

# FuturEnzyme WP7: Formulation and manufacturing of consumer products: sustainability and environmental assessments



36M Annual Meeting, Lipari

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- Outlook and discussion

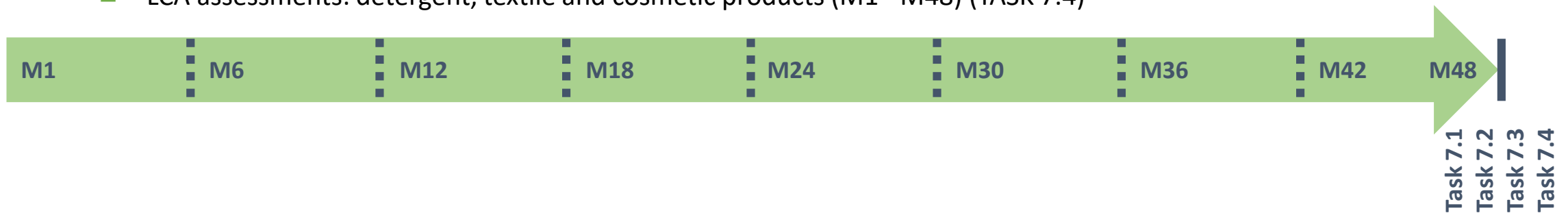


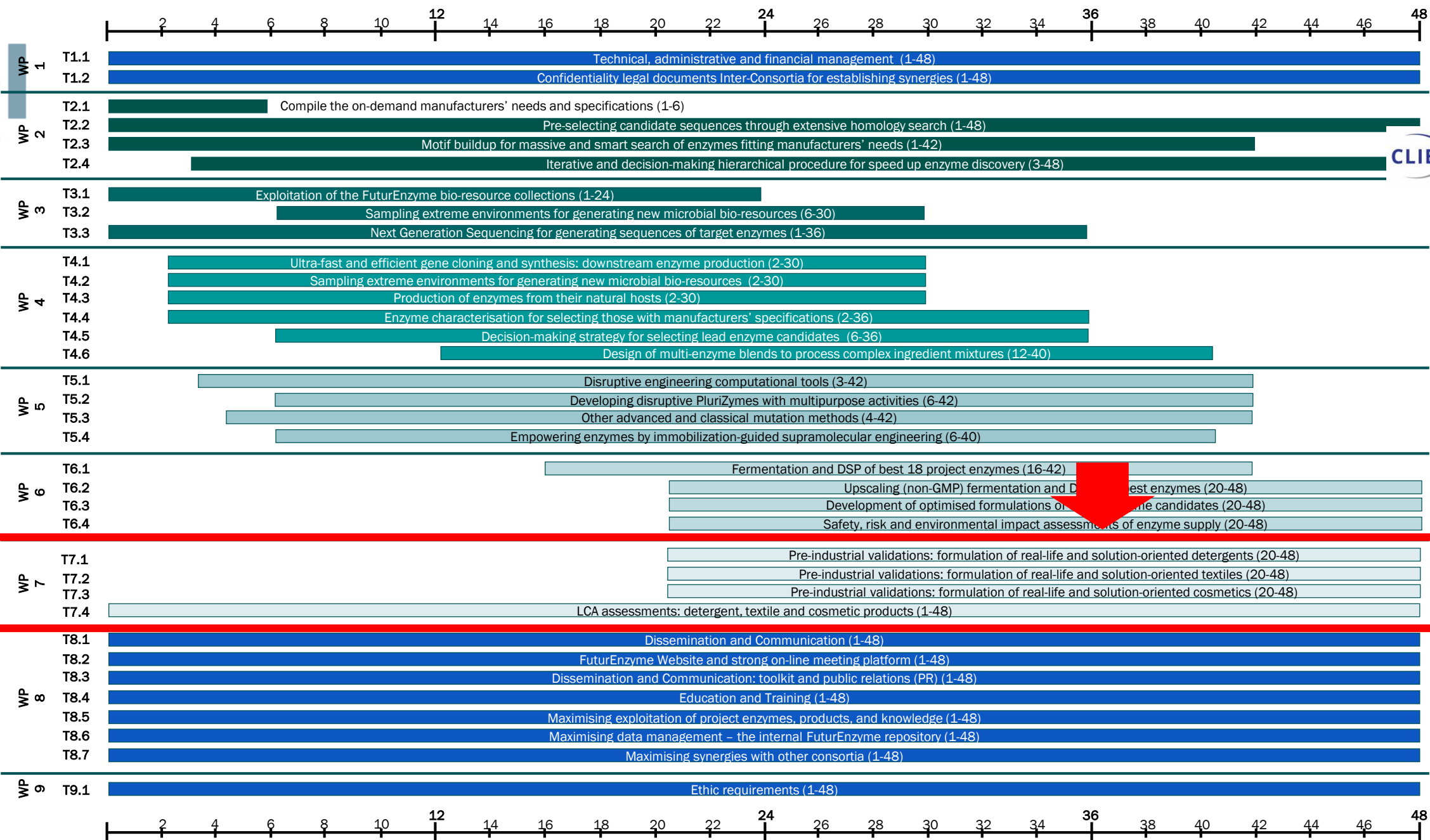
# WP7 - Formulation and manufacturing of consumer products: sustainability and environmental assessments



## OBJECTIVE

- **Validation** of enzyme performance and stability under industrially relevant conditions
- Upscaling of appropriately dimensioned **trials for the application** of enzymes to 3 project's sectors (detergents, textiles, cosmetics)
- **Life cycle assessment (LCA)** of newly developed enzyme-containing processes / products in comparison to conventional benchmark processes / products
- **TASKS**
  - Pre-industrial validations: formulation of real-life and solution-oriented detergents (M20 – M48) (TASK 7.1)
  - Pre-industrial validations: formulation of real-life and solution-oriented textiles (M20 – M48) (TASK 7.2)
  - Pre-industrial validations: formulation of real-life and solution-oriented cosmetics (M20 – M48) (TASK 7.3)
  - LCA assessments: detergent, textile and cosmetic products (M1 - M48) (TASK 7.4)







# WP7 – Deliverables and milestones



- First round of laundry tests completed (MS22)
- First round of textile tests completed (MS23)
- First tests for hydrolysis of hyaluronic acid (HAh) (MS24)

- First tests for producing HAh at gram scale (MS25)

- First trials for incorporating HAh into cosmetics (MS26)

- First report on product characteristics (MS27)

- First LCA report for the 3 FuturEnzyme products (MS28)

- Report on small/ medium validation trials of 18 best pre-selected enzymes (D7.1)

- A leading liquid and a unit dose cap detergent product with new enzymes integrated (D7.2)
- 3-4 Enzymatically functionalised leading textiles in more than DIN A4 size (D7.3)
- A leading cosmetic formulation with an enzyme-based HA-hydrolysis product integrated (D7.4)

- LCA report of the 3 real-life products (D7.5)



- Achieved in RP2
- For next RPs
- Deliverable
- Milestone





# WP7 Management M24 – M36

- 09/23 Industry meetings, round 4
- 11/23 Textiles results and standardisation meetings
- 01/24 Industry meetings, round 5
- 01/24 Roll-out Enzyme specification sheets
- 02/24 LCA info round
- 04/24 Industry meetings, round 6
- 04/24 Optimisation task force initiation (WP5)
- 08/24 Reporting RP2
- 08/24 Industry meeting, round 7

Enzyme specification sheet					
Original version:	23.10.2023	CSIC	Paula Vidal	p.vidal.ramon@csic.es	
Last update:	28.10.2023	CSIC	Paula Vidal	p.vidal.ramon@csic.es	
Enzyme:	#02	FE_Lip9			
Enzyme class:	List of <b>enzymes</b> or preparations (quantities for industrial tests have already been shared with partners):			Target application sector(s):	
Esterase / Lipase / <b>PKase</b>	1. B01   CSIC   Ec_InCel_Mix_pur (Lot: 11.09.2023)			<input checked="" type="checkbox"/> Detergents	
	2. B02   BIOSYNTH   Pa_ExtCel (Lot: 03908223SS0516)			<input checked="" type="checkbox"/> Textiles (specify)	
	3. B03   CSIC   Ec_InCel_ext (Lot: 08.11.2022)			<input type="checkbox"/> Oil removal	
				<input type="checkbox"/> Cosmetics	
				<input type="checkbox"/> Other:	
Enzyme origin					
Identifying partner:	CSIC	Type of expression:	Intracellular	Tested enzyme fraction:	Soluble enzyme
Original host:	E. coli BL21	Purification and formulation:	formulation	Measured activity (unit):	text
Further characteristics / comments: text					
Production batch: B01					
Producing partner:	CSIC	Type of expression:	Intracellular	Mode of application:	Soluble enzyme
Production host:	E. coli BL21	Purification and formulation:	Lyophilised Extract	Measured activity:	2.5 U/mg
Further characteristics / comments: Purified enzyme (His6-tag); activity measured for tributyrin (30°C, pH 8.0)					
Production batch: B02					
Producing partner:	Biosynth	Type of expression:	Secreted	Mode of application:	Soluble enzyme
Production host:	P. pastoris	Purification and formulation:	Lyophilised Supernatant	Measured activity:	0.21 U/mg
Further characteristics / comments: Activity measured for tributyrin (30°C, pH 8.0)					
Production batch: B03					
Producing partner:	CSIC	Type of expression:	Intracellular	Mode of application:	Soluble enzyme
Production host:	E. coli BL21	Purification and formulation:	Lyophilised Extract	Measured activity:	0.43 U/mg
Further characteristics / comments: Unpurified intracellular protein extract; activity measured for tributyrin (30°C, pH 8.0)					





# Lead Enzyme Candidates

- Total Lead candidates (incl. mutants): 48 (D: 19 / T: 17 / C: 25)
  - Not investigated further: 11 (4/2/12)
  - Planned for production: 13 (1/0/4)
  - In production: 7 (0/1/7)
- Produced: 1 (0/1/0)
- Shipped to partners: 11 (9/7/2)
- Tested by industry partners: 2 (2/2/0)
- Planned for scale-up: 4 (4/4/0)

## Enzyme specification sheets

- Available on OneDrive



Coordinative information for production and application tests													Production and application results												
ID	Enzyme	Activity	Partner	Host (Origin)	Partner (Production)	Host (Production)	Pre-validated	IP	Scale	Scale	Scale	Scale	Status	Last results (activity confirmed/not confirmed, indicate beneficiary)	Quantity of produced enzyme	Next steps (indicate beneficiary)	Planned optimization	Link to ESS	Comment						
1	Kut3	Esterase / Lipase	BANGOR	E. coli BL21(DE3)	Biosynth	P. pastoris	Yes	Yes	Yes	Yes	Yes	Yes	01 - Planned for production	Biosynth closed, no expression detected		Biosynth: achieve expression in Pichia									
2	FE_Lip3	Esterase / Lipase / PETase	CSC	E. coli BL21(DE3)	Biosynth	P. pastoris and E. coli	Yes	Yes	Yes	Yes	Yes	Yes	01 - Planned for production	CSC: good activity and stability Haskel: activity good but stability poor Schneider: no activity detected	CSC: 100 g BIO5/NTR: 55.9 g (Pichia)	Haskel: not planned for full-scale wash trials, mutant B02.2 in preparation T: IIT-ID testing higher concentration	Further variants?	<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>	Haskel Top4: Selected for full scale wash trials CV17, 2024						
2,2	FE_Lip3_mut	Esterase / Lipase / PETase	CSC	E. coli BL21(DE3)	Biosynth	P. pastoris	Yes	Yes	Yes	Yes	Yes	Yes	04 - Shipped to partners	Pichia clones generated, will be shipped once ready	Biosynth: 62.50 g	g-scale production, ship to partners				Mutant of B02_FE_Lip3 (single point mutation)					
3	FE_ID3	Esterase / Lipase	CSC	E. coli BL21(DE3)	Biosynth	P. pastoris	Yes	Yes	Yes	Yes	Yes	Yes	04 - Shipped to partners	CSC: Produced at g-scale BIO5/NTR: No successful expression	CSC: 2.0 g	CSC: Ship to Haskel									
4	FE_poker	Esterase / Lipase / PETase	CSC	E. coli BL21(DE3)	Biosynth	P. pastoris	Yes	Yes	Yes	Yes	Yes	Yes	06 - Tested by industry partners	UDUS: closed and expressed in Pichia, activity confirmed Haskel: poor performance in small-scale wash trials	20.36 g (Pichia)	D: not investigated further		<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>							
5	EntLip_Dim_800	Esterase / Lipase	UDUS	E. coli	Biosynth	P. pastoris	Yes	Yes	Yes	Yes	Yes	Yes	07 - Planned for Scale-up	UDUS: activity confirmed on Schoeller fabrics Biosynth: low enzyme content Schneider: ?? Haskel: stable in wash, low performance in small-scale wash trials	41.2 g (Pichia)	Haskel: planned for full-scale wash trials	T: activity? D: TBD	<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>	Haskel Top3: Selected for full scale wash trials CV17, 2024						
6	EntLip_Pave_TB035	Esterase / Lipase	UDUS	E. coli	Biosynth	P. pastoris	No	Yes	Yes	Yes	Yes	Yes	06 - Tested by industry partners	UDUS: minor activity towards the oils on the fabric Biosynth: low enzyme content Haskel: activity not confirmed, but high	176 g (Pichia)	Haskel: repeat small-scale tests? T: IIT-ID testing higher concentration	D: ?	<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>							
7	EntLip_PeLit	Esterase / Lipase	UDUS	E. coli	Biosynth	P. pastoris	No	Yes	Yes	Yes	Yes	Yes	01 - Planned for Scale-up	UDUS: minor activity towards the oils on the fabric Biosynth: low enzyme content Haskel: activity not confirmed, but high	59.80 g (Pichia)	Haskel: planned for full-scale wash trials	D: TBD	<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>	Haskel Top1: Selected for full scale wash trials CV17, 2024						
8	EntLip_TB030	Esterase / Lipase	UDUS	E. coli	Biosynth	P. pastoris	No	Yes	Yes	Yes	Yes	Yes	04 - Shipped to partners	UDUS: activity not verified (same as HEPE3 and Epi buffer) Biosynth: closed and expressed, activity confirmed	93.0 g (Pichia)	D: Haskel small-scale wash trials T: Wait for activity confirmation by IIT-ID	D: results available?	<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>							
9	Pico_FE_HLY2505	PETase	UDUS	E. coli	Biosynth	P. pastoris	Yes	Yes	Yes	Yes	Yes	Yes	01 - Planned for Scale-up	UDUS: EcoI-derived protein locus activity at high concentrations; activity on Schoeller fabrics confirmed. No activity in Haskel washing liquor; Haskel: stable in wash, reasonable performance Schneider: no activity detected	84.35 g (Pichia)	Haskel: planned for full-scale wash trials; FHMV: engineering for stability in washing liquor T: IIT-ID testing higher	D: TBD	<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>	Additional application in textile's end-of-life Haskel Top2: Selected for full scale wash trials CV17, 2024						
10	PEL_Pave_FE-H	PETase	UDUS		Biosynth		No	Yes	Yes	Yes	Yes	Yes	04 - Shipped to partners	Biosynth: closed and expressed in Pichia, activity confirmed	125 g (Pichia)	D: Haskel small-scale wash trials T: Wait for activity confirmation by IIT-ID									
11	Pico_FE-H	PETase	UDUS	E. coli	Biosynth	P. pastoris	No	Yes	Yes	Yes	Yes	Yes	04 - Shipped to partners	Biosynth: No SDS signal but high activity in detergent and textile application	152 g (Pichia)	D: Haskel small-scale wash trials T: Wait for activity confirmation by IIT-ID		<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>							
12	PEL_Pave_FE-H	PETase	UDUS		Biosynth	P. pastoris	No	Yes	Yes	Yes	Yes	Yes	04 - Shipped to partners	Biosynth: closed and expressed, activity confirmed	76.8 g (Pichia)	D: Haskel small-scale wash trials T: Wait for activity confirmation by IIT-ID		<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>							
13	GEN005	Esterase / Lipase	BANGOR	Mutagenesis	Biosynth	P. pastoris	Yes	Yes	Yes	Yes	Yes	Yes	04 - Shipped to partners	BANGOR: activity verified Biosynth: closed and expressed, activity verified	92.1 g (Pichia)	D: Haskel small-scale wash trials T: Wait for activity confirmation by IIT-ID		<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>							
14	GEN005	Cellulase	BANGOR		Biosynth	P. pastoris	Yes	Yes	Yes	Yes	Yes	Yes	04 - Shipped to partners	Biosynth: closed and expressed in Pichia, activity confirmed; B Bangor: sources. Biosynth can co-cult	53.4 g (Pichia)	D: Haskel small-scale wash trials T: Wait for activity confirmation by IIT-ID		<a href="https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472">https://clib-cluster.sharepoint.com/sites/clib-ess/SharedDocuments/FE_Lip3/FE_Lip3_01472</a>	Of interest for D if activity confirmed						





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- Advances and next steps WP 7.4
- Outlook and discussion





# Task 7.1: Detergents

- Task 1: Enzyme analysis and verification
- Task 2: Enzyme production at g-scale
- Task 3: Industrial application tests
- Total enzyme candidates: 19
  - 4 not investigated further / 9 shipped to partners / 2 tested by industry, no scale-up / **4 tested by industry, scale-up**



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# Task 7.1: Detergents



- All lipase candidates have been analysed with respect to:
  - Volumetric activity (Standard substrate, photometric)
  - In-wash stability in detergent liquor
  - Small scale wash performance: Miniaturized application test
- Best performing candidates have been selected, full-scale application tests in consumer relevant liquid detergents ongoing (05/2024):  
European wash conditions, 40 °C wash temperature
  - #07 EstLipPtEst1
  - #09 PHE-Paes-PE-H-Y250S
  - #05 EstLip-Dim\_#008
  - #02 Lip9





# Task 7.1: Detergents



## Activity of samples with artificial substrate

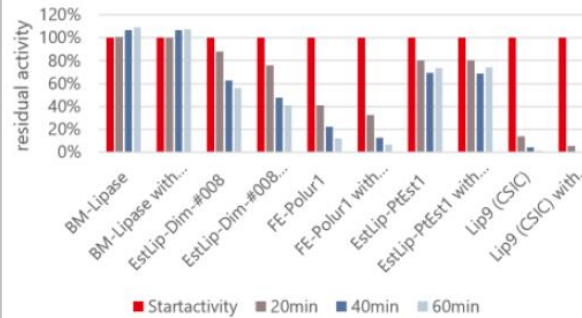
- The lyophilised samples were dissolved in 20% PG in the same protein-concentration as a technical benchmark (IP3461)

	LipaseU/ml
Benchmark Lipase	71500
EstLip-Dim#008	30
FE-Polur1	90
EstLip-PtEst1	0,2
Lip9 (CSIC-extrakt, <u>estimated</u> AEP 30%)	6400

## Stability in detergent liquor

- Residual activity in washing liquor (IP3461)

Stability in detergent wash liquor @40°C  
+/- Protease



t [min]	0	20	40	60
Benchmark-Lipase (15µl/L)	100%	101%	107%	109%
Benchmark-Lipase with protease	100%	100%	106%	107%
EstLip-Dim-#008 (0,1ml/5ml)	100%	88%	63%	56%
EstLip-Dim-#008 with protease	100%	76%	48%	41%
FE-Polur1 (0,1ml/5ml)	100%	41%	22%	12%
FE-Polur1 (0,1ml/5ml) with protease	100%	33%	13%	7%
EstLip-PtEst1 (0,2ml/0,4ml)	100%	80%	69%	74%
EstLip-PtEst1 (0,2ml/0,4ml) with protease	100%	80%	69%	74%
Lip9 (CSIC) (0,1ml/5ml)	100%	14%	4%	1%
Lip9 (CSIC) (0,1ml/5ml) with protease	100%	6%	0%	0%

## Washing performance in MWT („Mini Wash Trials“)

- Conditions of MWT (IP3462) (MWT challenging for Lipase, high deviations)
  - European detergent w/o enzymes, 6fold stain (CS46B), 40°C, 1h, 600rpm
  - Concentration row of lipase samples, pH checked before and after the measurement

BM-Lipase	Wash performance: Yes
EstLip-Dim#008	Poor wash performance
FE-Polur1	Poor wash performance
EstLip-PtEst1	Wash performance: Yes
Lip9 (CSIC-extrakt)	Poor wash performance
PHE-Paes-PE-H-Y250S	Wash performance: Yes





# Task 7.2: Textiles

- Task 1: Enzyme analysis and verification



- Task 2: Enzyme production at g-scale



- Task 3: Industrial application tests



- Total enzyme candidates: 17

- 2 not investigated further / 2 in production/produced / 9 shipped to partners / 4 tested by industry, no activity confirmed





## WP7

### Enzymes tested

#### Biosynth

EstLip\_Paes\_TB035  
PEH\_Paes\_PE-H\_Y250S  
FE\_Polur1  
EstLip\_Dim\_#008  
EstLip\_PtEst1  
PEH\_Pbau\_PE-H  
EstLip\_TBec304  
PEH\_Pform\_PE-H  
FE\_EH37  
PEH\_Poce\_PE-H  
GEN0105  
Lip9 Val161Ser  
Hyal\_HRDSV\_2334

#### Bangor

Pvec11  
GEN0105

#### BioC-CheM

*Vibrio* sp. IAMC-CNR#23

#### INOFEA

immobilized TB035  
immobilized TBec304

### Bacterial strains selected (detergents and textiles)

*Serratia quinivorans*  
*Psychrobacter celer*  
*Pseudomonas protegens*

#### Cosmetics

several strains with Hyal  
activity



### Assays

#### Detergents

- effect of  $\text{CaCl}_2$
- tributyrin, trioctanoin, soiled textiles
- wash liquor, genapol X-100
- analytical methods (GC-FID, GC-MS; UV-VIS)

#### Textiles

- analytical methods (GC-FID, GC-MS)
- removal of spinning oils in 61488, 3X58, 5237 from Schoeller
- degradation of dyeing liquids

#### Cosmetics

- analytical methods (diffusion on paper, BSA 232 nm, DNS)
- enzyme concentration
- temperature
- effect of  $\text{CaCl}_2$
- substrate concentration (HA, HA50 from Evonik)





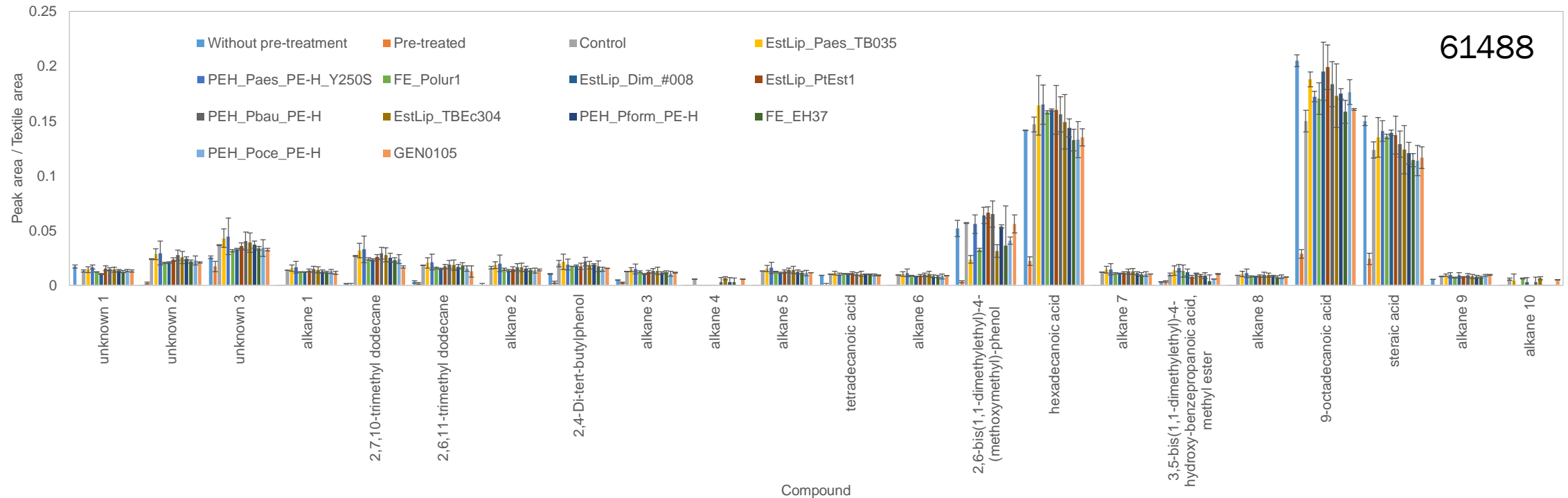
# Task 7.2: Textiles – Enzyme activity



## Example: Enzyme treatment of 61488 fabric made at Schoeller – spinning oils (no CaCl<sub>2</sub> added)

Assay: 200% of the weight of fabric was added as solution of enzyme at 12.5 g/L in TRIS-HCl 100 mM pH 8. The biodegradation was carried out at 30°C for 24h and at the end the fabrics were washed once with 1 g/L genapol X-100 and once with Milli-Q water.

Analysis: GC-FID and GC-MS



Composition of the spinning oils without treatment, after chemical treatment (at Schoeller), and after enzymatic treatment. The area of the peaks was corrected using an internal standard and normalized using the weight of fabric used.



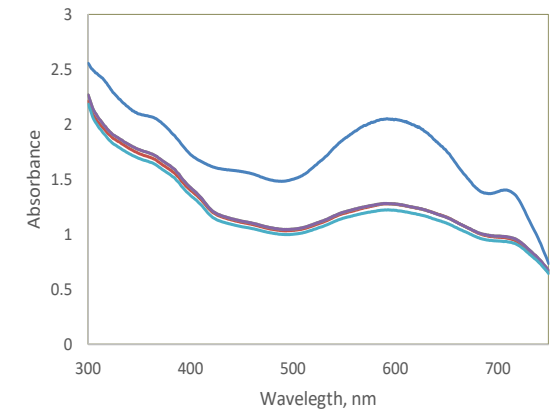
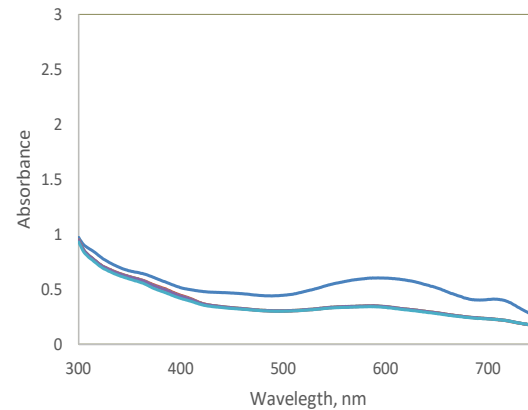
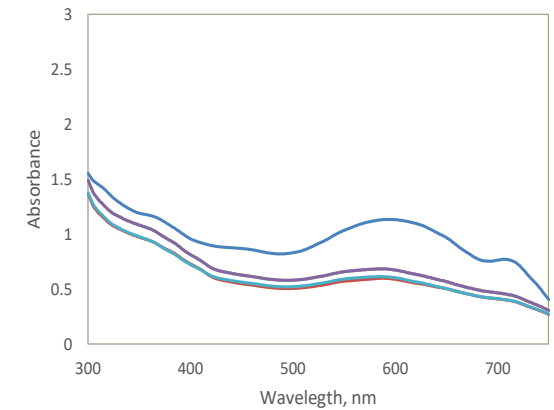
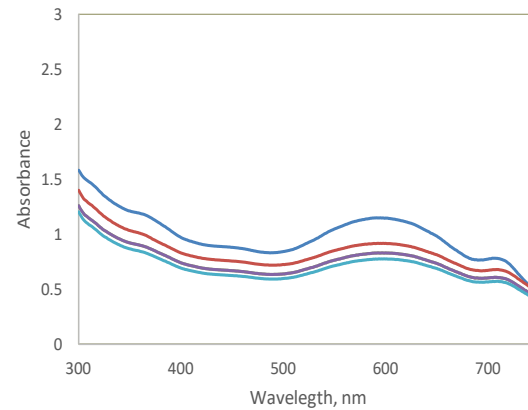
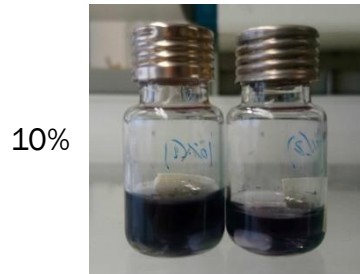
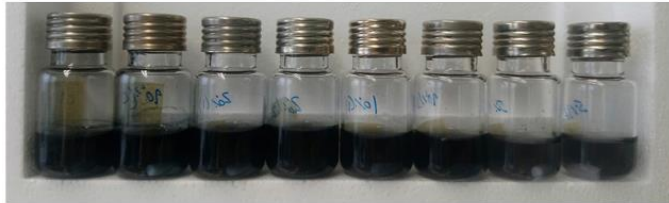
# Task 7.2: Textiles – dye removal



## Enzyme treatment of dyeing liquid after the dyeing process – enzyme Pvec11

Assay: Reactions carried out in 10 mL glass flasks with 20 mM acetate buffer pH 4.5 containing 1% CaCl<sub>2</sub>, with 5, 10 or 20% dyeing liquid after the dyeing process and 0.1 mg/mL of enzyme. The control shown contained 10% of dyeing liquid and no enzyme. Reaction time = 25h.

Analysis: UV-VIS



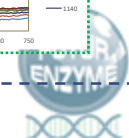
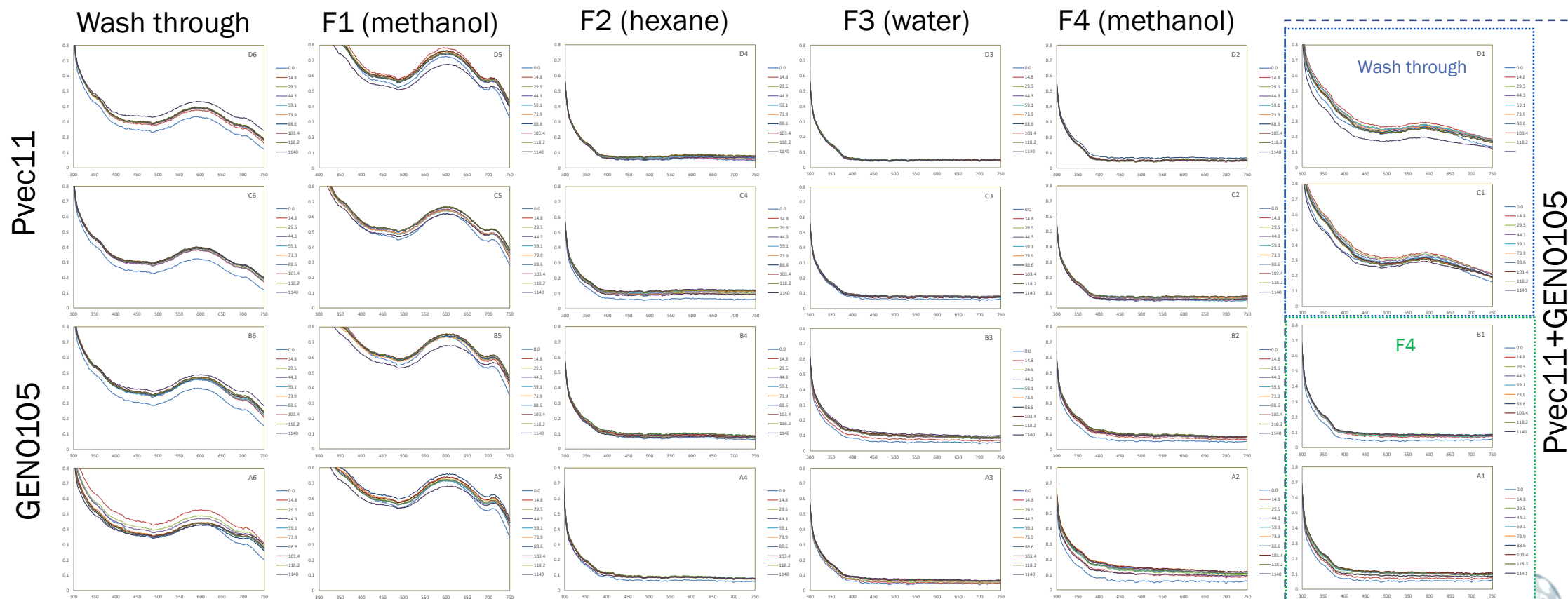


# Task 7.2: Textiles – dye removal (2 enzymes)



## Enzyme treatment of dyeing liquid after the dyeing process – enzymes Pvec11 and GEN0105

Assay: Reactions carried out with fractions collected after separation of dyeing liquid by SPE in silica gel.  
Reaction time = 20 h. Analysis: UV-VIS







# Task 7.2: Textiles – dye removal (2 enzymes)



## Enzyme treatment of dyeing liquid after the dyeing process – enzymes Pvec11 and GEN0105

Assay: Reactions carried out with fractions collected after separation of dyeing liquid by SPE in silica gel.  
Reaction time = 20 h. Analysis: UV-VIS

Peaks shown: 360, 600, 710 nm; 2h of reaction

Wash through

Wash through

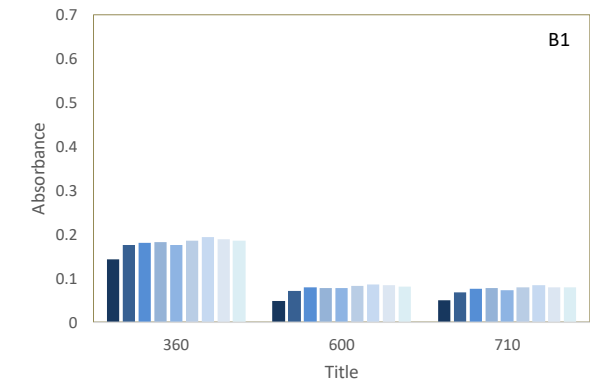
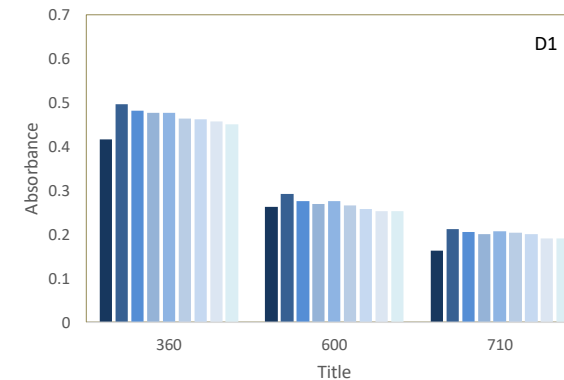
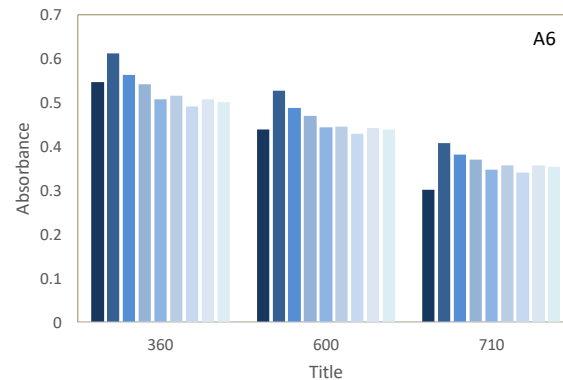
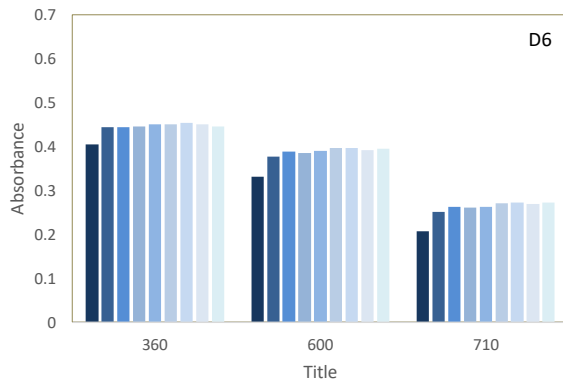
F3 (water)

Pvec11

GEN0105

Pvec11+GEN0105

Pvec11+GEN0105





# WP 7.2: Textiles



- Starting the experiments on textile samples with the received enzymes
  - Aim of the experiments was to remove the external substrates (silicons, spinning oils, etc.) from the raw samples
- In total four enzymes were examined (PEH-Paes and TB035) and the Lip9 is stopped due to its questionable activity
  - #06 EstLip\_Paes\_TB035
  - #09 Paes\_PE-H\_Y250S
  - #02 Lip9 Pure CSIC
  - #04 FE\_Polur1
- Quantitative and qualitative determination of the external substrates with delivered enzymes by Schoeller laboratories
  - Petroleum ether soluble substances - quantitative determination of the quantity
  - Qualitative determination





# WP 7.2: Textiles

## Experiments in the lab

Textile samples



Enzyme solution



Enzyme is really well soluble in water



Lab Foulard machine



Samples rested for 24 hours and washed out with no detergents





# WP 7.2: Textiles

## Results

Article Nr.	Composition	Raw		Original Schoeller Treatment		Enzymes without buffer				Enzymes with HEPES Buffer				No Enzyme			
						1-Est.Lip Paes TB 035		2-PEH-Paes PEH Y250S		3-PEH-Paes PEH Y250S		4-Lip9 Pure CSIC				5-Polur 1	
		A.Quantitative [%]*	B. Qualitativ [IR-Spectrum]**	A.Quantitative [%]*	B. Qualitativ [IR-Spectrum]**	A.Quantitative [%]*	B. Qualitativ [IR-Spectrum]**	A.Quantitative [%]*	B. Qualitativ [IR-Spectrum]**	A.Quantitative [%]*	B. Qualitativ [IR-Spectrum]**	A.Quantitative [%]*	B. Qualitativ [IR-Spectrum]**	A.Quantitative [%]*	B. Qualitativ [IR-Spectrum]**	A.Quantitative [%]*	B. Qualitativ [IR-Spectrum]**
3X58	100% PES	1.6	Fatty acid ester or emulsified Mineral oil / paraffin	0	Quantity too small for analysis	1.5	Paraffin, fatty acid ester, amide, little silicone	1.4	Paraffin, fatty acid ester, amide, little silicone	0.2	Fatty acid esters, possibly paraffin, silicone	0	No residue, no measurement possible	0.2	Paraffin, fatty acid esters, fatty acid amide, PES, silicone	0.3	Paraffin, PES, fatty acid amide, silicone
E03130	80%PA6 , 20%EL	1.9	Fatty acid ethoxylate, amide, silicone	0.1	Quantity too small for analysis	1.3	fatty acid esters, silicone	1.4	fatty acid esters (ethoxylate), little silicone	1.4	Possibly polyurethane	1.2	Possibly polyurethane	0.9	Fatty acid (ethoxylate), silicone, possibly a little PES	1.4	Possibly polyurethane
61488F1	92% PA, 8% EL	2.6	Fatty acid ethoxylate, amide (little amount)	0.2	Fatty acid ester, polyamide, silicone	1.8	mineral oil, silicone, little fatty acid ester	1.8	mineral oil, fatty acid esters, silicone	1.2	Fatty acid ethoxylate, little silicone	1.2	fatty acid esters, silicone	0.9	Mineral oil, silicone, fatty acid ester, fatty acid amide, possibly little PES	1.1	fatty acid esters, silicone





# WP 7.2: Textiles



## Conclusions and next steps

- The enzymes have not delivered any desired effect yet
- Any comments regarding the current test setup? The time frame in the Foulard machine seems to be surprisingly low for the partners, but this is the realistic procedure time available
- More experiments will be done with recently received enzymes (with/without buffer?)
  - #10 PEH\_Pbau\_PE-H
  - #08 EstLip\_TBec304
  - #11 PEH\_Pform\_PE-H
  - #21 FE\_EH37
  - #12 PEH\_Poce\_PE-H
  - #13 GEN0105





# WP 7.3: Cosmetics

- Task 1: Enzyme analysis and verification
- Task 2: Enzyme production at g-scale
- Task 3: Enzymatic HA hydrolysis
- Task 4: Industrial application tests
- Total enzyme candidates: 25
  - 12 not investigated further / 4 planned for production / 7 in production / 2 shipped to partners



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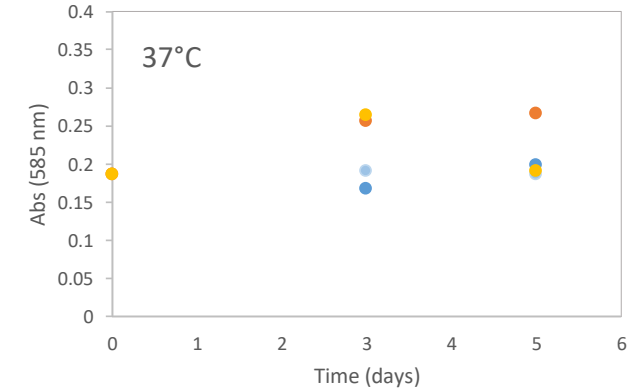
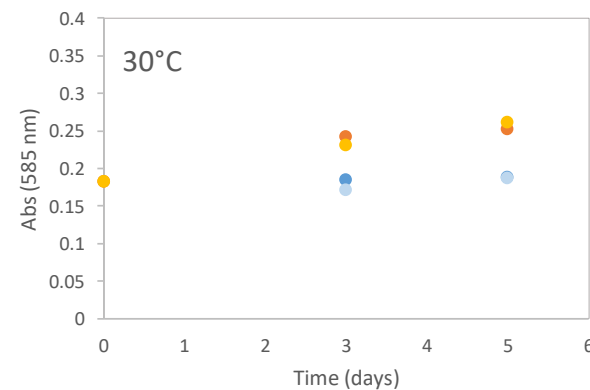
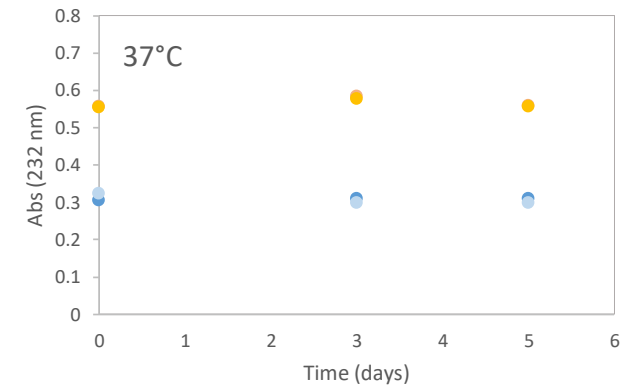
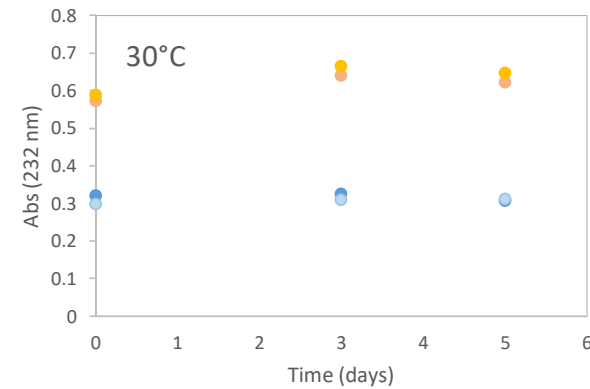
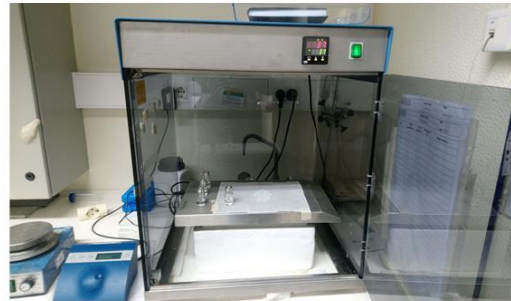
# Task 7.2: Cosmetics – influence of temperature



## Effect of temperature on enzyme activity - *Vibrio* sp. IAMC-CNR#23

Assay: carried out at 30 and 37°C in 10 mL glass reactors containing 4mL of 2 g/L HA50 in phosphate buffer pH 6.2 and 20 g/L *Vibrio* sp. IAMC-CNR#23.

Analysis: BSA (232 nm) and Morgan Elson (585 nm) methods

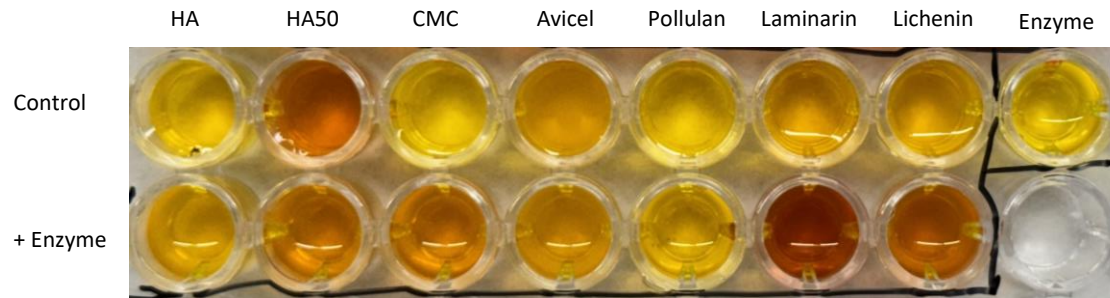




# WP 7.3: Cosmetics



- Hyal\_HRDSV\_2334 was confirmed to be active towards HYACARE (HMWHA) and HYACARE50(HA50). It is also active with Carboxymethylcellulose, laminarin and lichenan



- Workflow of the enzymatic process for the production of hyaluronic acid hydrolysis products have been set up, using HMWHA, as substrate. Degradation products have been identified by MS/MS

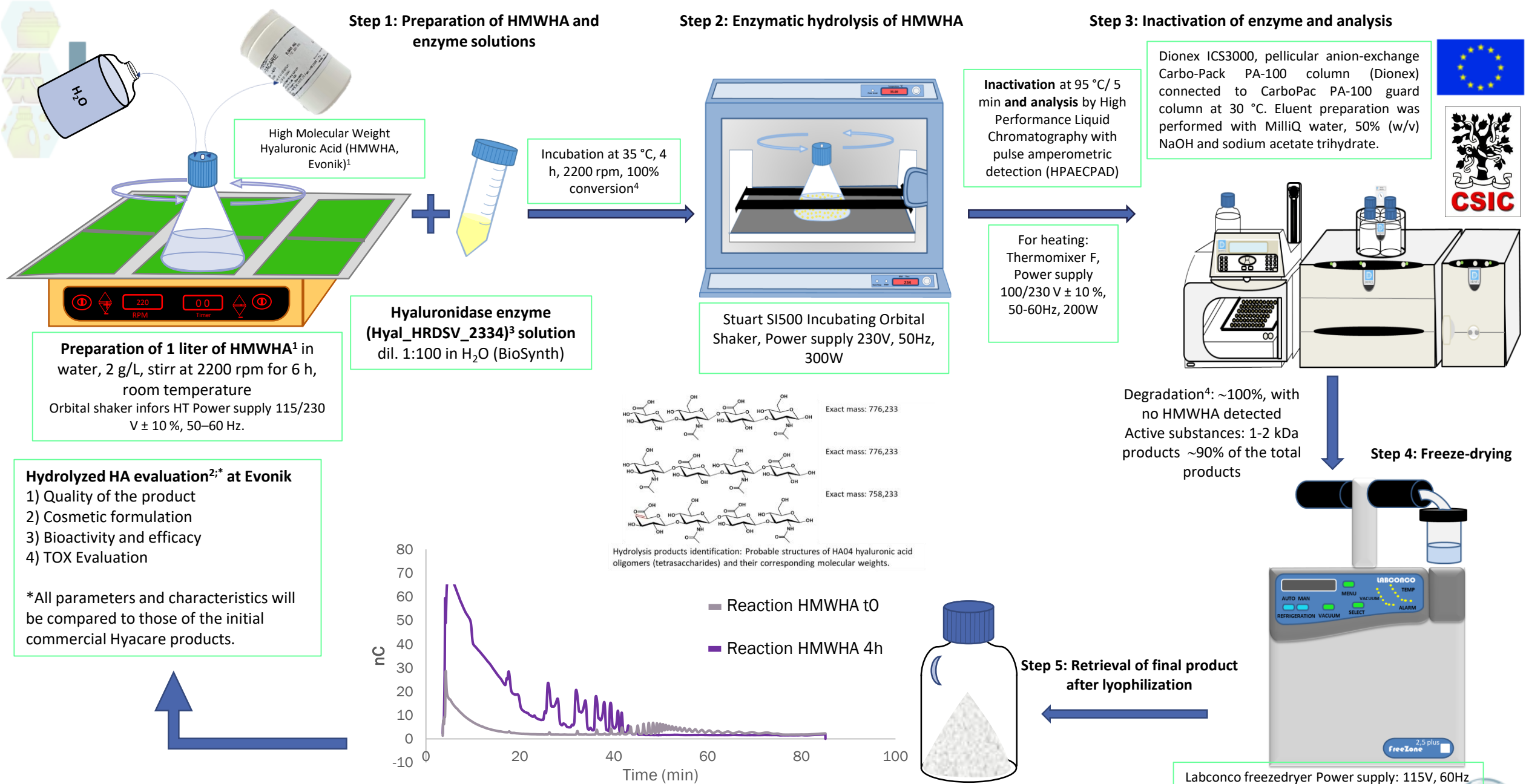




### Step 1: Preparation of HMWHA and enzyme solutions

### Step 2: Enzymatic hydrolysis of HMWHA

### Step 3: Inactivation of enzyme and analysis



<sup>1</sup>For both the final enzymatic process and for the production cost Evonik suggests to use HMWHA (600-900 kDa) as raw material instead of HA 50 (20-30 kDa)

<sup>2</sup>For analytics and the bioactivity screening 2 grams of the dried powder (> 90% active substance) are needed; for further performing different tests, in total at least 10- 25g will be needed.

<sup>3</sup>Lot: 03920323SS1127

<sup>4</sup>In our conditions, approx. 100% conversion is achieved, and thus we expect that starting from 2 g/L of of HMWHA, we will obtain approx. 1.8 g/L of 1-2 kDa hydrolysis products

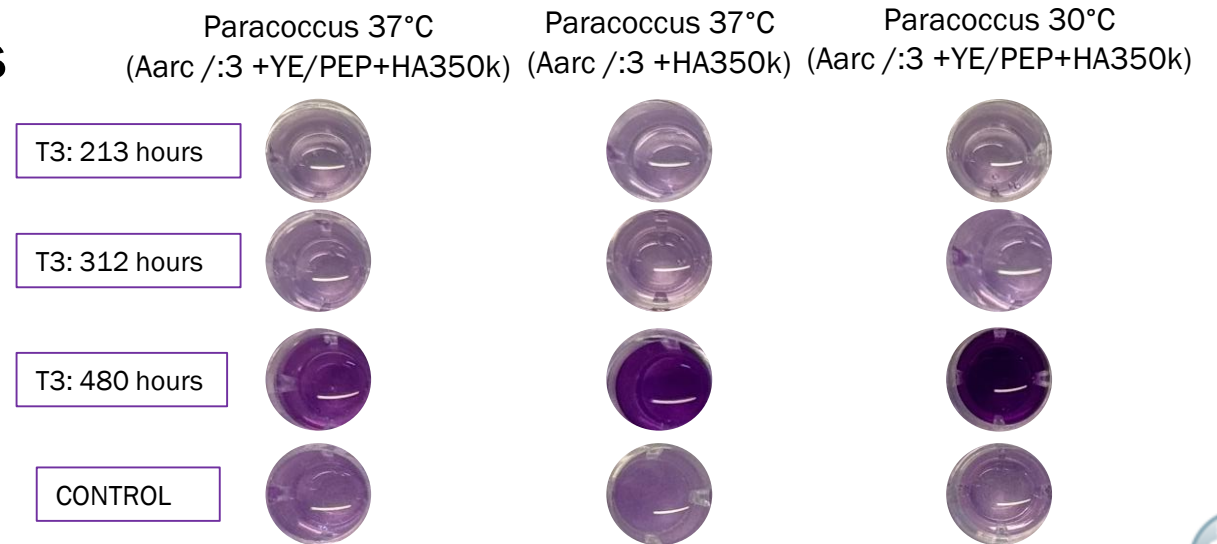


# WP 7.3: Cosmetics



- A total of 31 samples (Paracoccus, Hhyl1, Hhyl2 and M#18) provided by CNR have been analysed for signatures of hyaluronic acid degradation. Microbes were cultured at different conditions and times and samples have been analysed by colorimetric methods (BCA, DNS). 3 samples turned positive:
  - Paracoccus 37 °C (Aarc /:3 +YE/PEP+HA350k)
  - Paracoccus 37 °C (Aarc /:3 +HA350k)
  - Paracoccus 30 °C (Aarc /:3 +YE/PEP+HA350k)

→ We suggest focus on these strains





# Content

- General overview WP 7 and coordination
  - Project Timeline
  - Deliverables and Milestones
  - General progress in M24-M36
  - Lead Enzyme Candidates
- Advances and next steps WP 7.1-7.3
- Advances and next steps WP 7.4
- Outlook and discussion



# Task 7.4: LCA assessments – progress



## ■ Progress undertaken and outputs achieved since the Hamburg meeting:

### ■ Analysis of the **benchmark products** through:

- Individual meetings with technology / product providers among the project partners
- Data provided directly by the partners
- Patent and literature search

### LIQUID DETERGENT

Functional Unit: 7 kg of washed clothes

Activities completed:

- Life Cycle Inventory of the benchmark detergent and modelling completed
- The LCI is based mainly on literature data (PEF publication)
- LCI sent to Henkel for final validation

### VIRGIN PES TEXTILE

Functional Unit: 1 kg of dyed fabric (3x58 100% virgin PES) on roll

Activities completed:

- LCI for the benchmark almost finalized after several meetings and data exchange with Schoeller
- The LCI is based almost entirely on primary data provided by Schoeller

### FACE CREAM

Functional Unit: focus on the HayCare active ingredient but not defined yet

Activities completed:

- Some meetings with Evonik to define the Goal & Scope
- No many information received

**For the benchmarks of detegrent and textile, we plan to discuss the results of the analysis before the summer**





# Task 7.4: LCA assessments – next steps



- Including enzymes in the LCA analysis?
  - Discussing with BIOC-CHEM to include the enzymes in the analysis
  - For the benchmark: info based on Ecoinvent but implemented to be as close as to reality as possible
  
- Analysis of the innovative products for the **detergent** and **textile**
  - We can start to model the innovative products for the **detergent** (HENKEL) and the **textile** (SCHOELLER)
  - Starting from the benchmark, partners should collect data from their experimental activities highlighting the main differences -> we plan to organize a meeting with the two companies (and any other partners involved) in order to discuss the main innovations and discuss on how to implement the benchmark LCI
  - Data collection involves the reference industry for each product but also the other partners involved in the analysis
  - Evaluating the possibility to model the innovative enzymes (if we have some significant changes in the production)
  
- Finalizing the benchmark model for the **cosmetic**
  - We need at least a description of the industrial process and general input of main inputs and outputs (e.g. energy consumption, water consumption, amount of waste generated, ...)
  - As it is extremely difficult to find data in the literature, we cannot complete this analysis if we don't receive primary data from the partners





# Task 7.4: LCA assessments – guidelines



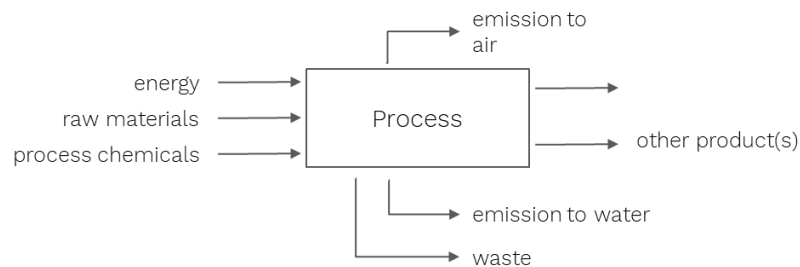
## ■ What is your contribution to the LCA?

- In order to assess the environmental impact of FuturEnzyme products and processes in the most accurate way as possible, we need the most accurate data. Primary data, YOUR data, are the basis for a realistic evaluation of our process.

## ■ How can you contribute?

- By supporting us in the completion of the **Life Cycle Inventory** for the innovative products
- The inventory is a data collection considering all the environmentally relevant flows for all the activities in the product system followed by documentation of the collected data.
- For the products for which the benchmark is already modelled, you may start by modifying the LCI of the benchmark, depending on the degree of the innovation
- We prepared two documents to support you in doing your inventory: **guidelines** and **inventory template**

### EXAMPLE

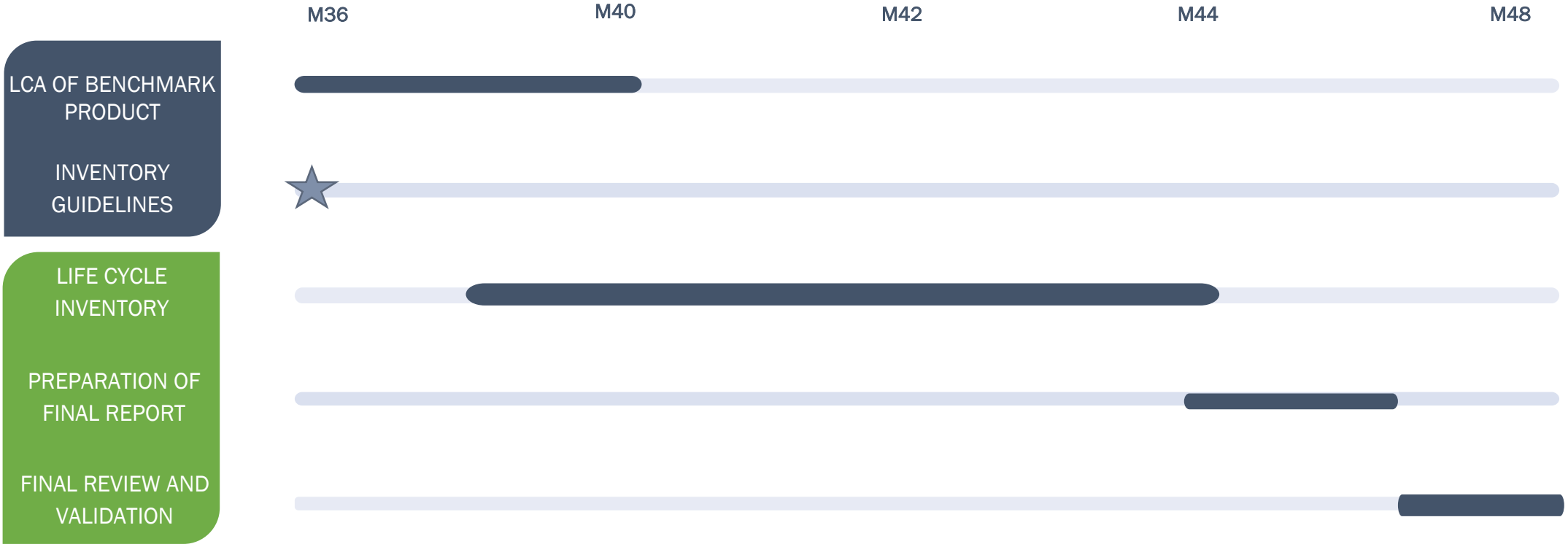


Input (to obtain 10g of product A)			Output		
Stirring energy	100	MJ	Product A	10	g
Heating energy	150	MJ	Waste water	15	l
Substrate	20	l	Used enzyme B	37	mg
Enzyme B	37	mg	CO <sub>2</sub>	25	g
Additive C	20	ml	NH <sub>3</sub>	10	g





# Task 7.4: LCA assessments – timeline of the activities





# Task 7.4: LCA assessments – main contacts



Product	Organization	Main contact	Additional contact(s) if needed
Detergent	HENKEL	<a href="mailto:christian.degering@henkel.com">christian.degering@henkel.com</a>	
Textile	SCHOELLER	<a href="mailto:Nazanin.Ansari@schoeller-textiles.com">Nazanin.Ansari@schoeller-textiles.com</a>	
Cosmetic	EVONIK	<a href="mailto:moniec.van-logchem@evonik.com">moniec.van-logchem@evonik.com</a>	<a href="mailto:xin.lu@evonik.com">xin.lu@evonik.com</a>
Enzyme	BIOCH-CHEM	<a href="mailto:fbeltrametti@bioc-chemsolutions.com">fbeltrametti@bioc-chemsolutions.com</a>	<a href="mailto:lmellere@bioc-chemsolutions.com">lmellere@bioc-chemsolutions.com</a>







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# Outlook

“Regarding application of enzymes in industry: the enzyme activity should be validated in industry processes and tested by labs in industry”

- Detergents: Full-scale wash trials @Henkel
  - Enzyme performance in full-scale wash trials, targets for optimisation
  - If available on-time: Testing of optimised variants
- Textiles: Find and apply working enzymes
  - PhD visit @Schoeller: IST-ID, CSIC, UDUS?
  - Positive performance under real-life conditions @Scholler
- Cosmetics: small-scale production and formulation of HA
  - Hydrolysis performance @CSIC, shipping to Evonik
  - Formulation and first tests @Evonik
- LCA: Data collection for innovative products' LCAs
- In-person Industry Meeting @Henkel/Evonik in Nov/Dec 2024?

# FuturEnzyme WP7: Formulation and manufacturing of consumer products: sustainability and environmental assessments



36M Annual Meeting, Lipari

Markus Müller (CLIB)

07/17/2024



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