Production of Organic Acids for Polyester Synthesis

Project acronym: POAP

Project no: EIB.12.007

Marta Tortajada

ERA-IB-2 final conference, Berlin, 16./17.02.2016

Project partners



- *POAP consortium:*

1. BIOPOLIS (SME), Spain
2. Thünen Institute (Research Institute) , Germany
3. Universidad Complutense de Madrid (University), Spain
4. ASA Special Enzymes (SME), Germany
5. H2Biyotek (SME), Turkey

   Middle East Technical University (University), Turkey
6. Ekodenge (SME), Turkey

- *Total project budget:* 1.562.500 €

Biopolis A8A METU ekodenge

Introduction

- Our aim is to develop efficient bioprocesses for the obtention of D-lactic acid and itaconic acid from citrus processing waste and wheat chaff as low-lignin agricultural wastes. Raw glycerol from biodiesel processing is considered as co-substrate.

-Pretreatment and hydrolysis of substrates

-Obtention and validation of novel hydrolases

-Screening and improvement of microbial catalysts

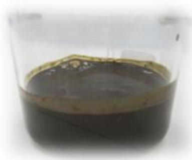
-Immobilization of microorganisms

-Process development: fermentation and SSF

-Purification methods and catalytic upgrade

-Life Cycle Assessment

Introduction




Citrus peel waste
Wheat chaff
Raw glycerol

Pretreatment
Hydrolysis

Screening,
Fermentation

Scale-up

Purification

D-lactic acid
Itaconic acid

Catalytic
upgrade
Polymerization

Life Cycle Assessment

Raw materials

	Wheat chaff
Lignin	27.6
Arabinose	**4.3**
Galactose	**1.3**
Xylose	**16.9**
Glucose	**23.6**
Ash&salts	13.8

	CPW
Lignin	6.5
Pectin	**18.6**
Arabinose	**6.6**
Galactose	**6.9**
Xylose	**2.1**
Glucose	**33.7**
Ash&salts	3.7

	Glycerol
pH	2.9
Glycerol	**88.0**
Methanol	0.0
FAME	-
Water	2
Ash&salts	10.0

PRETREATMENT | HYDROLYSIS | FERMENTATION | PURIFICATION | UPGRADING | POLYMERIZATION

LIFE CYCLE ASSESSMENT – LIFE COSTING CYCLE

MECHANICAL

Milling

- Ultracentrifugal mill
- Blade mill
- Cutting mill

Particle size

- 0.25, 0.75, 2, >2 μm
- 5-8 mm

THERMAL

Microwave

- 120ºC, 40 min
- 120ºC, 80 min

Steam stripping

Steam explosion

CHEMICAL

Acid

- HCl
- H_2SO_4

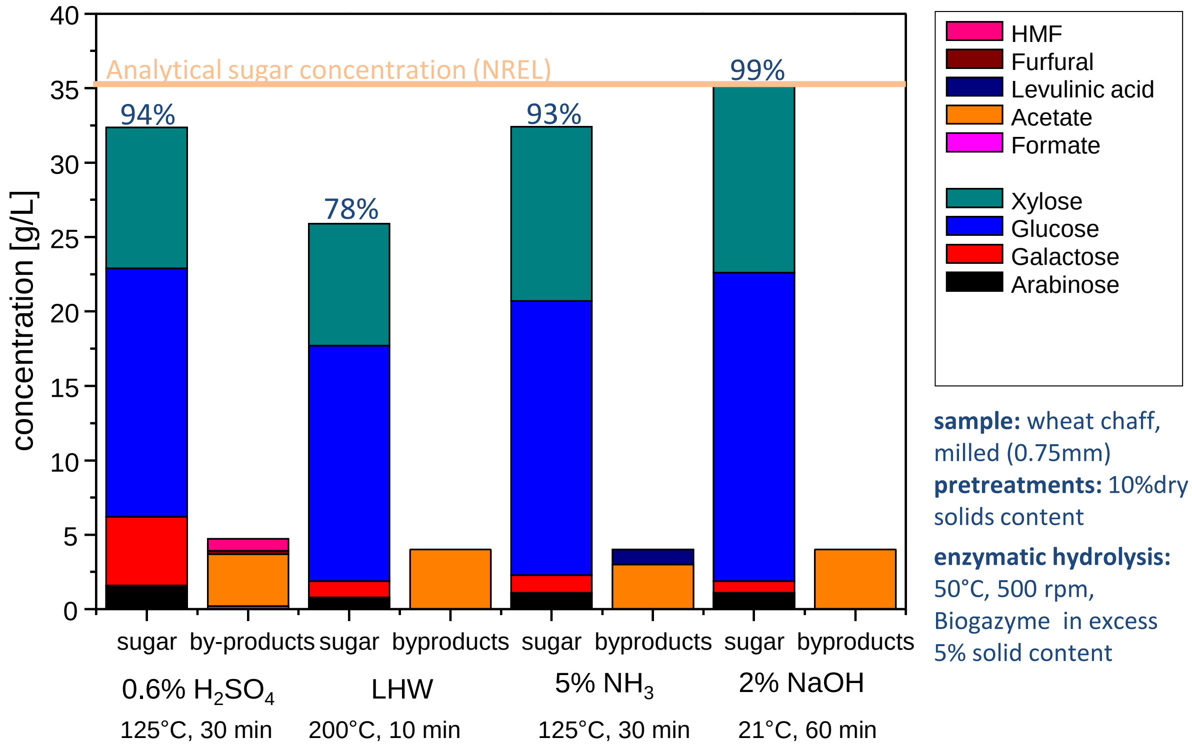
Neutral

- Liquid hot water
- Organo-Solv

Alkaline

- Ammonia solution
- Sodium hydroxide

- # WHEAT CHAFF

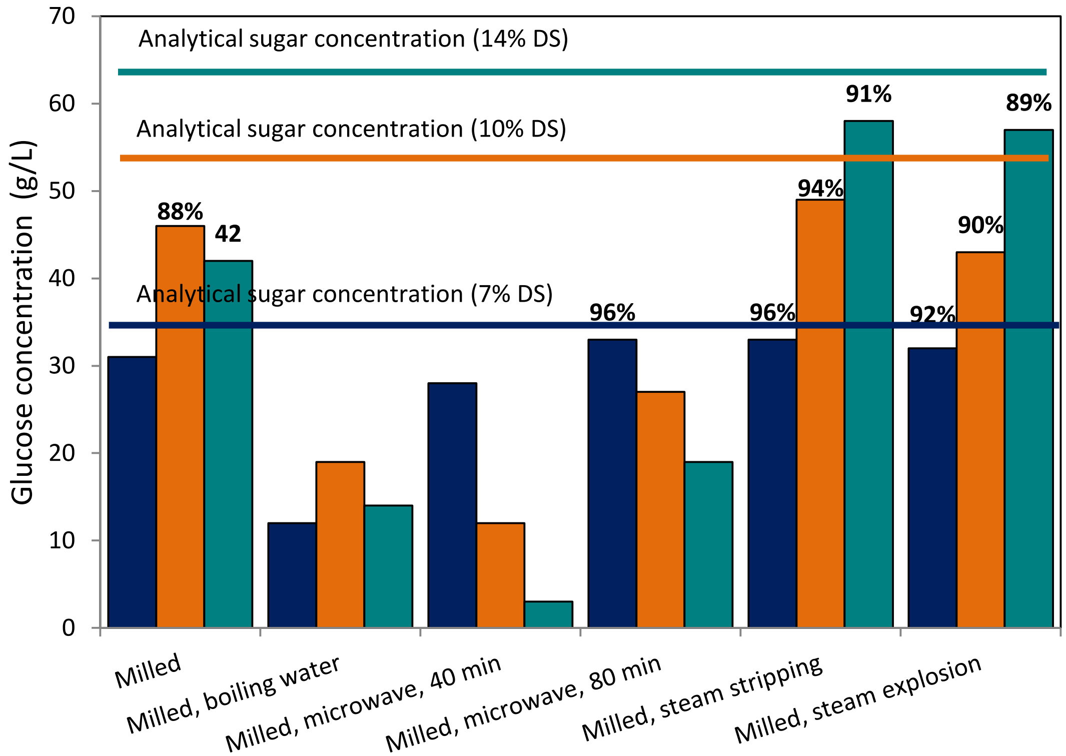


sample: wheat chaff, milled (0.75mm)
pretreatments: 10%dry solids content
enzymatic hydrolysis: 50°C, 500 rpm, Biogazyme in excess 5% solid content

Best pretreatment:
<0.75 µm particle size
No influence milling type
2% NaOH
21°C
60 min

→ Quantitative saccharification
→ Acetate as sole byproduct

• CITRUS PEEL WASTE

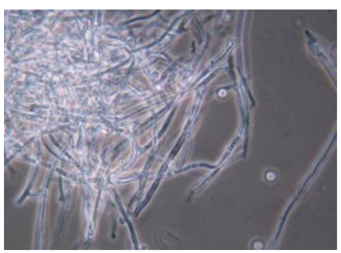


Best pretreatment:
5-8 mm particle size
Steam stripping
1 atm 40min
→ >90% saccharification yield
→ >90% limonene recovered after water treatments

Sample: CPW, blade-milled (5-8 mm)
Pretreatments: 6-14% dry solids content
66% humidity CPW (PD-CPW)
Enzymatic hydrolysis:
50 ºC, 300 rpm
Excess cellulase and pectinase

METU

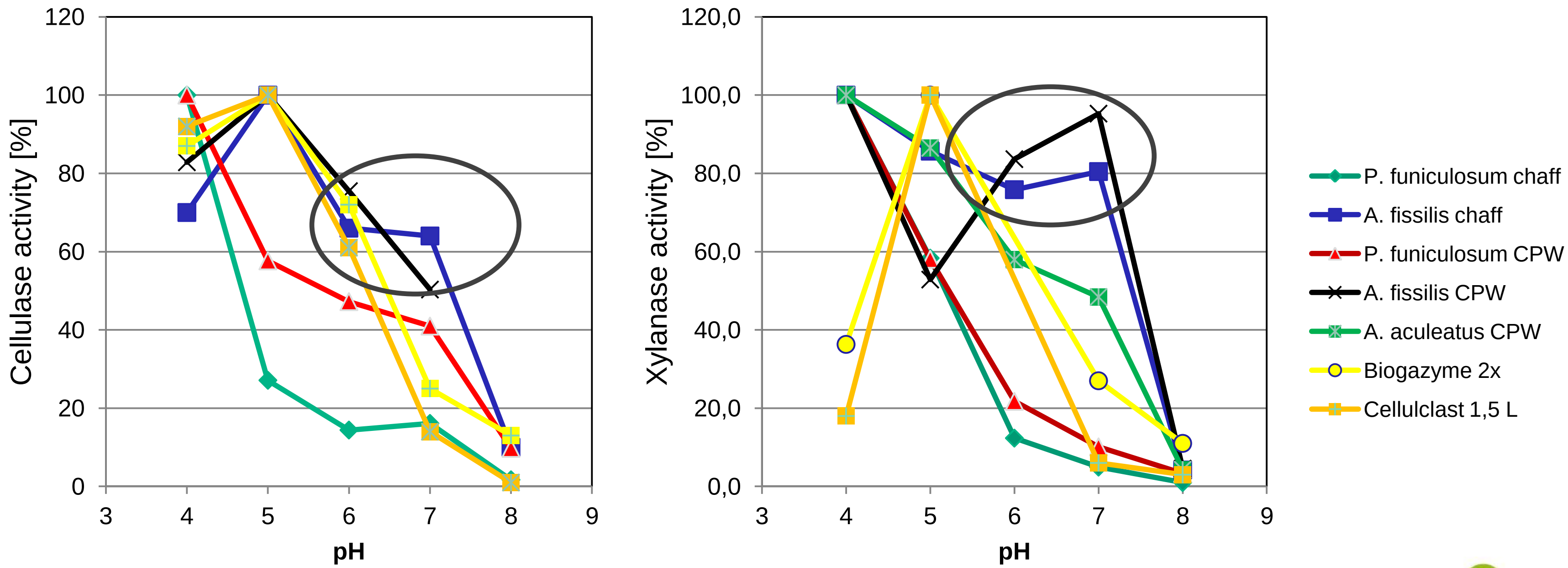
• NOVEL HYDROLASES



Lyophilizates	**Enzyme activity [units/g]**				
	exo-Cellulase	endo-Cellulase	ß-Glucosidase	Xylanase	Endo-pectinase
P. funiculosum chaff	5,73	1.125	4,7	718	
A. fissilis chaff	3,13	1.200	9,6	275	
A. aculeatus chaff	1,23	275	6,0	51	> 30
P. funiculosum CPW	8,16	1.880	8,3	560	> 600
A. fissilis CPW	5,39	1.730	1,1	583	0
A. aculeatus CPW	0,51	680	18,1	115	14,4

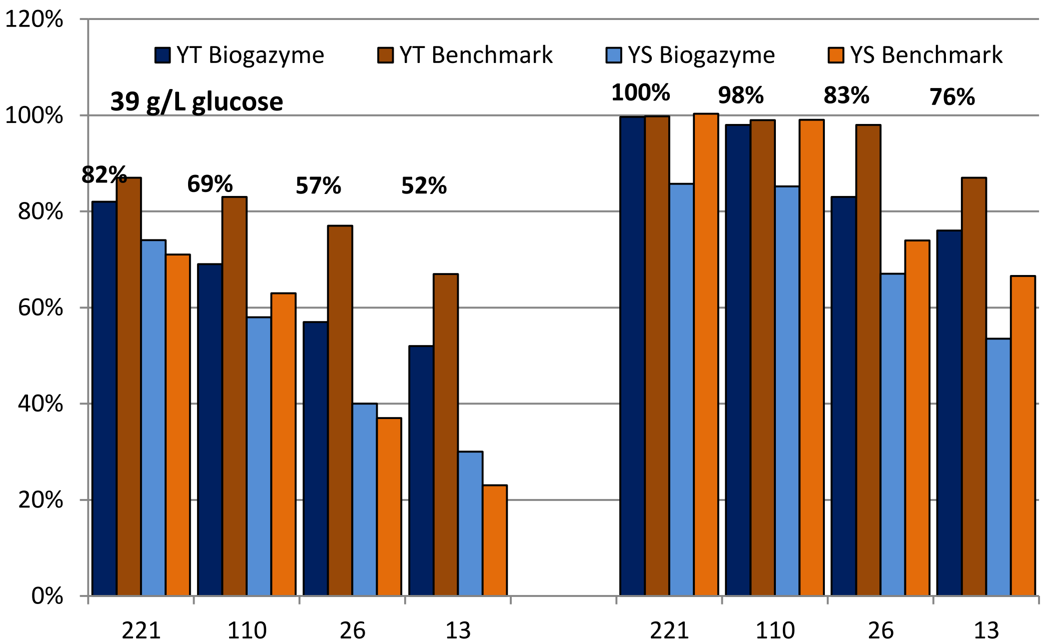
→ *Penicillium funiculosum* and *Aurantioporus fissilis* strains selected as producers.
→ Four different cellulase, hemicellulase and pectinase preparations produced and supplied.

• NOVEL HYDROLASES



→Characterization of new cellulase and hemicellulase preparations.
→pH profile of exo-cellulase and xylanase of *A. fissilis* shifted slightly to neutral values.
→Hydrolases seem to have higher thermostabiility

• CITRUS PEEL WASTE: SACCHARIFICATION



10% dry solid content
26 h batch saccharification

10% dry solid content
150 h batch saccharification

Optimized hydrolisis CPW (Taguchi design):

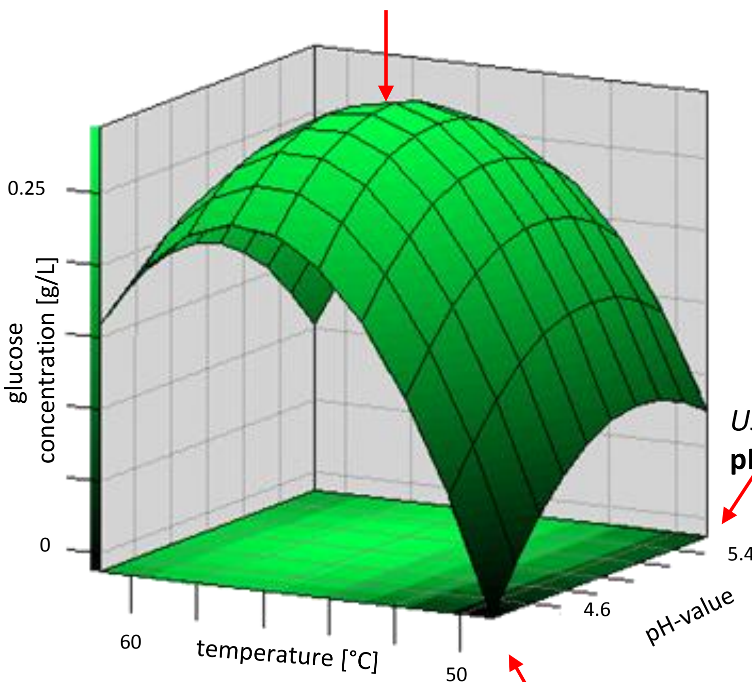
Benchmark
3 feedings
12 FPU/g DS
Pectinase/depectination required
70-90% YT at 6 FPU/g DS

Biogazyme
4-5 feedings
28 FPU/g DS
Pectinase/depectination required
70-80% YT at 6 FPU/g DS

→ Working conditions seem to favour SSF.

• WHEAT CHAFF: SACCHARIFICATION

Cellulase optimum of Biogazyme 2x : **pH 5, 55 °C**



Ustilago maydis: **pH 6, 30 °C**

Aspergillus terreus: **pH 3.4, 35 °C**

- SSF with two different itaconic acid producers was tested.
- SSF was not possible with *U. maydis, A. terreus* very low titer (< 2 g/L).
- Conditions of hydrolysis do not correspond with fermentation conditions of *A. terreus* and *U. maydis.*

→ SHF must be attempted in this case



• FERMENTATION – Screening set-up

Substrates used for screening and selection

Pure and raw glycerol from biodiesel processing
Synthetic hydrolysates
Ground chaff, chaff hydrolysate
Ground orange peels, orange peels hydrolysate

Screening strain sources

Bacteria (mesophilic strains, lactic acid bacteria), yeast and filamentous fungi
Culture and proprietary strain collections

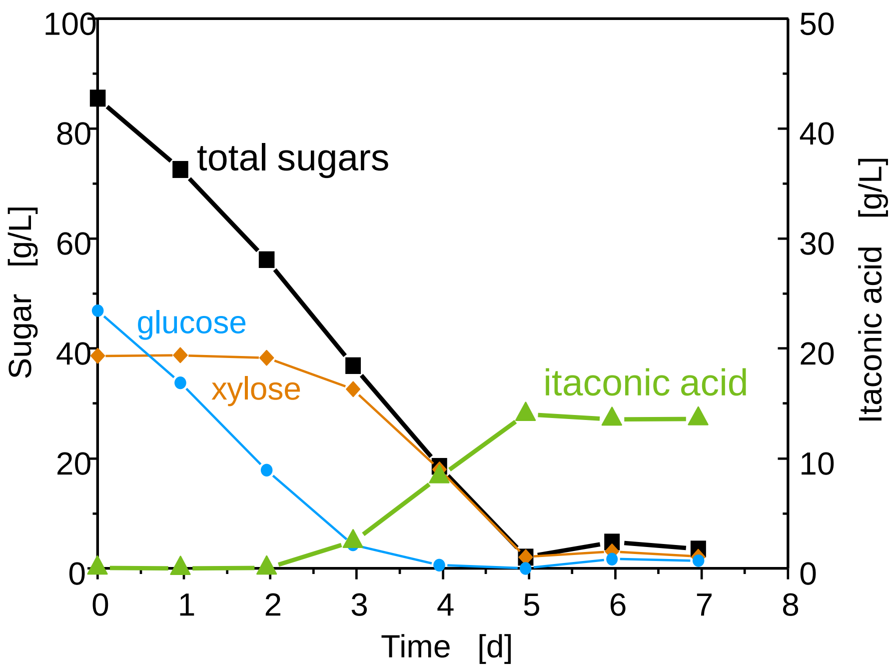
Screening criteria

Product yield and productivity
Performance in aerobic and anaerobic conditions (D-LA)
Tolerance towards product and substrate
Byproduct formation



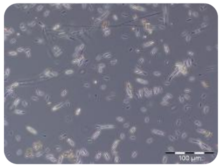
- # WHEAT CHAFF

***Ustilago maydis*, SHF**



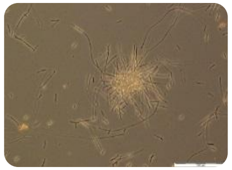
Pure glucose

- Itaconic acid: 28 g/L
- Productivity: 0.29 g/L/h
- Yield: 0.31 (w/w)



Wheat chaff hydrolysate

- Itaconic acid: 13.6 g/L
- Productivity: 0.09 g/L/h
- Yield: 0.16 (w/w)

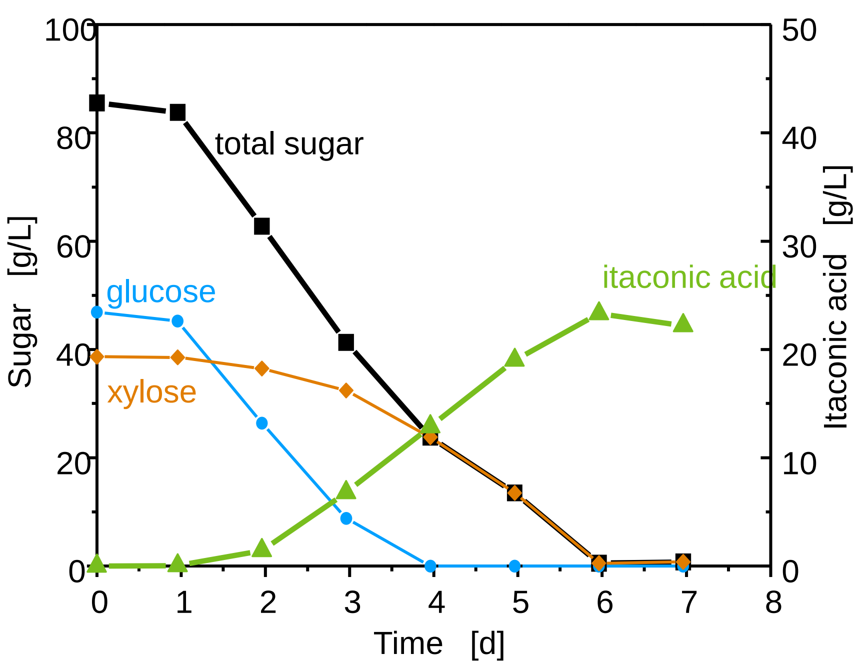


→ Change in morphology detected, presence of additional inhibitors.

→ Work in progress to further purify hydrolysate

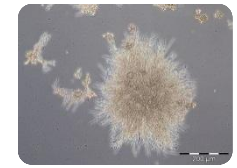
- WHEAT CHAFF

***Aspergillus terreus*, SHF**



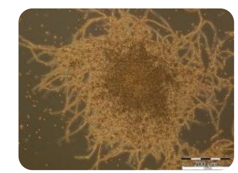
Pure glucose

- Itaconic acid: 54 g/L
- Productivity: 0.32 g/L/h
- Yield: 0.61 (w/w)



Wheat chaff hydrolysate

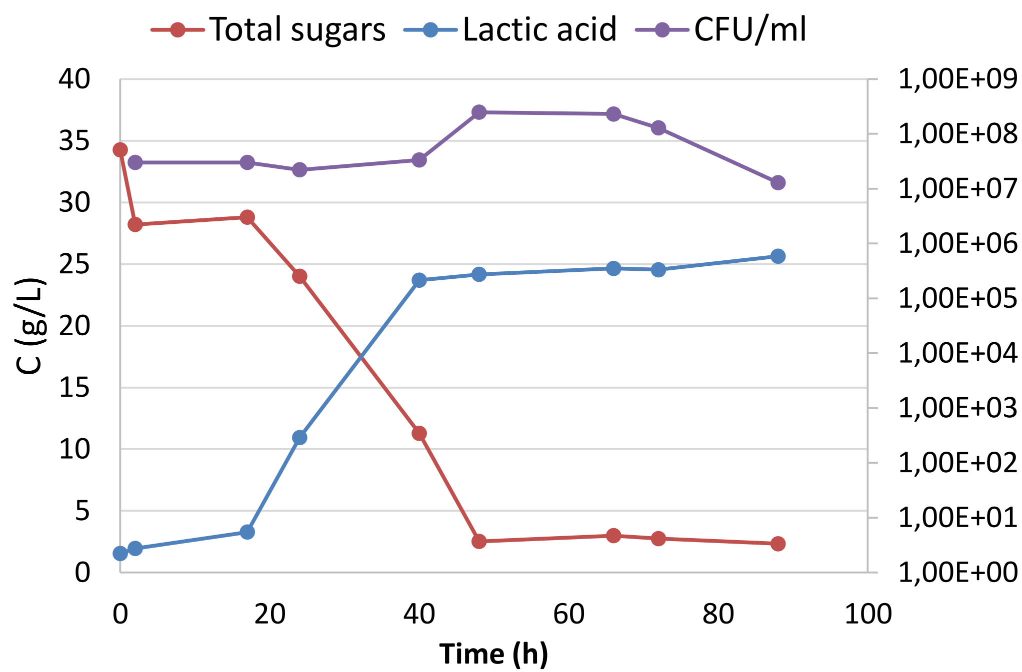
- Itaconic acid: 23.3 g/L
- Productivity: 0.16 g/L/h
- Yield: 0.27 (w/w)



→ 1.7 times higher IA titer than *U. maydis*
→ Low yield and sporulation pinpoint presence of inhibitors
→ Further purification ongoing to scale-up with *A. terreus*

• CITRUS PEEL WASTE

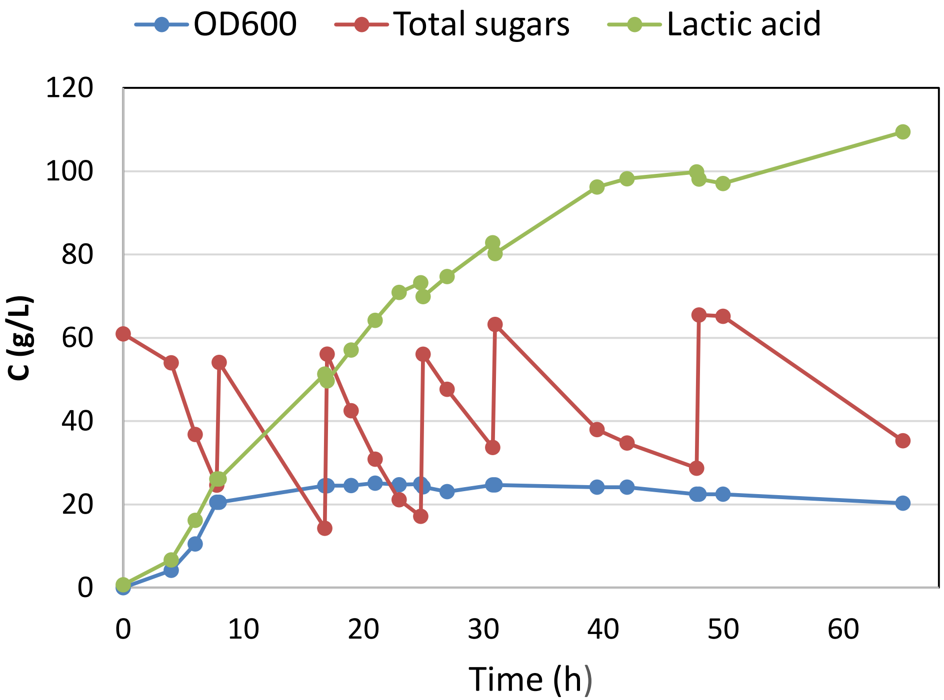
D-lactic acid > 99%



→ SHF at low total sugar and using hydrolysates in batch yields 70-80% D-lactic acid at 45 ºC

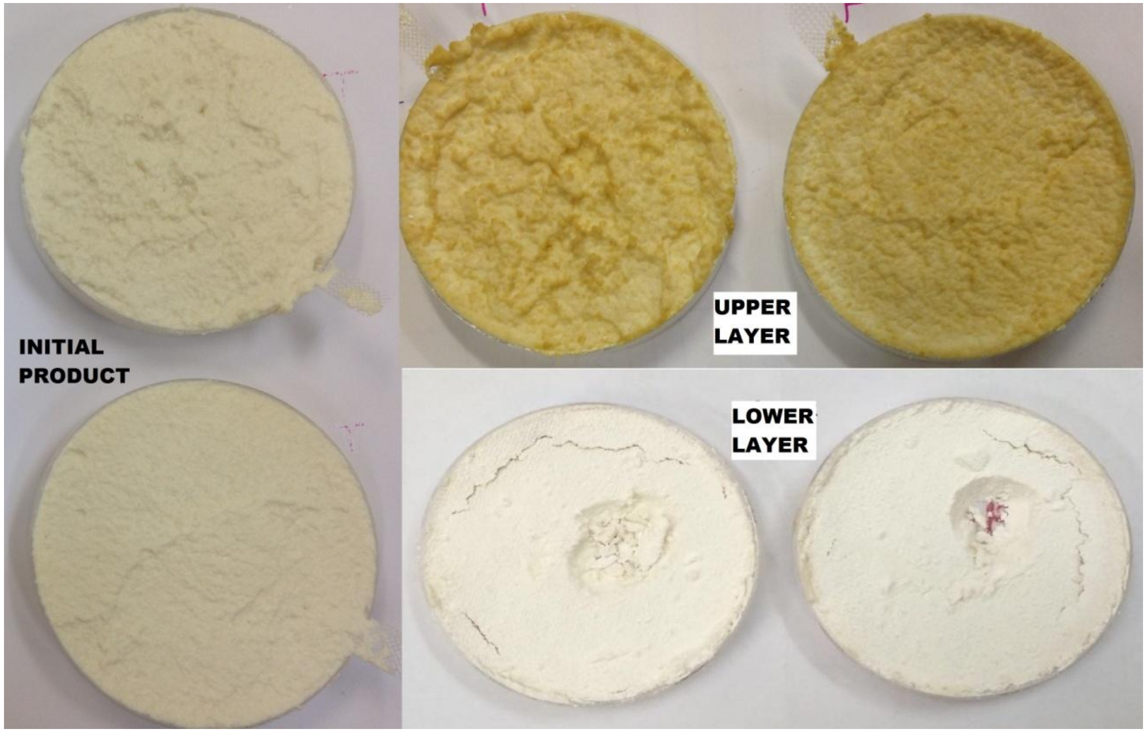
• CITRUS PEEL WASTE

D-lactic acid > 99%



→ Fed-batch operation enables reaching higher D-lactic acid titers.
→ Further optimization on going to select final scale-up set-up.

- Drying-induced elution of Ca-Lactate



→ Further improvement on going (impurities characterization)
→ *Patent pending*

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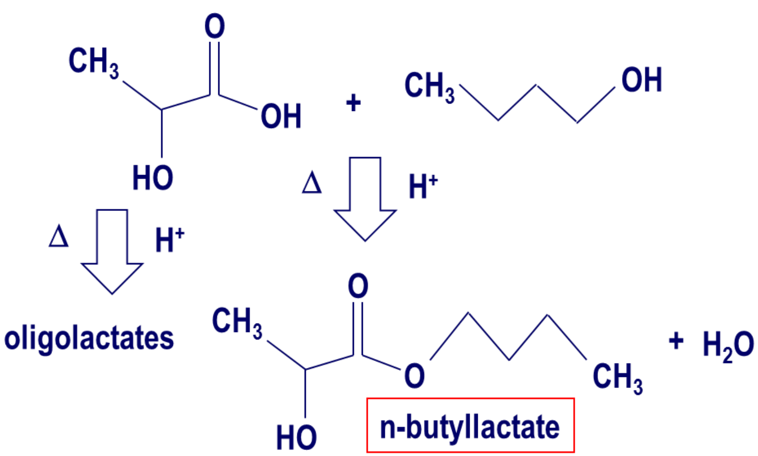
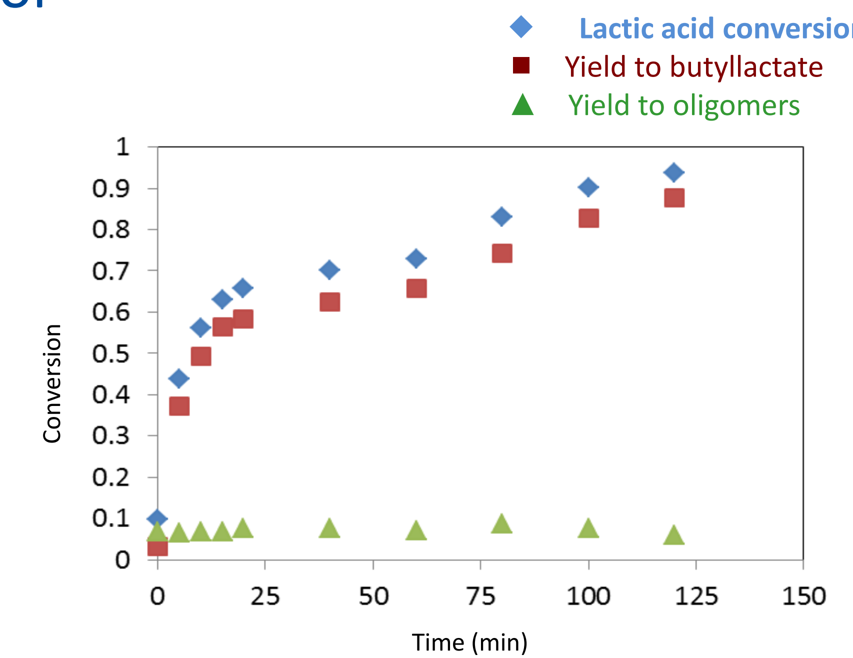
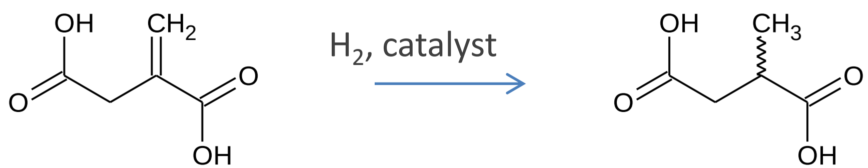
- Esterification with n-butanol



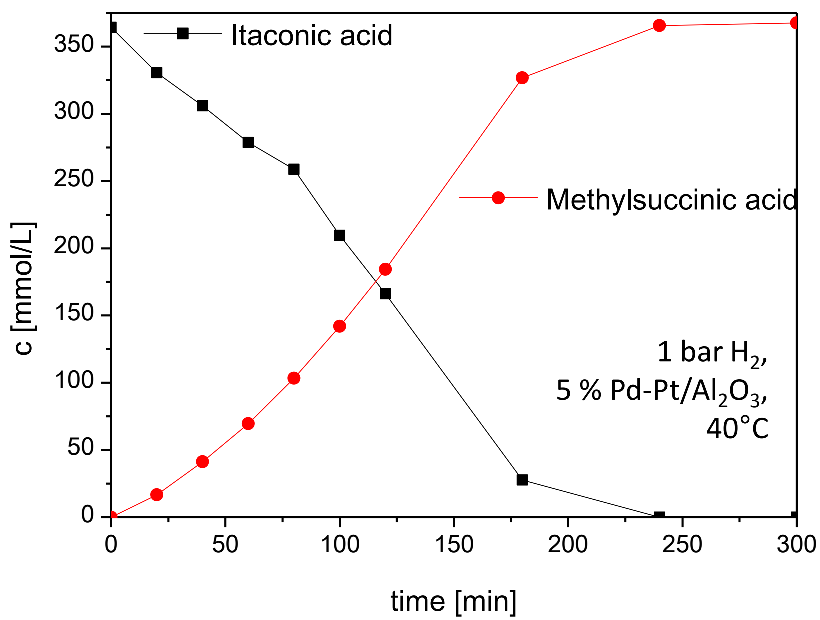


→ Reactive distillation and extraction is being developed for D-lactic acid purification
→ Conditions with homogeneous acid catalysts have been optimized

• UPGRADING



Itaconic acid (IA) Methylsuccinic acid (MSA)



Standard reaction conditions: 400 mL 5 % IA solution, H_2-pressure, 1 g 1-5 % metal/Al_2O_3, 40-80 °C

→ Most efficient catalyst: 5 % Pd-Pt/Al_2O_3 at 40 °C

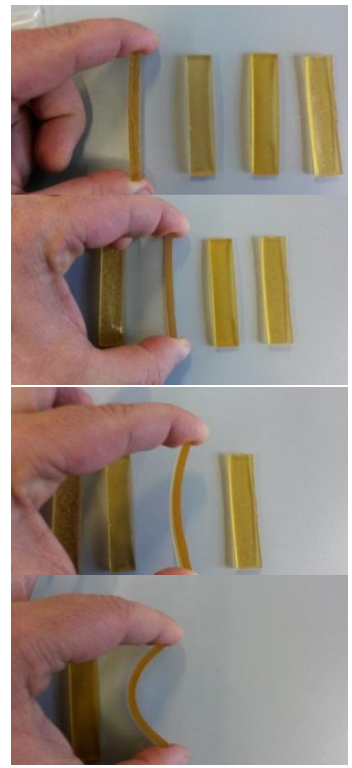
→ 100 % yield to methylsuccinic acid

• ITACONIC ACID BASED POLYESTERS

REACTION SCHEME
1. IA + MSA + PD –> (IA-PD)n-(MSA-PD)m
Opt. Crosslinking

IA[%]	MSA [%]	Tg [°C]
100	0	90-92
75	25	82-83
50	50	<25
25	75	<25

Protective layer with high elasticity for fiber-reinforced plastics possible, *e.g.* boat-parts, car-parts…



100 % Itaconic acid-PD
→inflexible

75 % Itaconic acid-
25 % Methylsuccinic acid-PD
→ inflexible

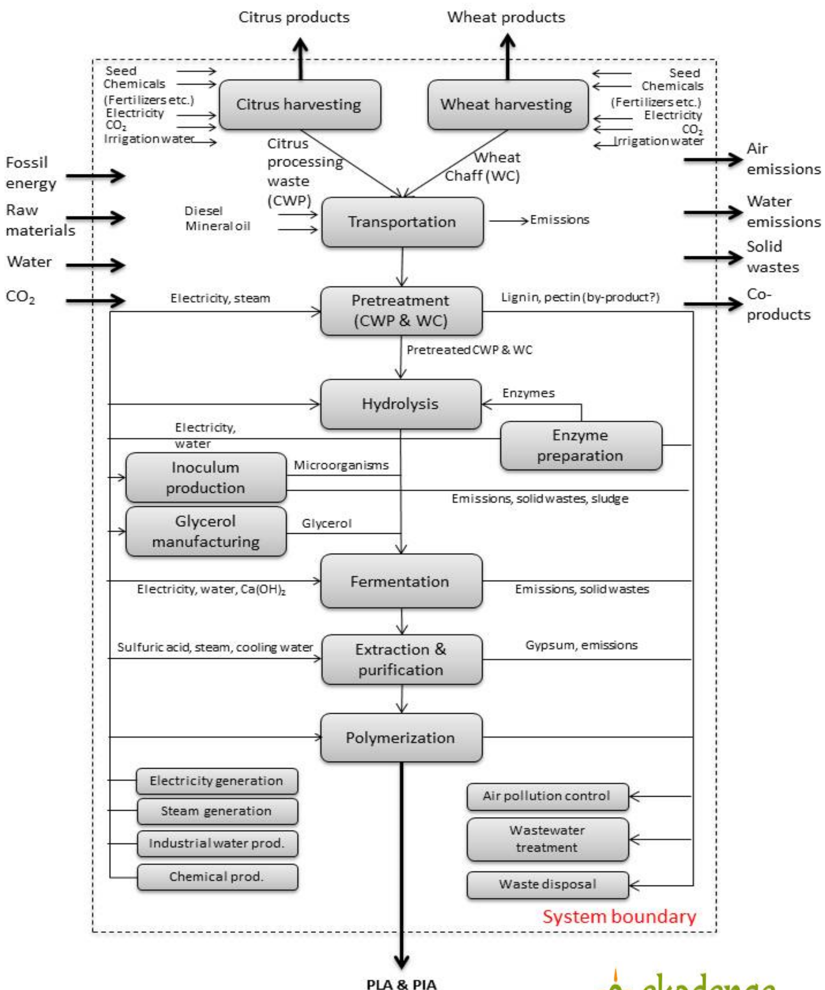
50 % Itaconic acid-
50 % Methylsuccinic acid-PD
→ flexible

25 % Itaconic acid-
75 % Methylsuccinic acid-PD
→ very flexible

PRETREATMENT HYDROLYSIS FERMENTATION PURIFICATION UPGRADING POLYMERIZATION

LIFE CYCLE ASSESSMENT – LIFE COSTING CYCLE

- Sustainability evaluated through LCA and LCC studies: environmental performance and comparison to selected benchmarks
- Functional units selected: PLA (LA) and PIA (IA)
- Cradle to Factory gate system boundaries
- Burden free raw materials as per agricultural residues





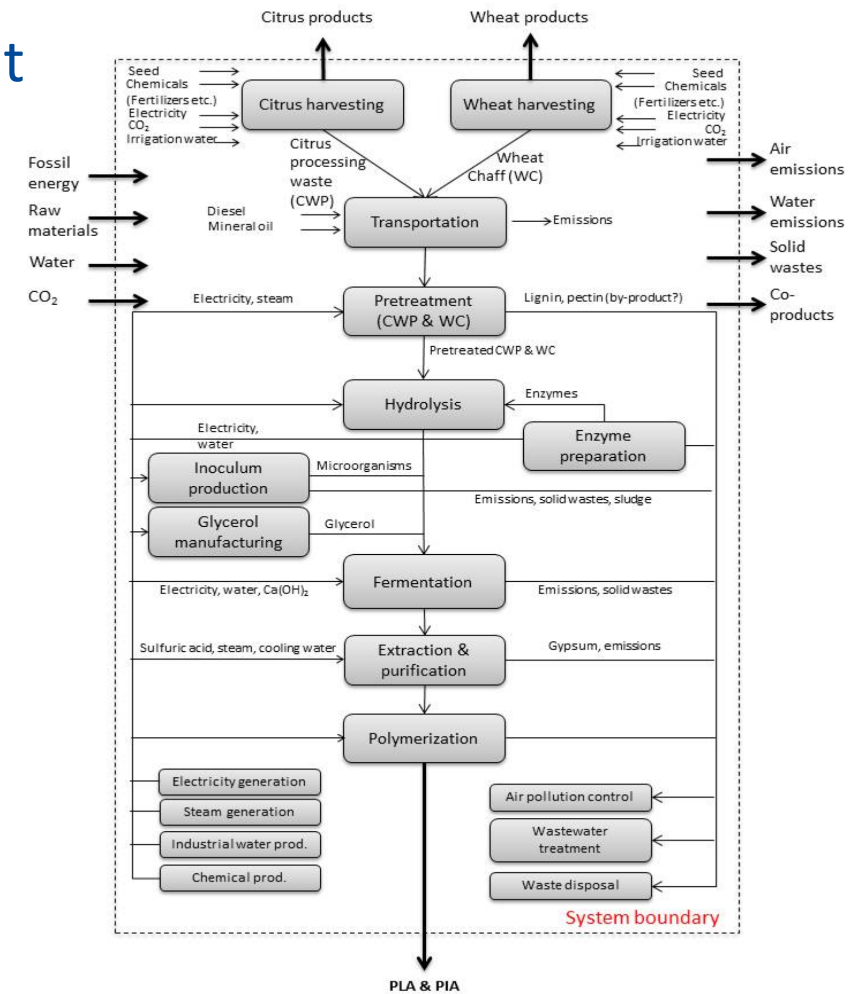
• Preliminary assessment

Benchmark (bio-PLA):
- GWP: 1.45 kgCO2/kg PLA
- Energy: 24.7-35,7 MJ/kg PLA
- Water: 9.9 kg/kg PLA

Benchmark (PIA-softwood):
- GWP: 1.32 kgCO2/kg PIA
- Energy: 14.9 MJ/kg PIA
- Water: 7.5 kg/kg PIA

Benchmark (PCL):
- GWP: 3.1-5.7 kgCO2/kg PCL

→ 57% energy > steam explosion
→ 3 kg/kg LA water consumption
→ Recycling of solvents



Summary

	Production of Organic Acids for Polyester Synthesis	Year 1				Year 2				Year 3				Year 4			
		3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12
WP1	Raw materials and fermentation substrates																
Task 1.1	Characterization of raw materials	■	■														
Task 1.2.	Optimization of raw glycerol pretreatment	■	■	■													
Task 1.3.	Optimization of lignin-poor biomass pretreatment	■	■	■													
Task 1.4.	Comparison of chemical and enzymatic hydrolisis for lignin poor biomass	■	■	■	■												
Task 1.5.	Optimization of the hydrolysis process for lignin-poor biomass	■	■	■	■	■	■	■	■								
WP2	Screening for enzymes and microorganisms																
Task 2.1	Development of new enzymes for the disintegration of biomass	■	■	■	■												
Task 2.2.	Screening for new hydrolases for the release of monosaccharides and sugar acids	■	■	■	■	■	■										
Task 2.3.	Improvement of selected enzymes by site-directed mutagenesis	■	■	■	■	■	■	■	■								
Task 2.4.	Development of special enzyme products by gene knock-out	■	■	■	■	■	■	■	■								
Task 2.5.	Characterization of new cellulases and hemicellulases		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Task 2.6.	Screening for MOs for D-lactic production using glycerol as substrate	■	■	■	■	■	■										
Task 2.7.	Screening for MOs for D-lactic production using hydrolisates as substrate	■	■	■	■	■	■										
Task 2.8.	Screening for MOs for itaconic production using glycerol as substrate	■	■	■	■	■	■										
Task 2.9.	Screening for MOs for itaconic production using hydrolisates as substrate	■	■	■	■	■	■										
WP3	Fermentation for D-lactic and itaconic acid production																
Task 3.1	Selection and improvement of biocatalyst for D-lactic and itaconic acid production				■	■	■	■	■								
Task 3.2.	Production of D-lactic with free and immobilized cells				■	■	■	■	■								
Task 3.3.	Production of itaconic acid with free and immobilized cells				■	■	■	■	■								
Task 3.4.	Simultaneous saccharification and fermentation for D-lactic acid production				■	■	■	■	■	■	■						
Task 3.5.	Simultaneous saccharification and fermentation for itaconic acid production				■	■	■	■	■	■	■						
WP4	Process development and scale-up																
Task 4.1	Selection of most suited feedstock/strain/fermentation combination (LA and IA)						■	■	■								
Task 4.2.	Kinetic analysis and process modelling (LA and IA)						■	■	■								
Task 4.3.	Optimization of fermentation processes (LA and IA)						■	■	■	■	■	■	■	■	■	■	■
Task 4.4.	Scale up fermentation under optimized conditions (LA and IA)									■	■	■	■	■	■	■	■
WP5	Product isolation, purification and upgrading																
Task 5.1	Development of product isolation and purification techniques (LA and IA)							■	■	■	■	■	■	■	■	■	■
Task 5.2.	Catalytic upgrading of monomers by derivatisation and production of polymers	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
WP6	Process economics and sustainability																
Task 6.1	Definition of the goal and system boundaries	■	■	■	■												
Task 6.2.	Data collection and inventory analysis	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Task 6.3.	Evaluation of environmental impact, energy and cost balance									■	■	■	■	■	■	■	■
WP7	Project management																

Summary



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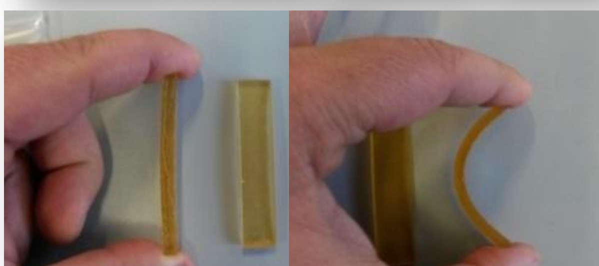
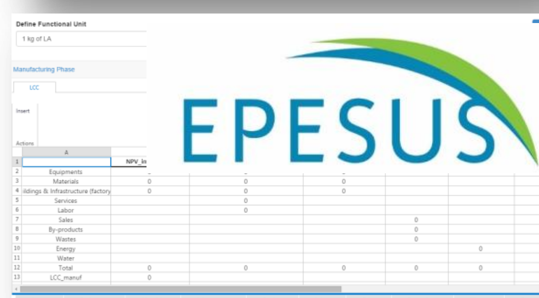
WP7	Project management																

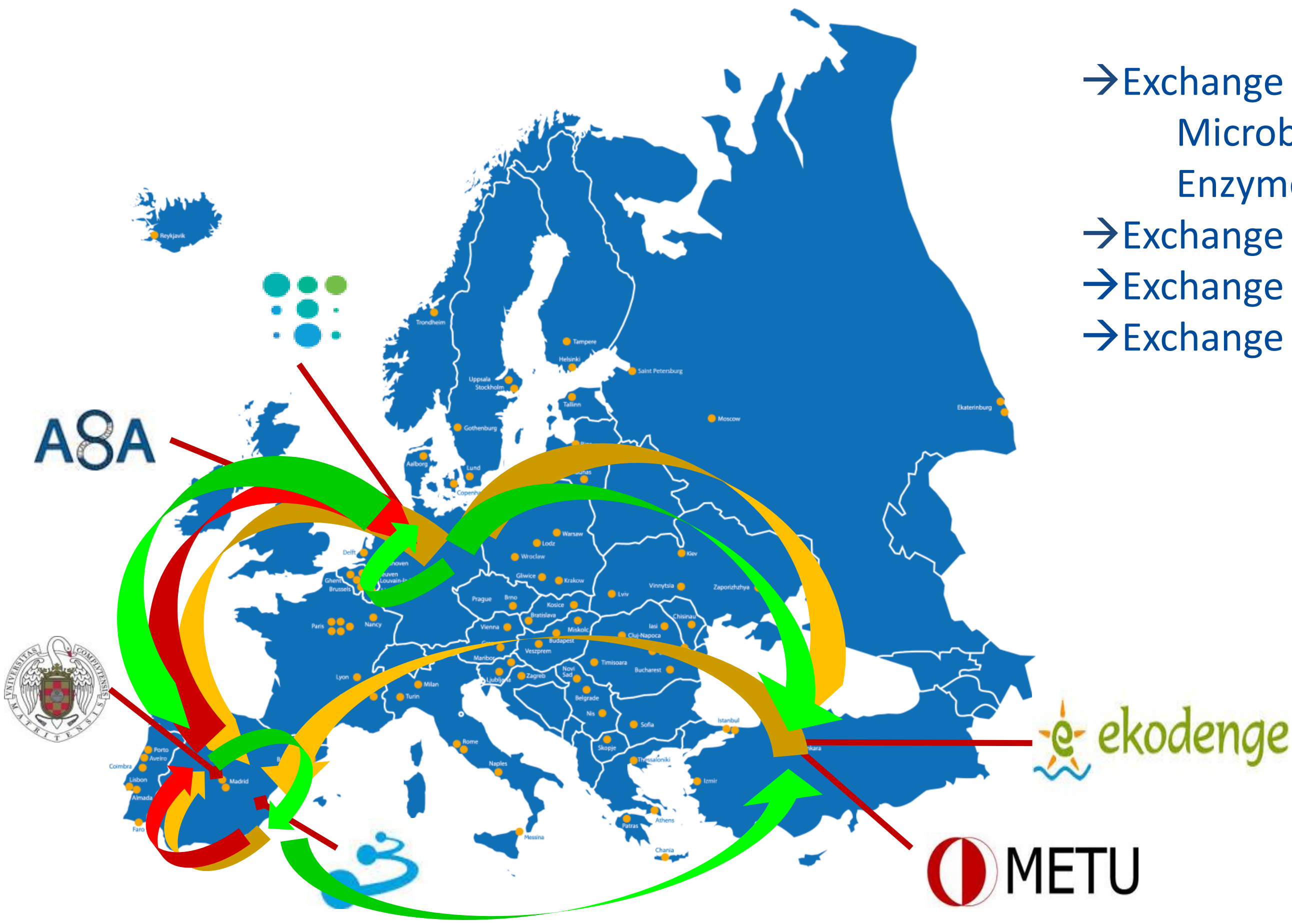
Project outcome





General Evaluation

- *Benefits of international collaboration*



→Exchange of catalysts
Microbial strains
Enzymes
→Exchange of raw materials
→Exchange of researchers
→Exchange of know-how

General Evaluation

- *Dissemination*

1. Krull, S. *et al*. (Thünen Institut) Biotechnological itaconic acid production from hydrolysates (Poster), 3rd European Congress of Applied Biotechnology, Nice, France, September 2015
2. Oetken J. *et al*. (Thünen Institut). Optimization of common chemical pretreatments to enhance the enzymatic hydrolysis of wheat chaff (conference abstract, submitted) 12th International Conference on Renewable Resources & Biorefineries, Genth, Belgium, May 2016.
3. Krull, S. *et al*. (Thünen Institut) Biotechnological itaconic acid production from wheat chaff hydrolysate. (conference abstract, submitted) 12th International Conference on Renewable Resources & Biorefineries, Genth, Belgium, May 2016.
4. De la Torre, I. *et al*. (UCM, Biopolis) "Effect of several thermal and mechanical pretreatments on solid composition and saccharification of orange peel wastes". Poster communication. Biorefinerias2016, Concepcion (Chile). November 2015.
5. De la Torre, I. *et al*. (UCM, Biopolis) "Orange peel waste enzymatic saccharification: batch and fed-batch operation optimisation". BioIberoamerica International Congress, submitted. Salamanca (Spain). June 2016.
6. De la Torre, I. et al. (UCM, Biopolis) "Effect of operational conditions on lactic acid production in model solutions resembling orange peel hydrolysates". BioIberoamerica International Congress. Salamanca (Spain), submitted. June 2016.
7. Pocan, P. et al. (H2Byotek) "Enzymatic hydrolysis of corn cob, orange and pomegranate peels: a comparative study" submitted.
8. Bustamante, D. *et al*. (Biopolis), "Strain screening and improvement for D-lactic acid production", ANQUE·ICCE·BIOTEC 2014, Madrid (Spain) July 2014.
9. Bustamante, D. *et al* (Biopolis, UCM) **"**Production of D-lactic acid from orange peel waste: strain selection and optimization**"** ". BioIberoamerica International Congress, submitted. Salamanca (Spain). June 2016.

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