BIOTECH RESEARCH AND INNOVATION HACK 2021

Mid-term seminar of the projects from 2. call of ERA CoBioTech

Project name: Microbial conversion of lignin to monomers for bio-based plastics using synthetic biology

Project acronym: MILIMO

Name: Prof Tim Bugg (University of Warwick, UK)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

27.09.2021

Project partners

Partners

Prof Tim Bugg, University of Warwick, UK (Co-ordinator) –lignin biotransformation
Dr Eduardo Diaz, CSIC, Madrid, Spain – microbial genetics of *Pseudomonas putida*
Dr Stéphanie Baumberger, INRA, Paris, France – lignin characterisation
Prof Ralf Takors, University of Stuttgart, Germany – scale-up, biochem engineering
Biome Bioplastics (SME, Director Paul Mines), UK – industry partner
Nova Institute GmbH (Germany) – sustainability & life cycle analysis

- Total project budget: 1.0 M Euros
- Project start: 2nd March 2020
- Contact: T.D.Bugg@warwick.ac.uk

Introduction

- Project objectives

Major unsolved problem in industrial biotechnology: **how to convert aromatic biopolymer lignin into aromatic chemicals**. Aim is to generate **pyridine-dicarboxylic acids** from lignin using metabolic engineering, to be used as monomers for polyester bioplastic synthesis.

- Scientific approach

WP1 Enhancing lignin depolymerisation

WP2 Redesigning metabolic pathways

WP3 Synthetic microbial consortia

WP4 Large scale fermentation (g to kg)

WP5 Sustainability, life cycle analysis

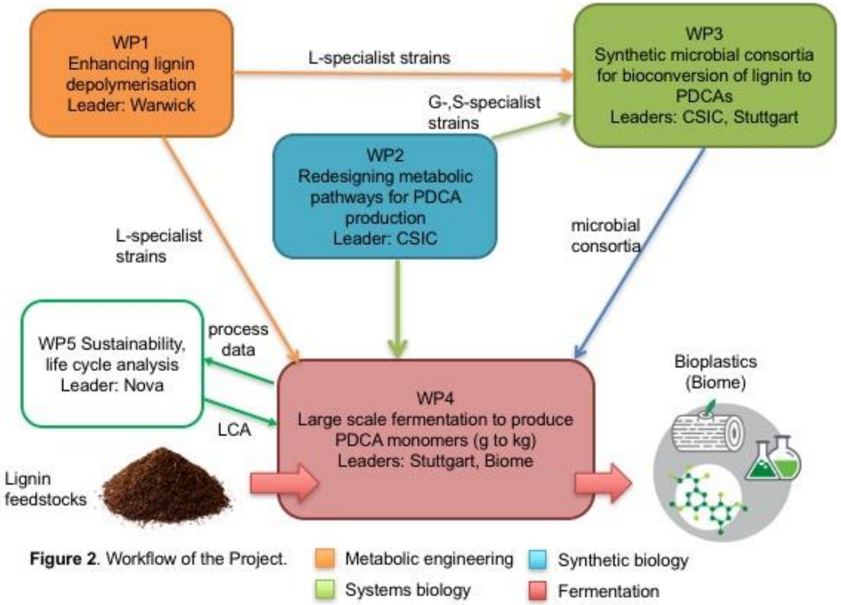


Figure 2. Workflow of the Project.

Background to Project

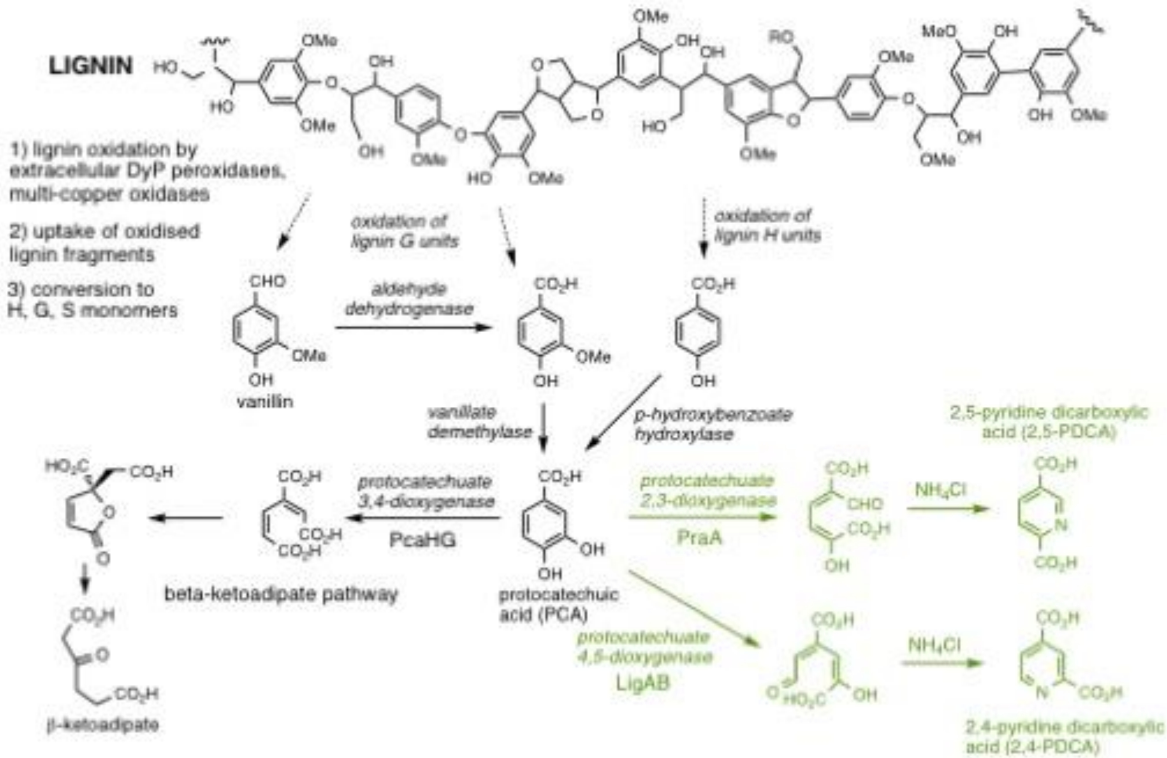


Figure 1. Conversion of lignin to PDCA monomers

2015 Insertion of *ligAB* or *praA* genes into *Rhodococcus jostii* produces 2,4-PDCA or 2,5-PDCA in 80-125 mg/L from 1% wheat straw in 7 days.

2015-2018 Gene deletion of *pcaHG*, chromosomal expression of *ligAB* genes, overexpression of *dyp2* gene produces 2,4-PDCA in 300 mg/L in 40 hr.

Project plan

WP1 Enhancing lignin depolymerisation in *Rhodococcus jostii* RHA1 and *Pseudomonas putida* KT2440

- Insertion of modules containing lignin-oxidising genes (months 1-12)
- Protein secretion into periplasm/cell surface (months 1-18)

WP2 Redesigning metabolic pathways for synthesis of PDCA monomers

- Additional metabolic routes to PDCAs, specialists for conversion of G- or H-lignin (months 12-24)
- Gene deletion of competing pathways (months 12-24)

WP3 Synthetic microbial consortia for PDCA production

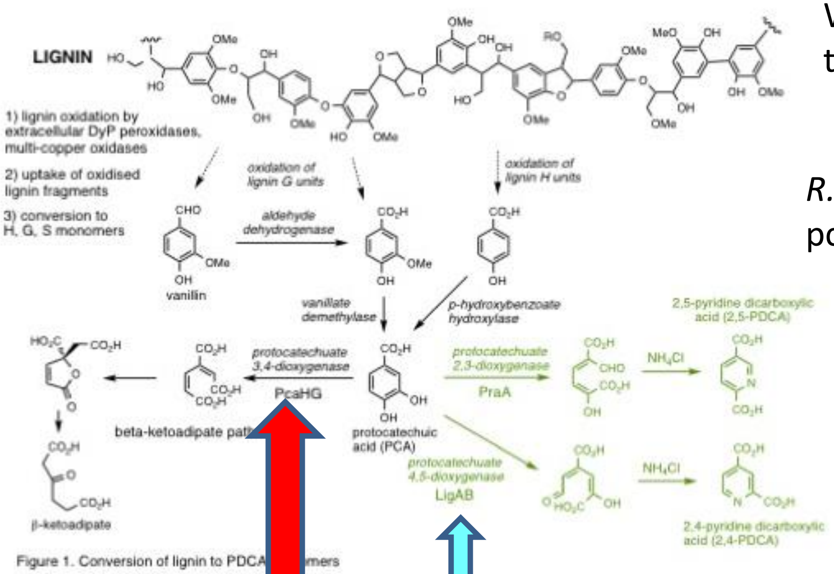
- Use of co-cultures or consortia for conversion of lignin to PDCAs (months 18-36)

WP4 Large scale fermentation

- Downstream processing (months 1-12)
- bioconversion at g to kg scale (months 12-36)

WP5 Sustainability, life cycle analysis (months 12-36)

Rhodococcus jostii RHA1 production strains

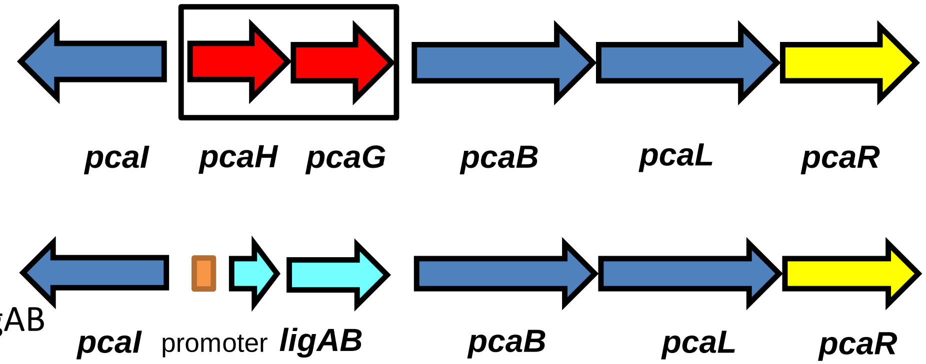


pcaHG genes deleted

ligAB genes inserted, with promoter

R. jostii Wild-type

R. jostii pcaHG::ligAB



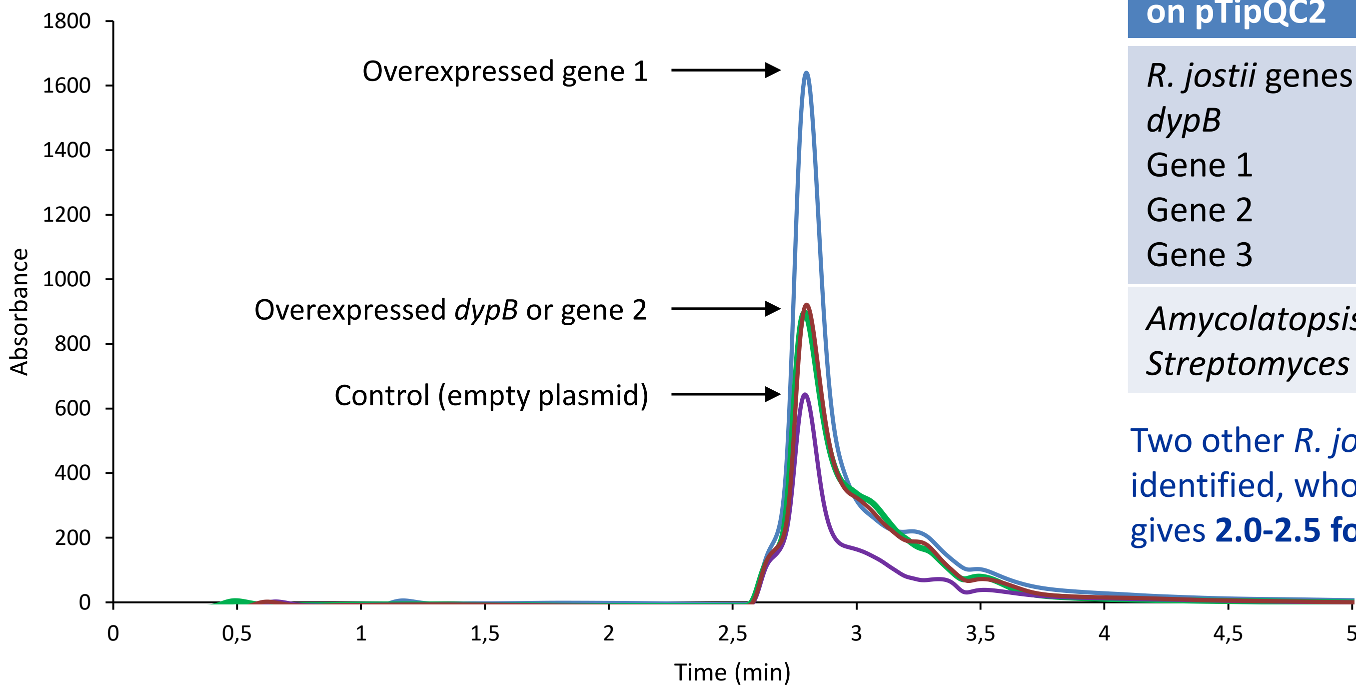
Promoter	Type	Inducer	PDCA titre 1% wheat straw	PDCA titre 1% Green Value lignin
PicI PnitA Ptpc5	Inducible Inducible constitutive	methanol ε-caprolactam - *with pTipQC2-dyp2*	ND 79 mg/L 290 mg/L *330 mg/L*	70 mg/L 100 mg/L 164 mg/L *240 mg/L*

- Constitutive Ptpc5 promoter is effective for gene expression in *R. jostii* RHA1
- Inducible PnitA promoter also active
- Overexpression of *Amycolatopsis dyp2* gives enhanced PDCA titre
- Test overexpression of other lignin-oxidizing genes

E.M. Spence, L. Calvo-Bado, P. Mines, and T.D.H. Bugg, *Microb. Cell Fact.*, **20**, article 15 (2021).

WP1. Overexpression of lignin-oxidising genes in *Rhodococcus jostii RHA1* production strain (Warwick)

Production of 2,4-PDCA from *Rhodococcus jostii pcaHG::ligAB* (ptpc5) containing pTipQC2-empty (control) or lignin-oxidising genes, grown for 7 days in M9/1% Green Value lignin



Gene overexpressed on pTipQC2	Fold increase in 2,4-PDCA
R. jostii genes:	
dypB	1.4
Gene 1	2.5
Gene 2	1.4
Gene 3	2.0
Amycolatopsis dyp2	1.5
Streptomyces SLAC	1.4

Two other *R. jostii* genes have been identified, whose overexpression gives **2.0-2.5 fold increase in PDCA titre**.

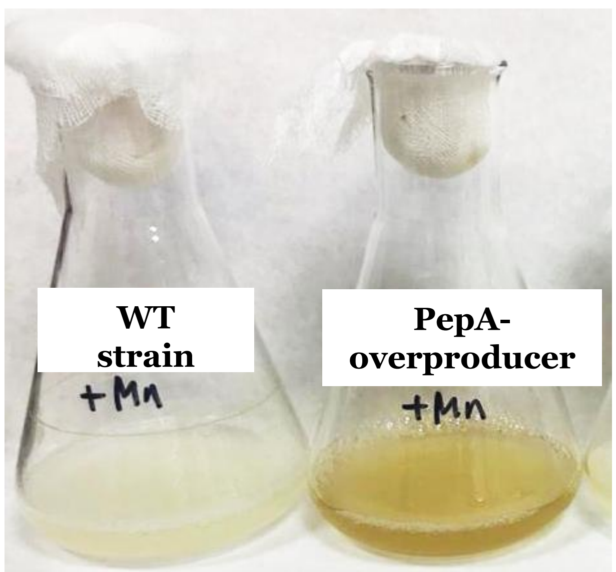
WP1. *P. putida* PepA is an extracellular manganese oxidase induced in the presence of lignin (CIB)

A

Genes coding PepA and its T1SS are highly induced in the presence of lignin

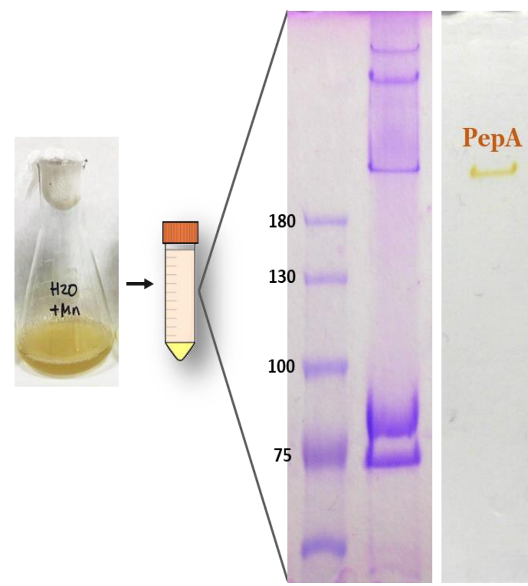
B

The enzyme generates Mn oxides

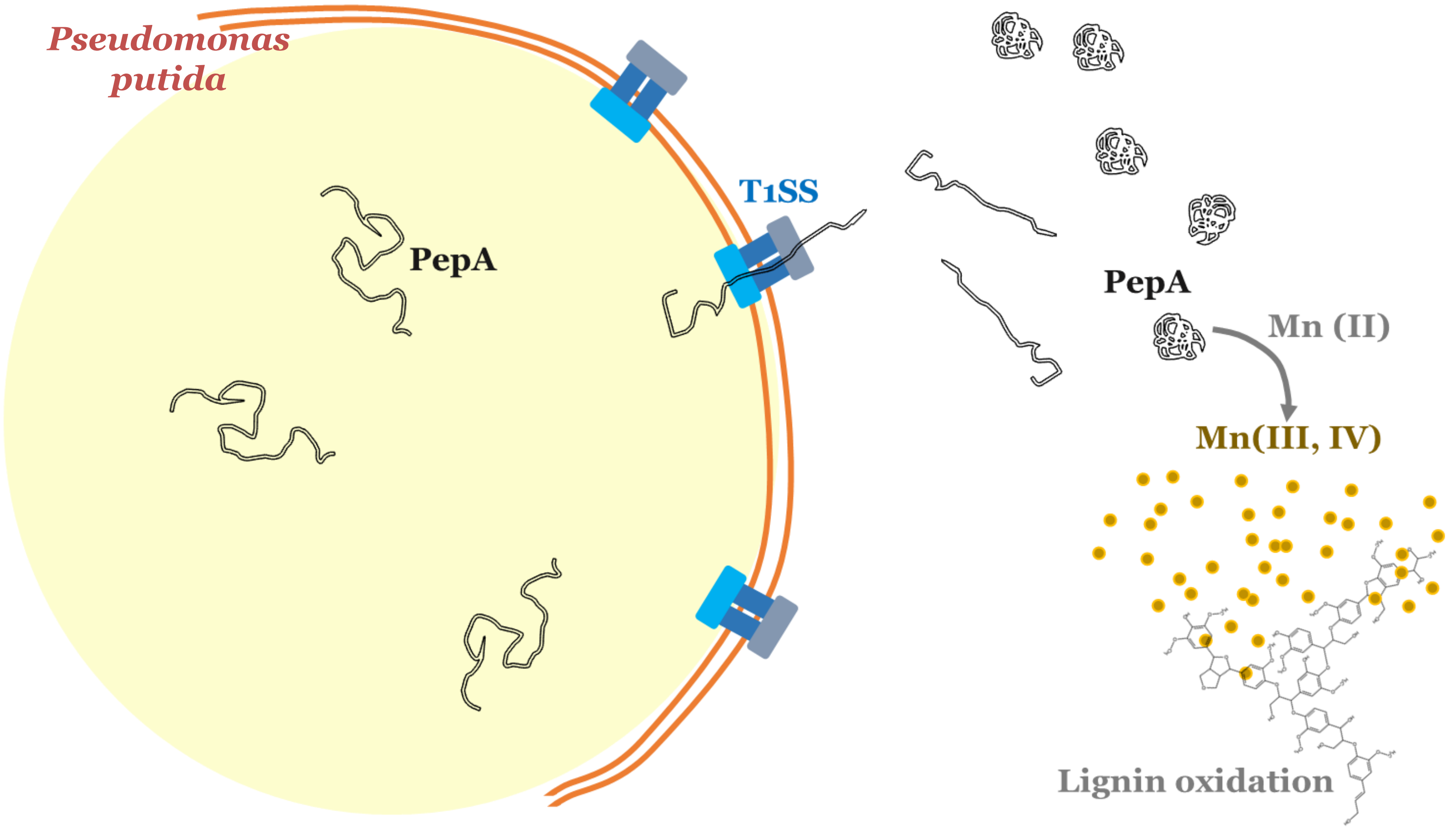


C

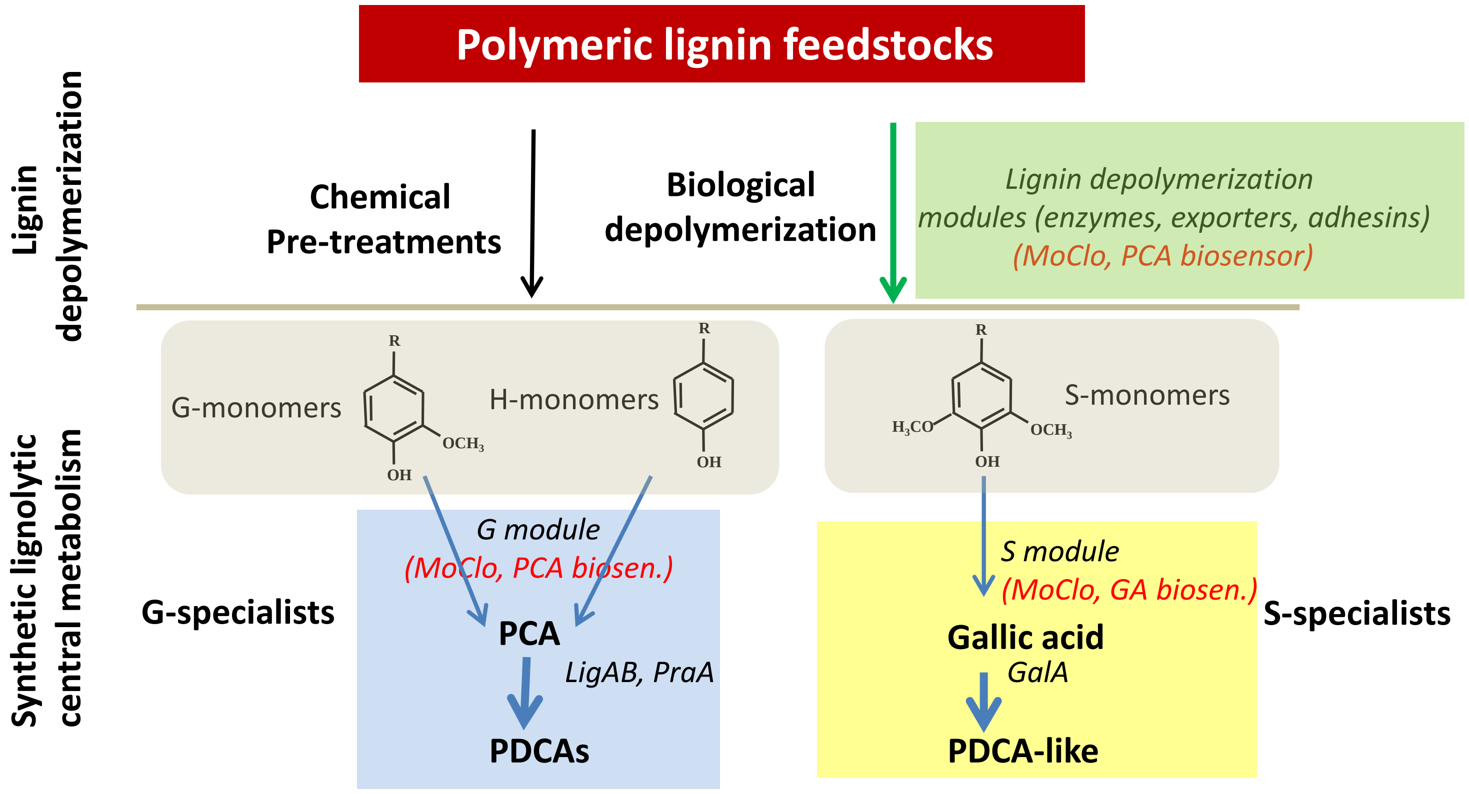
PepA is an extracellular enzyme



WP1. *P. putida* PepA is an extracellular manganese oxidase induced in the presence of lignin (CIB)

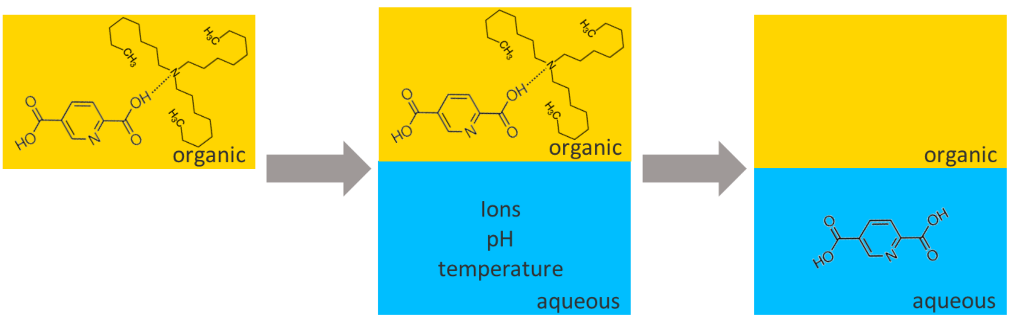


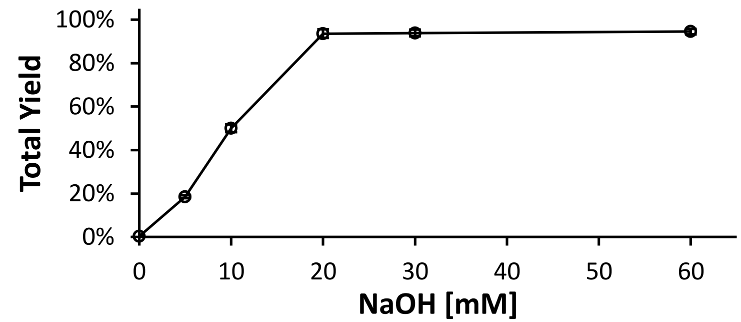
WP2. Design of G- and S-specialist *P. putida* strains for degradation of softwood vs hardwood lignins (CIB)



WP4. Downstream processing of PDCA product via reactive extraction (Stuttgart)

Theory



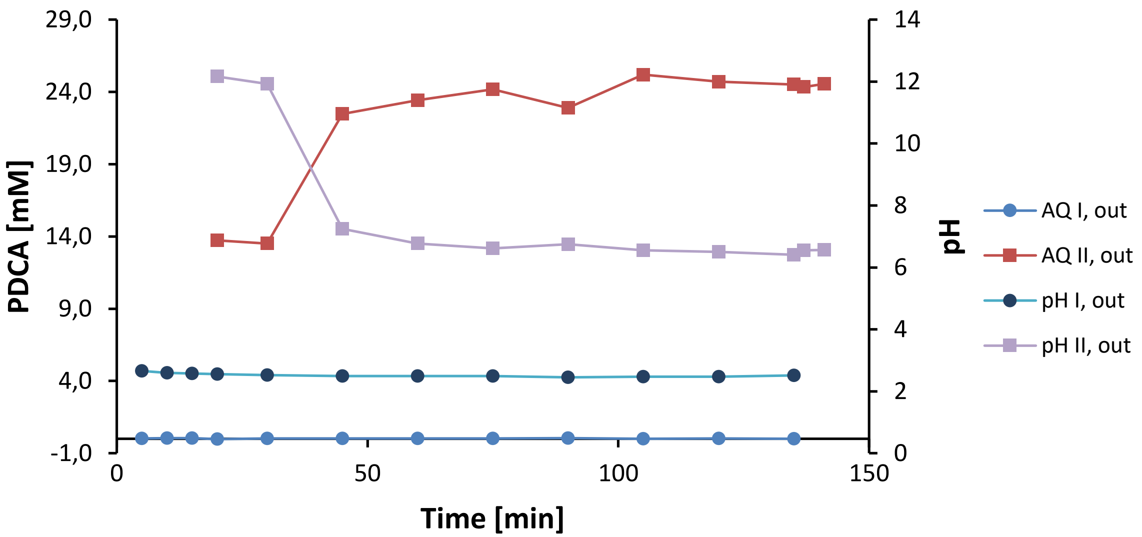


- Reactive extraction = Physical extraction coupled with a chemical “reaction”
- Complex formation between product (PDCA) in aqueous phase and extractant in organic phase
- Recovery of PDCA from organic phase is achieved by pH shift through base (NaOH) addition

- Trioctylamine found to be effective as an extractant.
- Complete recovery with 20 mM NaOH (pH 12.3)
- No impact of volume and temperature on recovery performance
- Recycling of organic phase after product recovery is possible

WP4. Downstream processing of PDCA product via reactive extraction (Stuttgart)

Scale-up of reactive extraction in centrifugal extractors:



- Reactive extraction in litre-scale with centrifugal extractors
- Two serial centrifugal extractors allow simultaneous extraction and back-extraction
- Recycling of the organic phase between both extractors reduces organic solvent requirement and process costs
- Complete extraction of 2,5-PDCA from aqueous donor solution

Route to application.
Biodegradable tree shelter project

Biome Bioplastics has started a new collaboration with Suregreen, a leading UK based supplier of tree shelters, to end the plastic pollution caused by tree shelters.

Tree shelters are used to protect young trees and hedges from predation by animals. They are widely used by the forestry industry as a well-proven and economic route to limiting losses in the first 5 years after planting.

Most tree shelters are made from oil-based and non-biodegradable plastics and are not collected at the end of their life with the result they litter the landscape with microplastic pieces (UK estimate 2500 tonnes per year). Plans to significantly increase tree planting as part of the UK's push to net zero emissions will increase these littering problems without an innovative solution. We began work on this challenge in 2020 with a design brief of designing shelters that provide market-leading protection to growing trees.

https://biomebioplastics.com/tree-shelter/

BIOME BIOPLASTICS

sure green

Project outcomes so far

Publications

- 1. “Metabolic engineering of *Rhodococcus jostii* RHA1 for production of pyridine-dicarboxylic acids from lignin” E.M. Spence, L. Calvo-Bado, P. Mines, and T.D.H. Bugg, *Microb. Cell Fact.*, **20**, article 15 (2021).
- 2. “Assessment strategy for bacterial lignin depolymerisation: Kraft lignin and synthetic lignin bioconversion with *Pseudomonas putida*” E. Rouches, H. Gomez-Alvarez, A. Majira, Z. Martin-Moldes, J. Nogales, E. Diaz, T.D.H. Bugg, S. Baumberger, *Bioresource Technol. Reports*., **15**, article 100742 (2021).
- *Poster presentation*. “Bioplastic production from lignin – Development of a novel downstream processing for biotechnologically produced PET replacement” J. Notheisen, R. Takors, Himmelfahrtstagung on Bioprocess Engineering 2021 – New Bioprocesses, New Bioproducts 10 – 12 May 2021.

Meetings

- No face-to-face meetings due to COVID-19 pandemic
- Online Project meetings in April 2020, October 2020, May 2021.

Contact details

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