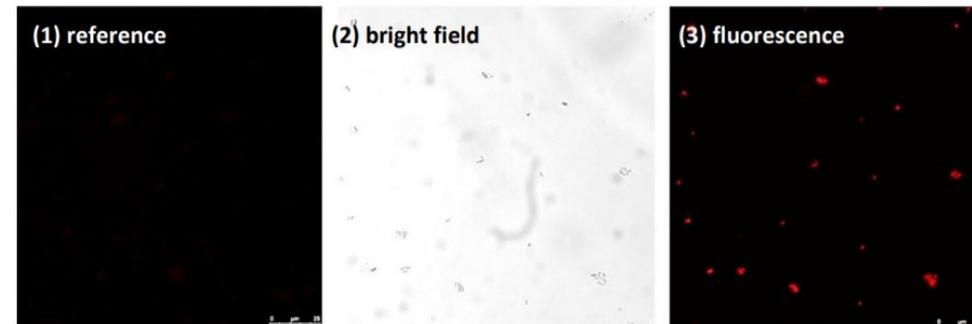
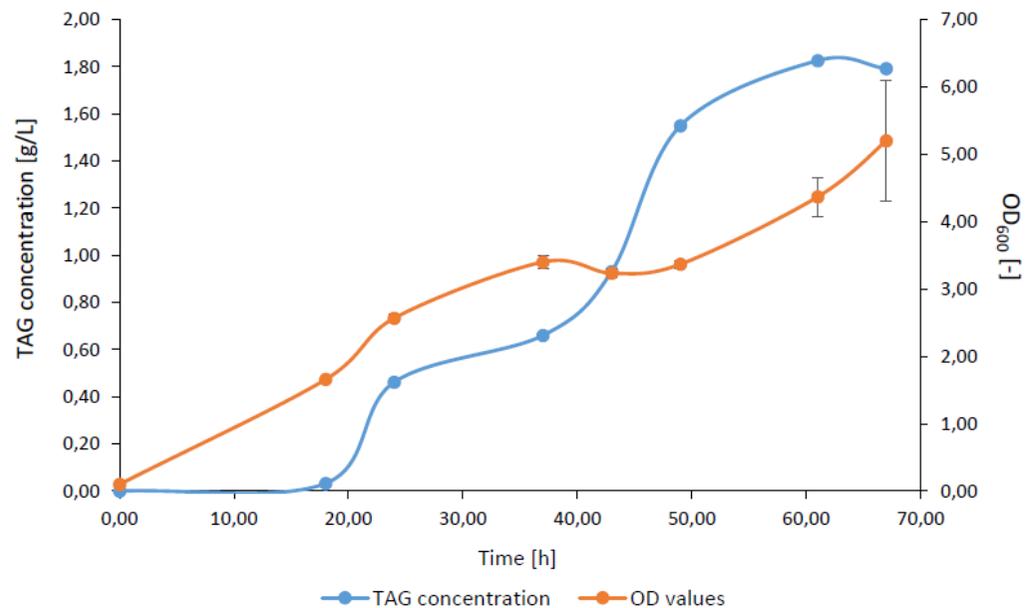


Figure 14: *R. opacus* TAG production with Delft+PBAT medium using fluorescence microscopy. Picture (1) represents a fluorescence recording of the reference culture with no N-limitation; picture (2) is a bright field microscopy record of a N-limited culture; picture (3) is a fluorescence recording of the same culture. Pictures were recorded after 72h of cultivation.

Vielen Dank für die Aufmerksamkeit!



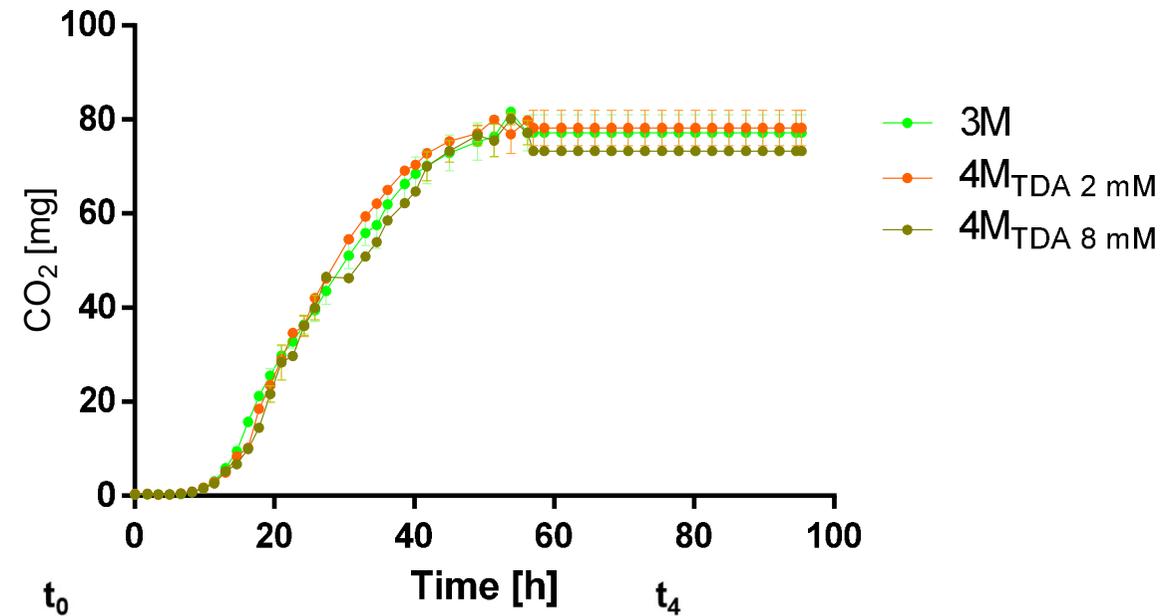
EUROPÄISCHE UNION
Investition in unsere Zukunft
Europäischer Fonds
für regionale Entwicklung



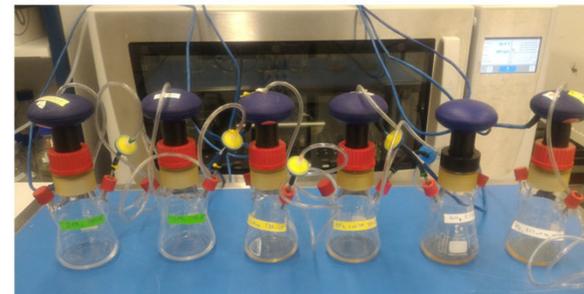
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The toxicity test of TDA on *P. capeferrum* TDA1



t_0



t_4

