FuturEnzyme
WP7: Formulation and manufacturing of consumer products: sustainability and environmental assestments

24M Annual Meeting, Hamburg

Markus Müller (CLIB)

07/06-07/2023

Project funded by the European Union's Horizon 2020
Research and Innovation Programme under grant agreement No [101000327]

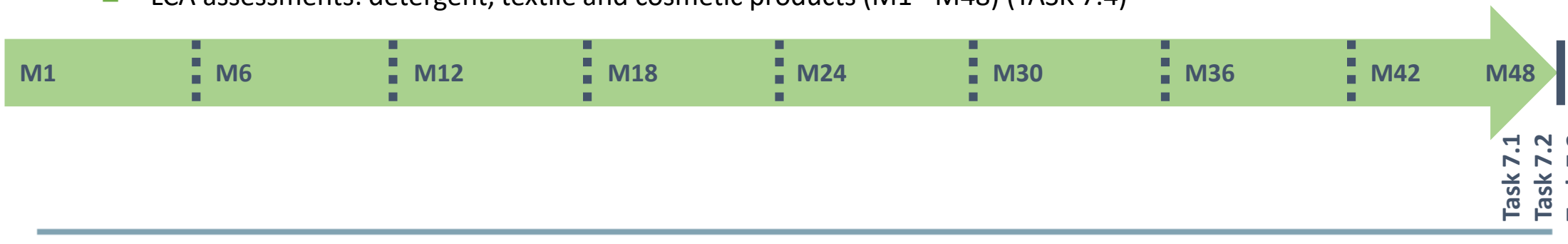
Content

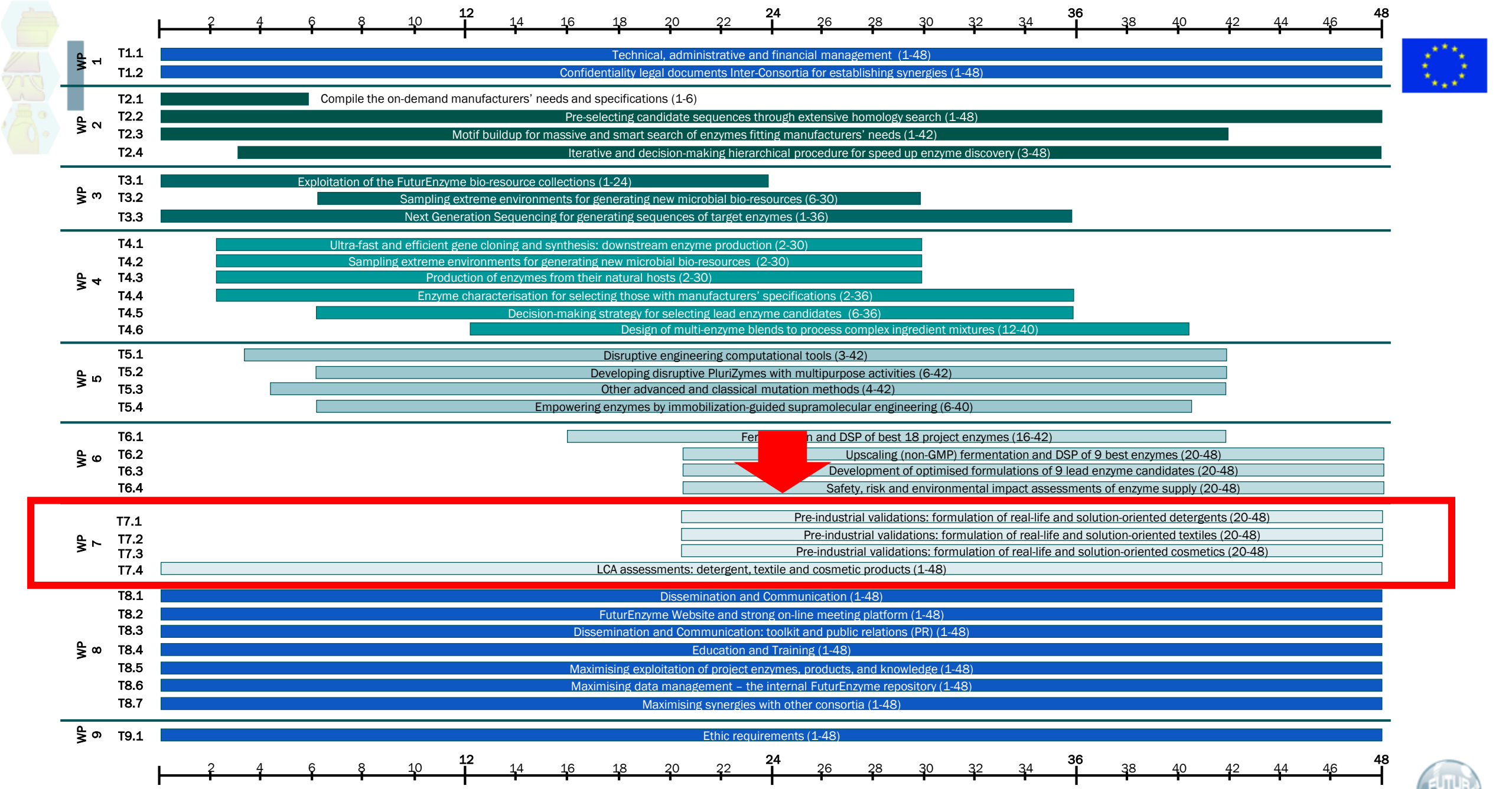
- General overview WP 7 and coordination
  - Project Timeline
  - Lead Enzyme Candidates
  - Material Transfers
  - Nagoya Templates
- Advances and next steps WP 7.1-7.3
- Advances and next steps WP 7.4
- Open questions and discussion

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)




2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
WP 1
T1.1 Technical, administrative and financial management (1-48)
T1.2 Confidentiality legal documents Inter-Consortia for establishing synergies (1-48)
WP 2
T2.1 Compile the on-demand manufacturers' needs and specifications (1-6)
T2.2 Pre-selecting candidate sequences through extensive homology search (1-48)
T2.3 Motif buildup for massive and smart search of enzymes fitting manufacturers' needs (1-42)
T2.4 Iterative and decision-making hierarchical procedure for speed up enzyme discovery (3-48)
WP 3
T3.1 Exploitation of the FuturEnzyme bio-resource collections (1-24)
T3.2 Sampling extreme environments for generating new microbial bio-resources (6-30)
T3.3 Next Generation Sequencing for generating sequences of target enzymes (1-36)
WP 4
T4.1 Ultra-fast and efficient gene cloning and synthesis: downstream enzyme production (2-30)
T4.2 Sampling extreme environments for generating new microbial bio-resources (2-30)
T4.3 Production of enzymes from their natural hosts (2-30)
T4.4 Enzyme characterisation for selecting those with manufacturers' specifications (2-36)
T4.5 Decision-making strategy for selecting lead enzyme candidates (6-36)
T4.6 Design of multi-enzyme blends to process complex ingredient mixtures (12-40)
WP 5
T5.1 Disruptive engineering computational tools (3-42)
T5.2 Developing disruptive PluriZymes with multipurpose activities (6-42)
T5.3 Other advanced and classical mutation methods (4-42)
T5.4 Empowering enzymes by immobilization-guided supramolecular engineering (6-40)
WP 6
T6.1 Fer n and DSP of best 18 project enzymes (16-42)
T6.2 Upscaling (non-GMP) fermentation and DSP of 9 best enzymes (20-48)
T6.3 Development of optimised formulations of 9 lead enzyme candidates (20-48)
T6.4 Safety, risk and environmental impact assessments of enzyme supply (20-48)
WP 7
T7.1 Pre-industrial validations: formulation of real-life and solution-oriented detergents (20-48)
T7.2 Pre-industrial validations: formulation of real-life and solution-oriented textiles (20-48)
T7.3 Pre-industrial validations: formulation of real-life and solution-oriented cosmetics (20-48)
T7.4 LCA assessments: detergent, textile and cosmetic products (1-48)
WP 8
T8.1 Dissemination and Communication (1-48)
T8.2 FuturEnzyme Website and strong on-line meeting platform (1-48)
T8.3 Dissemination and Communication: toolkit and public relations (PR) (1-48)
T8.4 Education and Training (1-48)
T8.5 Maximising exploitation of project enzymes, products, and knowledge (1-48)
T8.6 Maximising data management – the internal FuturEnzyme repository (1-48)
T8.7 Maximising synergies with other consortia (1-48)
WP 9
T9.1 Ethic requirements (1-48)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48

WP7 Management M12 - M24

- 08/22 LCA Benchmark meetings
- 09/22 Industry meetings, round 1
- 11/22 Decision meeting on first Lead enzyme candidates
- 01/23 Industry meeting, round 2
- 02/23 Reporting RP1
- 05/23 Industry meeting, round 3

→ 09/23 Industry meeting, round 4

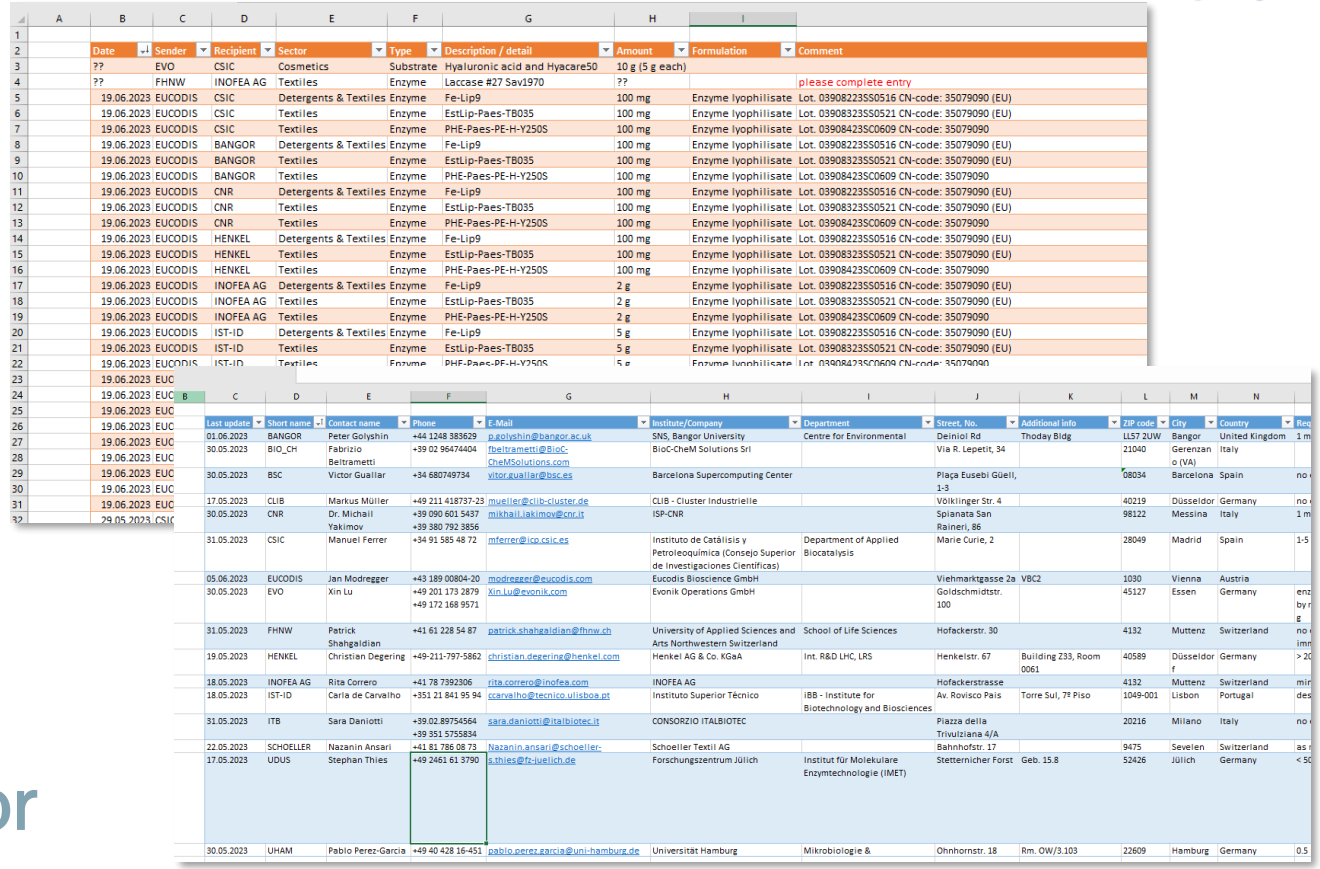
Lead Enzyme Candidates

ID	Enzyme	Partner	Priority	Signal peptide	Homology (%[3]	Application
1	Kest3 (lipase)	Bangor	No	No	33.87	Detergent
2	FE_Lip9 (lipase)	CSIC	Yes	Yes	99.45	Detergent, textile
3	FE_ID9 (lipase)	CSIC	Yes	No	100	Detergent
4	FE_polur1 (lipase)	CSIC	Yes	No	97.3	Detergent
5	EstLip_Dim_#008 (lipase)	UDUS	Yes	No	100	Detergent
6	EstLip_Paes_TB035 (lipase)	UDUS	Yes	Yes	41.98	Detergent
7	EstLip_PtEst1 (lipase)	UDUS	Yes	No	58.63	Detergent
8	EstLip_TBEc304 (lipase)	UDUS	No	No	62.54	Detergent
9	PEH_Paes_PE-H_Y250S (PETase)	UDUS	Yes	Yes	62.88	Detergent, textile
10	PEH_Pbau_PE-H (Lipase, PETase)	UDUS	No	Yes	61.70	Detergent, textile
11	PEH_Pform_PE-H (Lipase, PETase)	UDUS	No	Yes	69.08	Textile
12	PEH_Poce_PE-H (Lipase, PETase)	UDUS	No	Yes	62.5	Detergent, textile
13	GEN0105 (Lipase, PETase)	Bangor	No	No	61.69	Detergent
14	GEN0095 (cellulase)	Bangor	No	No	52.5	Textile
15	VD_PL9 (hyaluronidase)	CNR	Yes	Yes	88.89	Cosmetic
16	VD_PL22 (hyaluronidase)	CNR	No	No	69.23	Cosmetic
17	VA_PL9 (hyaluronidase)	CNR	No	Yes	32.38	Cosmetic
18	Hyal_HRDSV_2334 (hyaluronidase)	CNR	No	No	100	Cosmetic
19	V. diabolicus V4 (hyaluronidase)	CNR	Yes	Yes	-	Cosmetic
20	V. alginolyticus #23 (hyaluronidase)	CNR	Yes	Yes	-	Cosmetic
21	FE_EH37 (esterase)	CSIC	No	No	49.09	Predictive tools
22	FE_Lip5 (lipase)	CSIC	No	No	43.52	Detergent
23	TR_2E_2 (PluriZyme)	CSIC	No	No	66.74	All
24	EH_{1AB1C} (PluriZyme)	CSIC	No	No	64.19	All
25	X11_mut1 (PluriZyme)	CSIC	No	No	64.78	All
26	I8AMQ8 (Peroxidase)	Bangor	Yes	No	<50%	Textile
27	Sav1970 (laccase)	Bangor	Yes	No	<50%	Textile

Material Transfers

- List of shipping adresses available in OneDrive
- List of completed material transfers available
  - Substrates for enzyme reaction
  - Reference materials / benchmarks
  - Kits
  - Enzymes (only Leads?)

→ Keep lists up-to-date and/or inform CLIB!



Date	Sender	Recipient	Sector	Type	Description / detail	Amount	Formulation	Comment
??	EVO	CSIC	Cosmetics	Substrate	Hyaluronic acid and Hyacare50	10 g (5 g each)		
??	FHNW	INOFEA AG	Textiles	Enzyme	Laccase #27 Sav1970	??		please complete entry
19.06.2023	EUCODIS	CSIC	Detergents & Textiles	Enzyme	Fe-Lip9	100 mg	Enzyme lyophilisate	Lot. 03908223SS0516 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	CSIC	Textiles	Enzyme	EstLip-Paes-TB035	100 mg	Enzyme lyophilisate	Lot. 03908323SS0521 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	CSIC	Textiles	Enzyme	PHE-Paes-PE-H-Y250S	100 mg	Enzyme lyophilisate	Lot. 03908423SC0609 CN-code: 35079090
19.06.2023	EUCODIS	BANGOR	Detergents & Textiles	Enzyme	Fe-Lip9	100 mg	Enzyme lyophilisate	Lot. 03908223SS0516 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	BANGOR	Textiles	Enzyme	EstLip-Paes-TB035	100 mg	Enzyme lyophilisate	Lot. 03908323SS0521 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	BANGOR	Textiles	Enzyme	PHE-Paes-PE-H-Y250S	100 mg	Enzyme lyophilisate	Lot. 03908423SC0609 CN-code: 35079090
19.06.2023	EUCODIS	CNR	Detergents & Textiles	Enzyme	Fe-Lip9	100 mg	Enzyme lyophilisate	Lot. 03908223SS0516 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	CNR	Textiles	Enzyme	EstLip-Paes-TB035	100 mg	Enzyme lyophilisate	Lot. 03908323SS0521 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	CNR	Textiles	Enzyme	PHE-Paes-PE-H-Y250S	100 mg	Enzyme lyophilisate	Lot. 03908423SC0609 CN-code: 35079090
19.06.2023	EUCODIS	HENKEL	Detergents & Textiles	Enzyme	Fe-Lip9	100 mg	Enzyme lyophilisate	Lot. 03908223SS0516 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	HENKEL	Textiles	Enzyme	EstLip-Paes-TB035	100 mg	Enzyme lyophilisate	Lot. 03908323SS0521 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	HENKEL	Textiles	Enzyme	PHE-Paes-PE-H-Y250S	100 mg	Enzyme lyophilisate	Lot. 03908423SC0609 CN-code: 35079090
19.06.2023	EUCODIS	INOFEA AG	Detergents & Textiles	Enzyme	Fe-Lip9	2 g	Enzyme lyophilisate	Lot. 03908223SS0516 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	INOFEA AG	Textiles	Enzyme	EstLip-Paes-TB035	2 g	Enzyme lyophilisate	Lot. 03908323SS0521 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	INOFEA AG	Textiles	Enzyme	PHE-Paes-PE-H-Y250S	2 g	Enzyme lyophilisate	Lot. 03908423SC0609 CN-code: 35079090
19.06.2023	EUCODIS	IST-ID	Detergents & Textiles	Enzyme	Fe-Lip9	5 g	Enzyme lyophilisate	Lot. 03908223SS0516 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	IST-ID	Textiles	Enzyme	EstLip-Paes-TB035	5 g	Enzyme lyophilisate	Lot. 03908323SS0521 CN-code: 35079090 (EU)
19.06.2023	EUCODIS	IST-ID	Textiles	Enzyme	PHE-Paes-PE-H-Y250S	5 g	Enzyme lyophilisate	Lot. 03908423SC0609 CN-code: 35079090

Last update	Short name	Contact name	Phone	E-Mail	Institute/Company	Department	Street, No.	Additional info	ZIP code	City	Country
01.06.2023	BANGOR	Peter Golyshin	+44 1248 383629	p.golyshin@bangor.ac.uk	SNS, Bangor University	Centre for Environmental	Deiniol Rd	Thoday Bldg	LL57 2UW	Bangor	United Kingdom
30.05.2023	BIO_CH	Fabrizio Beltrametti	+39 02 96474404	fbeltrametti@BioC-CheMSolutions.com	BioC-CheM Solutions Srl		Via R. Lepetit, 34		21040	Gerenzano (VA)	Italy
30.05.2023	BSC	Victor Guallar	+34 680749734	vitor.guallar@bsc.es	Barcelona Supercomputing Center		Plaça Eusebi Güell, 1-3		08034	Barcelona	Spain
17.05.2023	CLIB	Markus Müller	+49 211 418737-23	mueller@clib-cluster.de	CLIB - Cluster Industrielle		Völklinger Str. 4		40219	Düsseldor	Germany
30.05.2023	CNR	Dr. Michail Yakimov	+39 090 601 5437 +39 380 792 3856	mikhail.iakimov@cnr.it	ISP-CNR		Spianata San Raineri, 86		98122	Messina	Italy
31.05.2023	CSIC	Manuel Ferrer	+34 91 585 48 72	mferrer@icp.csic.es	Instituto de Catálisis y Petroleoquímica (Consejo Superior de Investigaciones Científicas)	Department of Applied Biocatalysis	Marie Curie, 2		28049	Madrid	Spain
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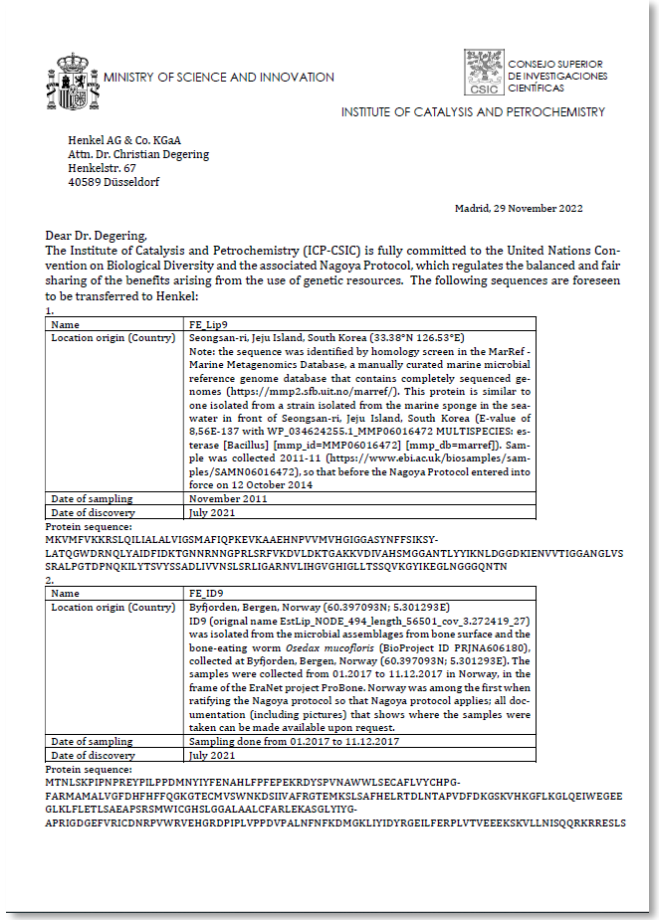
31.05.2023	FHNW	Patrick Shahgaldian	+41 61 228 54 87	patrick.shahgaldian@fhnw.ch	University of Applied Sciences and Arts Northwestern Switzerland	School of Life Sciences	Hofackerstr. 30		4132	Muttenz	Switzerland
19.05.2023	HENKEL	Christian Degering	+49-211-797-5862	christian.degering@henkel.com	Henkel AG & Co. KGaA	Int. R&D LHC, LRS	Henkelstr. 67	Building Z33, Room 0061	40589	Düsseldorf	Germany
18.05.2023	INOFEA AG	Rita Correro	+41 78 7392306	rita.correro@inofea.com	INOFEA AG		Hofackerstrasse		4132	Muttenz	Switzerland
18.05.2023	IST-ID	Carla de Carvalho	+351 21 841 95 94	ccarvalho@tecnico.ulisboa.pt	Instituto Superior Técnico	iBB - Institute for Biotechnology and Biosciences	Av. Rovisco Pais	Torre Sul, 7º Piso	1049-001	Lisbon	Portugal
31.05.2023	ITB	Sara Daniotti	+39.02.89754564 +39 351 5755834	sara.daniotti@italbiotec.it	CONSORZIO ITALBIOTEC		Piazza della Trivulziana 4/A		20216	Milano	Italy
22.05.2023	SCHOELLER	Nazanin Ansari	+41 81 786 08 73	Nazanin.ansari@schoeller-s.thies@fz-juelich.de	Schoeller Textil AG		Bahnhofstr. 17		9475	Sevelen	Switzerland
17.05.2023	UDUS	Stephan Thies	+49 2461 61 3790		Forschungszentrum Jülich	Institut für Molekulare Enzymtechnologie (IMET)	Stetternicher Forst	Geb. 15.8	52426	Jülich	Germany
30.05.2023	UHAM	Pablo Perez-Garcia	+49 40 428 16-451	pablo.perez.garcia@uni-hamburg.de	Universität Hamburg	Mikrobiologie &	Ohnhornstr. 18	Rm. OW/3.103	22609	Hamburg	Germany

Nagoya Protocol

- Templates available in OneDrive
- File Nagoya form **before** sending samples to:
  - Henkel
  - Evonik
  - Schoeller
- Other industry partners:
  - BioC-CheM-Solutions: No Nagoya forms required (Italy is not part of the Nagoya protocol)
  - Eucodis: Template will be provided in the next months, required before project ends
  - INOFEA: Template available but so far no forms required



MINISTRY OF SCIENCE AND INNOVATION

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

INSTITUTE OF CATALYSIS AND PETROCHEMISTRY

Henkel AG & Co. KGaA
Attn. Dr. Christian Degering
Henkelstr. 67
40589 Düsseldorf

Madrid, 29 November 2022

Dear Dr. Degering,
The Institute of Catalysis and Petrochemistry (ICP-CSIC) is fully committed to the United Nations Convention on Biological Diversity and the associated Nagoya Protocol, which regulates the balanced and fair sharing of the benefits arising from the use of genetic resources. The following sequences are foreseen to be transferred to Henkel:

1.

Name	FE_Lip9
Location origin (Country)	Seongsan-ri, Jeju Island, South Korea (33.38°N 126.53°E) Note: the sequence was identified by homology screen in the MarRef - Marine Metagenomics Database, a manually curated marine microbial reference genome database that contains completely sequenced genomes (https://mmp2.sfb.uit.no/marref/). This protein is similar to one isolated from a strain isolated from the marine sponge in the seawater in front of Seongsan-ri, Jeju Island, South Korea (E-value of 8.56E-137 with WP_034624255.1_MMP06016472 MULTISPECIES: esterase [Bacillus] [mmp_id=MMP06016472] [mmp_db=marref]). Sample was collected 2011-11 (https://www.ebi.ac.uk/biosamples/samples/SAMN06016472), so that before the Nagoya Protocol entered into force on 12 October 2014
Date of sampling	November 2011
Date of discovery	July 2021

Protein sequence:
MKVMFVKKRSLQLIALALVIGSMAFIQPKEVKAAEHNPVVMVHGIGGASYNFFSIKSY-
LATQGWDRNQLYAIDFIDKTGNNRNNGPRLSRFVKDVLDKTGAKKVDIVAHSMGGANTLYYIKNLDGGDKIENVVTIGGANGLVS
SRALPGTDPNQKILYTSVYSSADLIVVNSLSRLIGARNVLIHGVGHIGLLTSSQVKGYIKEGLNGGGQNTN

2.

Name	FE_ID9
Location origin (Country)	Byfjorden, Bergen, Norway (60.397093N; 5.301293E) ID9 (orignal name EstLip_NODE_494_length_56501_cov_3.272419_27) was isolated from the microbial assemblages from bone surface and the bone-eating worm *Osedax mucofloris* (BioProject ID PRJNA606180), collected at Byfjorden, Bergen, Norway (60.397093N; 5.301293E). The samples were collected from 01.2017 to 11.12.2017 in Norway, in the frame of the EraNet project ProBone. Norway was among the first when ratifying the Nagoya protocol so that Nagoya protocol applies; all documentation (including pictures) that shows where the samples were taken can be made available upon request.
Date of sampling	Sampling done from 01.2017 to 11.12.2017
Date of discovery	July 2021

Protein sequence:
MTNLSKPIPNPREYPILPPDMNYIYFENAHLFPFEPEKRDYSPVNAWWLSECAFLVYCHPG-
FARMAMALVGFDHFHFFQGKGTECMVSWNKDSIIVAFRGTEMKSLSAFHELRTDLNTAPVDFDKGSKVHKGFLKGLQEIWEGEE
GLKLFLETLSAEAPSRSMWICGHSLGGALAALCFARLEKASGLYIYG-
APRIGDGEFVRICDNRPVWRVEHGRDPIPLVPPDVPALNFNFKDMGKLIYIDYRGEILFERPLVTVEEEKSKVLLNISQQRKRRESLS

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WP 7.1: Detergents

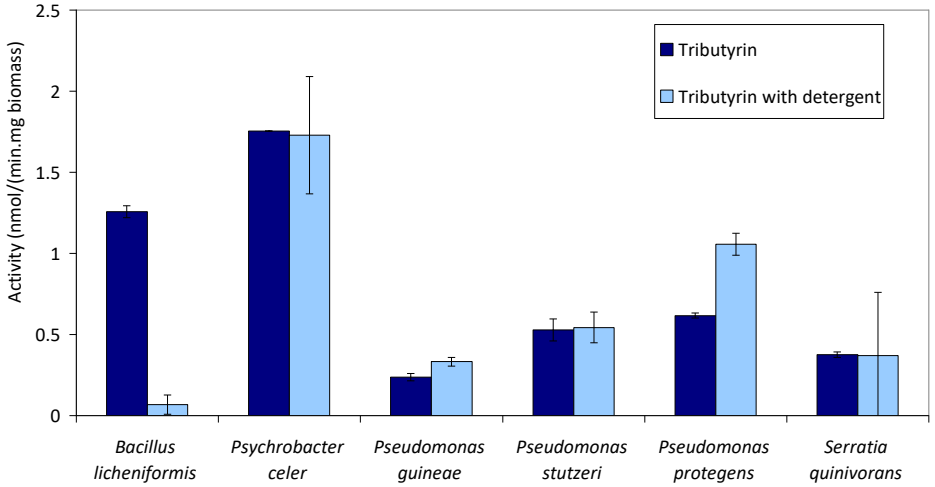
- Establishment of quick activity analysis of lipases
  - Strain/enzyme determines optimal reaction conditions
  - Activity measurement established based on IST-ID isolates
- First three enzyme samples transferred from Eucodis to Henkel (100 mg each) & IST-ID (5 g each)
  - Standard analytics and small-scale wash trials initiated @Henkel
- Technical discussion between Henkel and IST-ID on small-scale wash trials @IST-ID

  → Next: Testing of three enzymes under simulated washing cycle (textile „rubbing“ reactor under development)

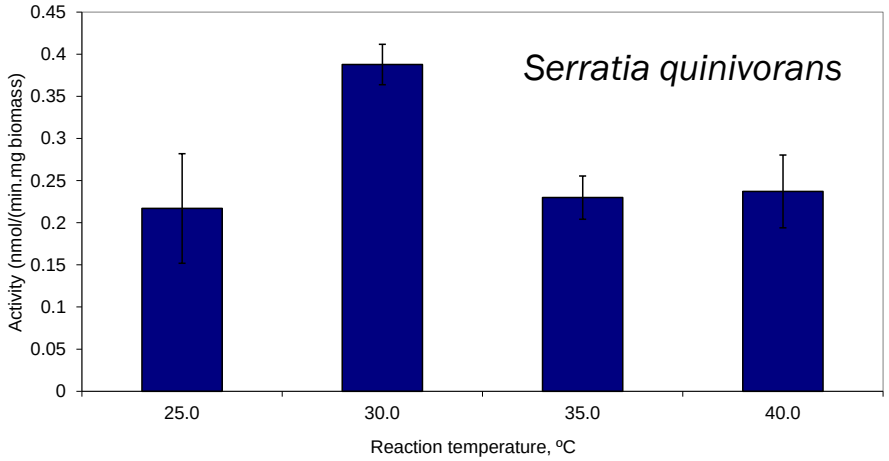
WP 7.1: Lipase activity of isolates for application in detergents

IST-ID
Associação do Instituto Superior Técnico para a Investigação e Desenvolvimento

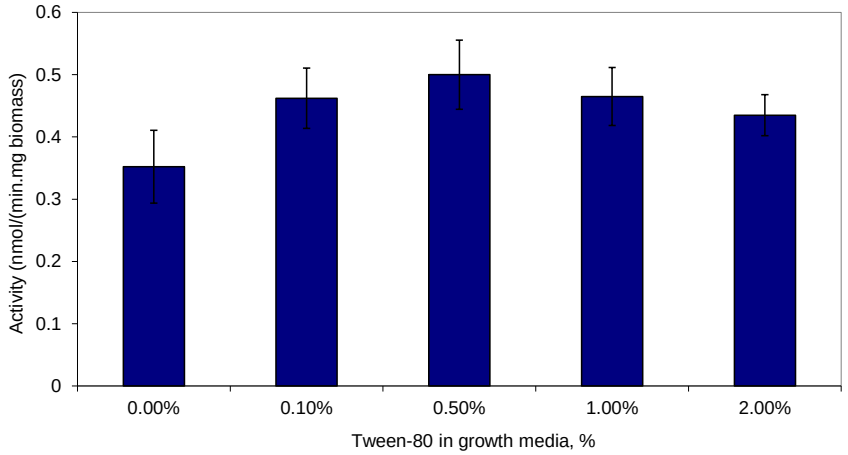
Effect of detergent



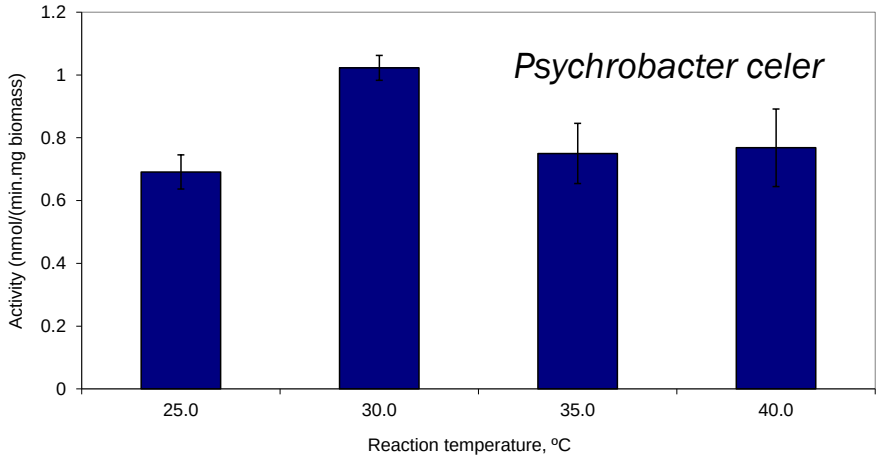
Effect of temperature



Effect of Tween 80



Effect of temperature



Road towards application tests „Detergents“

Enzyme selection	Enzyme production and formulation	Enzyme verification	Application tests
#01 Kest3 |L| Bangor		#02 FE_Lip9 |L| CSIC	
	#03 FE_ID9 |L| CSIC		
	#04 FE_polur1 |L| CSIC		
	#05 EstLip_Dim_#008 |L| UDUS		
	#07 EstLip_PtEst1 |L| UDUS	#06 EstLip_Paes_TB035 |L| UDUS	
#08 EstLip_TBEc304 |L| UDUS			
		#09 PEH_Paes_PE-H_Y250S |P| UDUS	
#10 PEH_Pbau_PE-H |L-P| UDUS			
#12 PEH_Poce_PE-H |L-P| UDUS			
#13 GEN0105 |L-P| Bangor			
#22 LE_Lip5 |L| CSIC			

L = lipase | P = PETase | C = cellulase | H = hyaluronidase | E = esterase | Z = PluriZyme | X = peroxidase | A = laccase red = priority

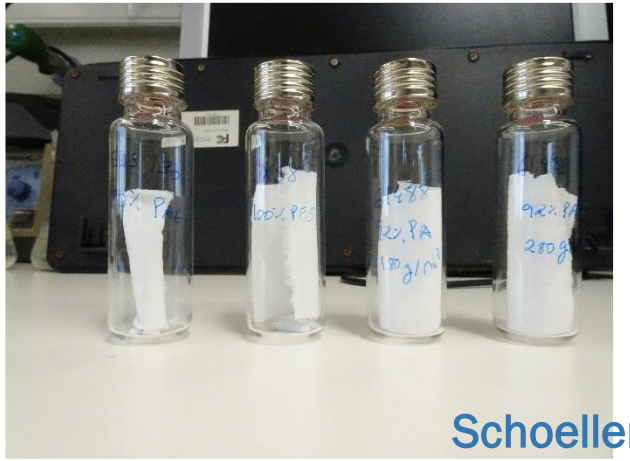
WP 7.2: Textiles

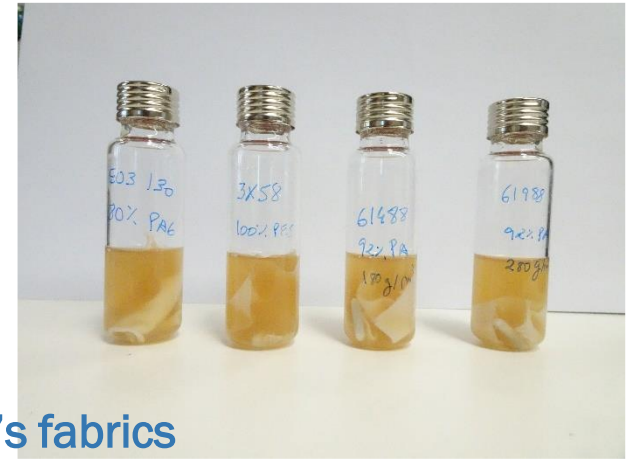
- Application 1: Spinning oil removal
  - Analysis of enzyme activity based on TPA concentration and contact angle
- Application 2: Excess dyestuff removal
  - Bio_CH: Successful laccase production and activity measurement (*Coriolopsis gallica*)
  - IST-ID: Anaysis of dyeing liquid after dyeing process: Many compounds, complicated analyis (hexane, chloroform, acetone, DMSO, MTBE, petroleum ether, …)

  →Focus on one exemplary combination fabric/dyestuff (black + PA66)

- First three enzyme samples transferred from Eucodis to Schoeller (10 g each) & IST-ID (5 g each)
  - Exchange between partners for first testing conditions @Schoeller
  - Next: Three enzymes and IST-ID strains tests in both applications

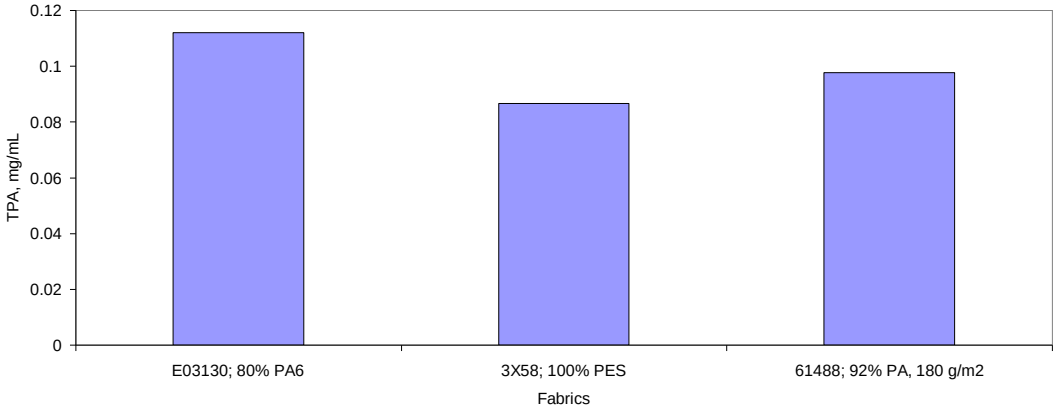
WP 7.1: Lipase activity of isolates for application in textiles



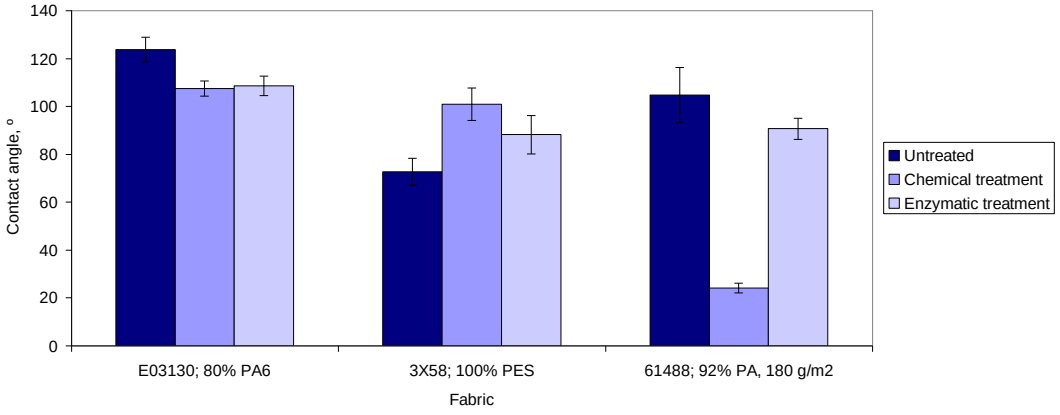


Schoeller's fabrics

Terephthalic acid (TPA) concentration following hydrolysis of polymeric fabrics for 24h



Contact angle (hydrophobicity/wettability)
> 90° → hydrophobic



Road towards application tests „Textiles“

Enzyme selection	Enzyme production and formulation	Enzyme verification	Application tests
#10 PEH_Pbau_PE-H |L-P| UDUS #11 PEH_Pform_PE-H |L-P| UDUS #12 PEH_Poce_PE-H |L-P| UDUS #14 GEN0095 |C| Bangor #26 I8AMQ8 |X| Bangor #27 Sav1970 |A| Bangor		#02 FE_Lip9 |L| CSIC #09 PEH_Paes_PE-H_Y250S |P| UDUS #XX *C. gallica* |A| Bio_CH	

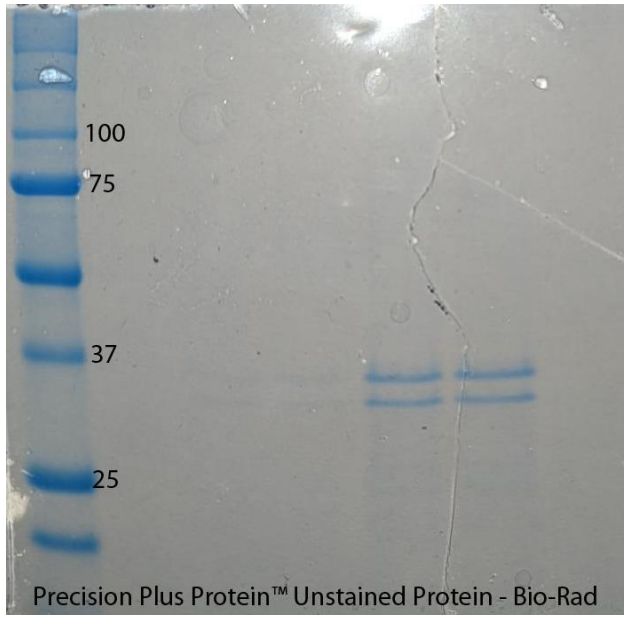
L = lipase | P = PETase | C = cellulase | H = hyaluronidase | E = esterase | Z = PluriZyme | X = peroxidase | A = laccase red = priority

WP 7.3: Cosmetics

- Bio_CH: Cultivation of Lead enzyme #20: (*V. alginolyticus* #23)
  - 15 L cultivation, medium screening, low measured activity on Hyacare substrate
- Establishment of quick activity analysis of hyaluronidases
  - Transfer of methods IST-ID → Bio_CH, Eucodis
  - DNS method
  - Paper-based method (optimised)
- Detailed analysis of the chain length of HA degradation products
  - HPLC-based (CSIC)
  - Laser light scattering (IST-ID) – established based on own isolates
- Next: Transfer of hyaluronidase candidates from Eucodis/Bio_CH to IST-ID and CSIC, optimisation of hydrolysis reaction

Fermentation Hyaluronidase 15 L

- Activity peaks in 48 hours
- Concentration performed on 30 KDa ultrafiltration membranes
- No signal on SDS-PAGE coherent with the estimated Hyaluronidase size
- Other fermentations in progress
- Samples to be sent to CSIC



Road towards application tests „Cosmetics“

Enzyme selection	Enzyme production and formulation	Hydrolysis reaction and formulation	Application tests
#16 VD_PL22 |H| CNR	#15 VD_PL9 |H| CNR		
#17 VA_PL9 |H| CNR			
#18 Hyal_HRDSV_2334 |H| CNR			
#19 V. diabolicus V4 |H| CNR			
	#20 V. alginolyticus #23 |H| CNR		

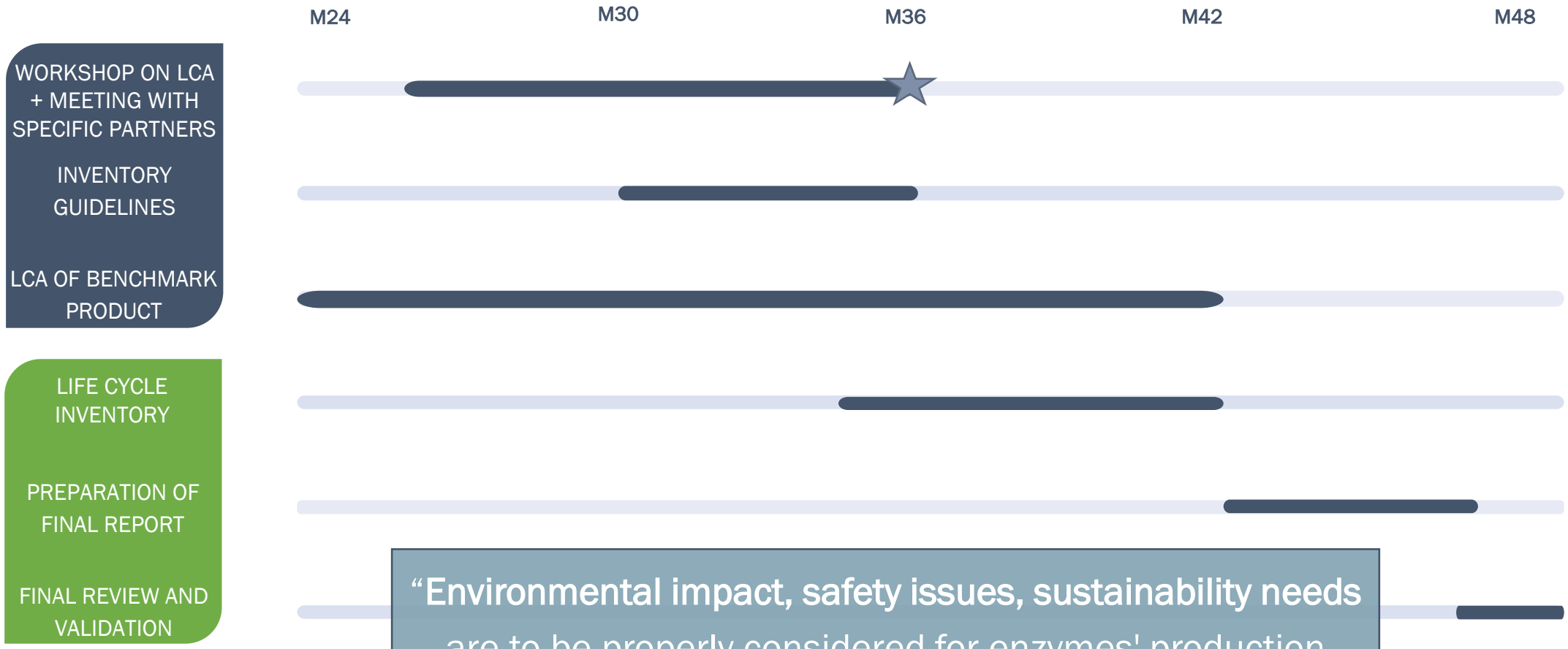
L = lipase | P = PETase | C = cellulase | H = hyaluronidase | E = esterase | Z = PluriZyme | X = peroxidase | A = laccase red = priority

WP 7.4: Life Cycle Assessment (LCA)

- Objective
  - To evaluate the environmental impact of the new products compared to the inovative products and the benchmark ones already on the market
- Progress undertaken and outputs achieved
  - Analysis of the benchmark products through:
    - Individual meetings with technology / product providers among the project partners
    - Patent and literature search

	LIQUID DETERGENT	VIRGIN PES TEXTILE	FACE CREAM
✓	Goal and scope Inventory -> validated by Henkel	Goal and scope Several meetings with Schoeller to validate some data	Analysis of the literature to find more information (e.g. existing LCA)
⏭	Working on modeling the end of life scenario Completing the impact assessment for the benchmark detergent	Working on completing a first draft of the inventory	Working on defining the Goal and Scope of the benchmark product

WP 7.4: Life Cycle Assessment (LCA)



"**Environmental impact, safety issues, sustainability needs** are to be properly considered for enzymes' production technology and also for their further applications."

Content

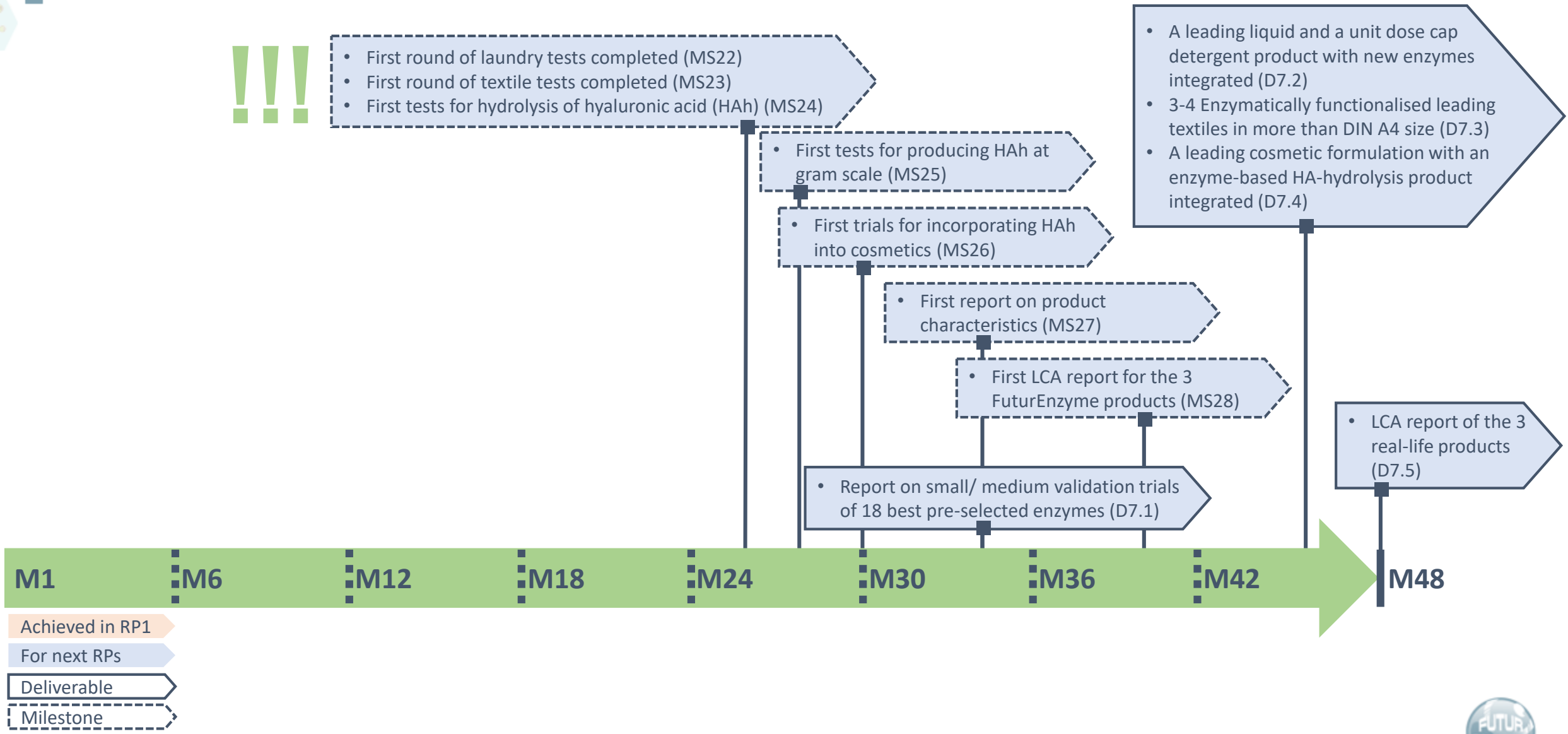
- General overview WP 7 and coordination
  - Project Timeline
  - Lead Enzyme Candidates
  - Material Transfers
  - Nagoya Templates
- Advances and next steps WP 7.1-7.3
- Advances and next steps WP 7.4
- Outlook and open questions/discussion

Outlook

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

- Detergents: Small-scale wash trials @Henkel, IST-ID
  - Enzyme performance on real substrates
  - Feedback to WP6 and definition of targets for optimisation
- Textiles: Enzyme reaction optimisation by IST-ID
  - Method transfer to Schoeller, application tests for first produced enzymes
  - How to proceed with laccase? Add it to the list of Lead candidates?
- Cosmetics: Enzyme transfer Bio_CH/Eucodis → CSIC
  - Verification of detailed enzyme activity measurements
  - Optimisation of Hyacare hydrolysis conditions
- LCA: Workshops on Benchmark and FuturEnzyme processes

WP7 – Deliverables and milestones



Open Discussion

- New enzyme candidates to be added to the Lead enzyme list?
- Suggestion: Status updates for each enzyme candidate in OneDrive to maintain overview: Development state, last results, next steps, …
- Issues with accessing OneDrive folder – Troubleshooting and how to proceed?
  - www.sli.do / #FE24M

"The **success** of the project **equals** to the **industrial application** of prepared enzymes. The consortium needs to make sure that prepared enzymes (the main enzymes but also side ones) will be **available to the market**"

FuturEnzyme
WP7: Formulation and manufacturing of consumer products: sustainability and environmental assestments

24M Annual Meeting, Hamburg

Markus Müller (CLIB)

07/06-07/2023

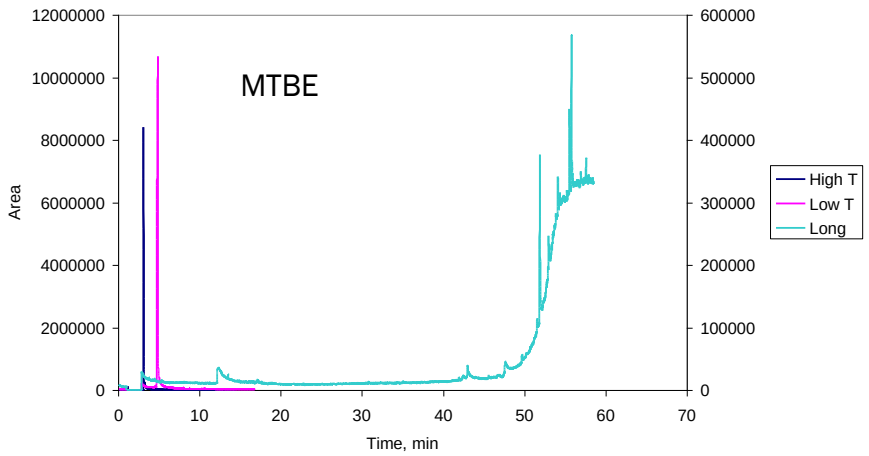
Project funded by the European Union's Horizon 2020
Research and Innovation Programme under grant agreement No [101000327]

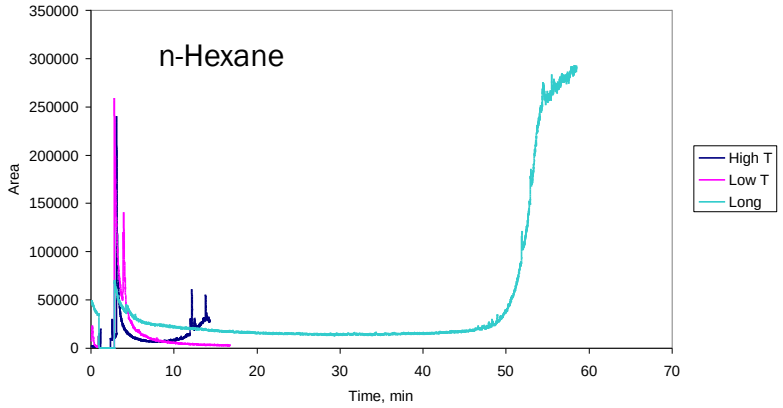
Backup

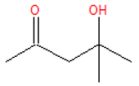
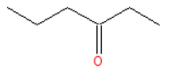
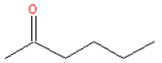
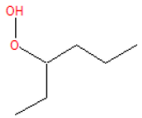
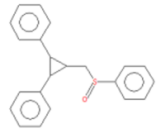
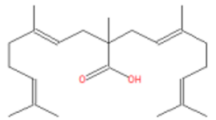
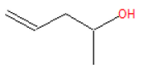
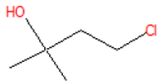
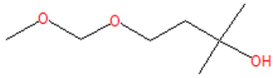
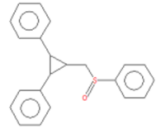
WP7 - Formulation and manufacturing of consumer products: sustainability & environmental assessments. (22 PM)

Schoeller's "dyeing liquid after dyeing process"

GC-MS analysis – different solvents and temperature programmes were necessary to determine which compounds are present

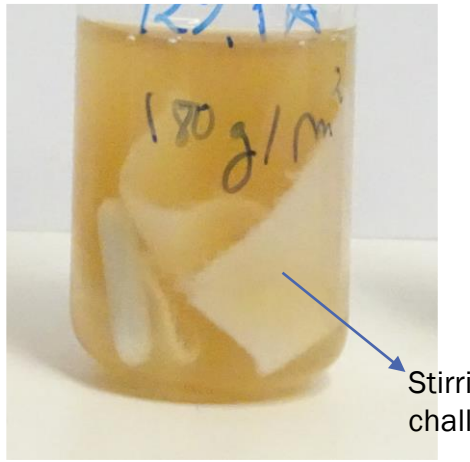




Acetone	4-hydroxy-4-methyl-2-pentanone 	n-Hexane	3-Hexanone  2-Hexanone  Hydroperoxide, 1-ethylbutyl 	MTBE	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide  2,6,9,12,16-Pentamethylheptadeca-2,6,11,15tetraene-9-carboxylic acid 
Chloroform	4-Penten-2-ol  1,1-Dimethyl-3-chloropropanol 	Petroleum ether	Toluene  1-(Methoxymethoxy)-3-methyl-3-hydroxybutane 	Ethyl acetate	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide 

WP7 - Formulation and manufacturing of consumer products: sustainability & environmental assessments. (22 PM)

Schoeller's "challenges"



Stirring is challenging

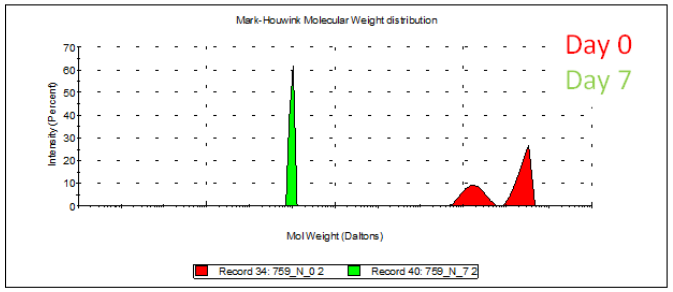
Several compounds to be degraded simultaneously

WP7 - Formulation and manufacturing of consumer products: sustainability & environmental assessments. (22 PM)

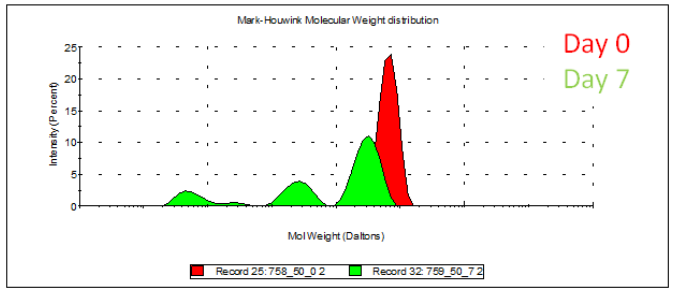
Hyaluronidase activity of isolates for application in cosmetics (laser light scattering measurements)

Stutzerimonas stutzeri

HA - Evonik

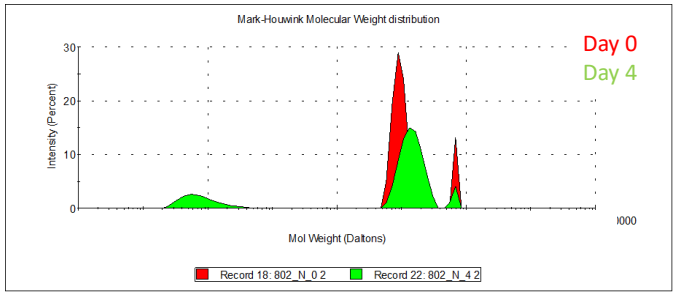


HA50 - Evonik

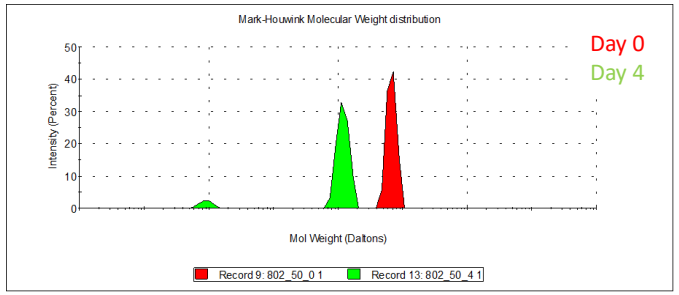


Martelella sp.

HA - Evonik



HA50 - Evonik



Screening of cultivation media from the BCSMedDat database

- Media BCS365 and BCS366 identified as suitable for growth and hyaluronidase (extracellular production)
- Hyacare used as inducer

Medium	OD600 max	Hyaluronidase activity (IU/ml)	Notes
DSM2216	5,8	0,023	control
BCS365	4,6	0,028	
BCS366	8,7	0,028	

Hyaluronidase assay – notes on methodology

- Activity assay adapted from Dorfman, A. (1955) Methods in Enzymology, Volume I, 166-173 – current
- Activity assay adapted from Zhao et al. (2022) Anal. Chem. 2022, 94, 4643−4649 – in progress
- Activity considerably lower than reported in studies with other strains/sources. Qualitatively much evident
- pH has been modified from the method
- Study performed on the Hyacare substrates supplied by Evonics

Fermentation of *Coriolopsis gallica*

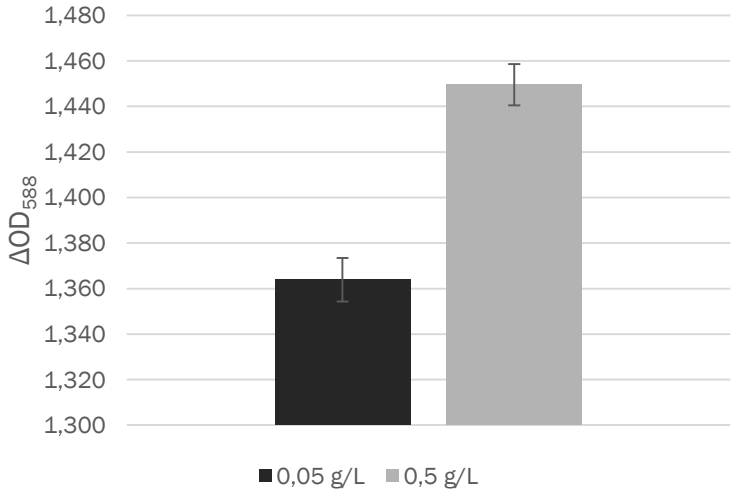
- High level of laccase activity
- Affordable fermentation and DSP process
- Concentration performed by ultrafiltration and (NH4)2SO4 precipitation
- Oxydation of wastewater dyes from Sholler

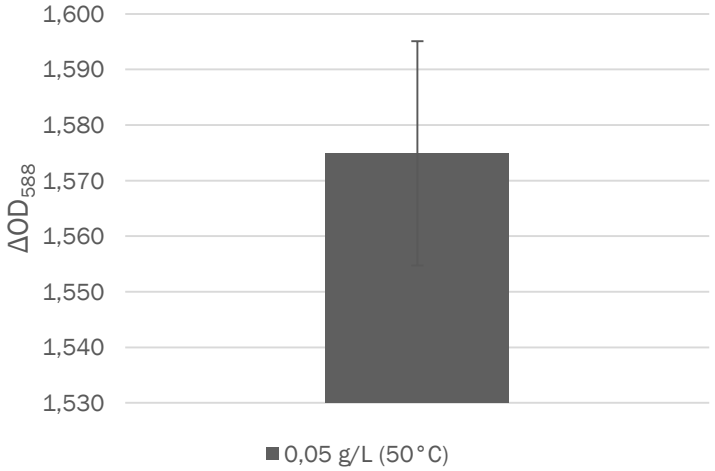
Fermentation *Coriolopsis gallica*

- Dye degradation test (Bangor)
- 10% liquid dye (Sholler)
- The assay was performed in 1mL solutions obtained by addition of 100 µL of dye, 100 µL of enzyme stock solution and 800 µL of ultrapure water. Absorbance changes were measured 588 nm after incubation at 30°C or 50°C for 50 minutes.

Fermentation *Coriolopsis gallica*

- samples treated with 0,05 µg/µL of laccases showed absorbance decrease of 75±1% while samples treated with 0,5 µg/µL of enzyme showed decrease of 79±1%.
- using 0,05 µg/µL of enzyme solution and an incubation temperature of 50°C absorbace decrease of 87±1% was achieved
- A greysh color is retained by the solution after treatment
- The tested laccase has proved to be efficient also in the presence of tensioactives (Tween 20 and Tween 80).





Conclusions and outlook

Hyaluronidase

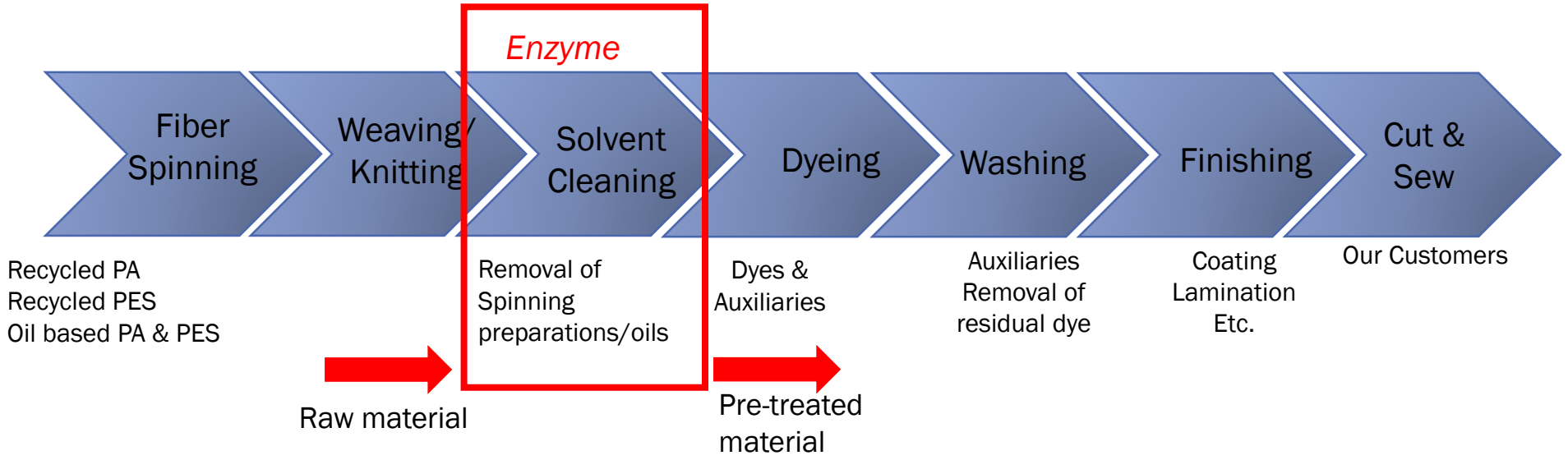
- Production feasible and relative easy in an industrial medium
- Other inducers?
- Enzyme concentrate to be delivered to CSIC

Laccase

- Efficient in wastewater decoloration tests
- Next steps?

Priority definition

Priority 1: Cleaning of synthetic materials (PES, PA and EL)



Problem:

- Removing of silicon/mineral oils
- Small fine dtex EL can't be solvent cleaned (they will be destroyed), and additives can't be fully removed by water/surfactant process
- Residual spinning oils will generate emissions during the drying and fixation steps
- These residual oils can have negative impact on the subsequent dyeing/finishing

Goal by the newly-developed enzymes:

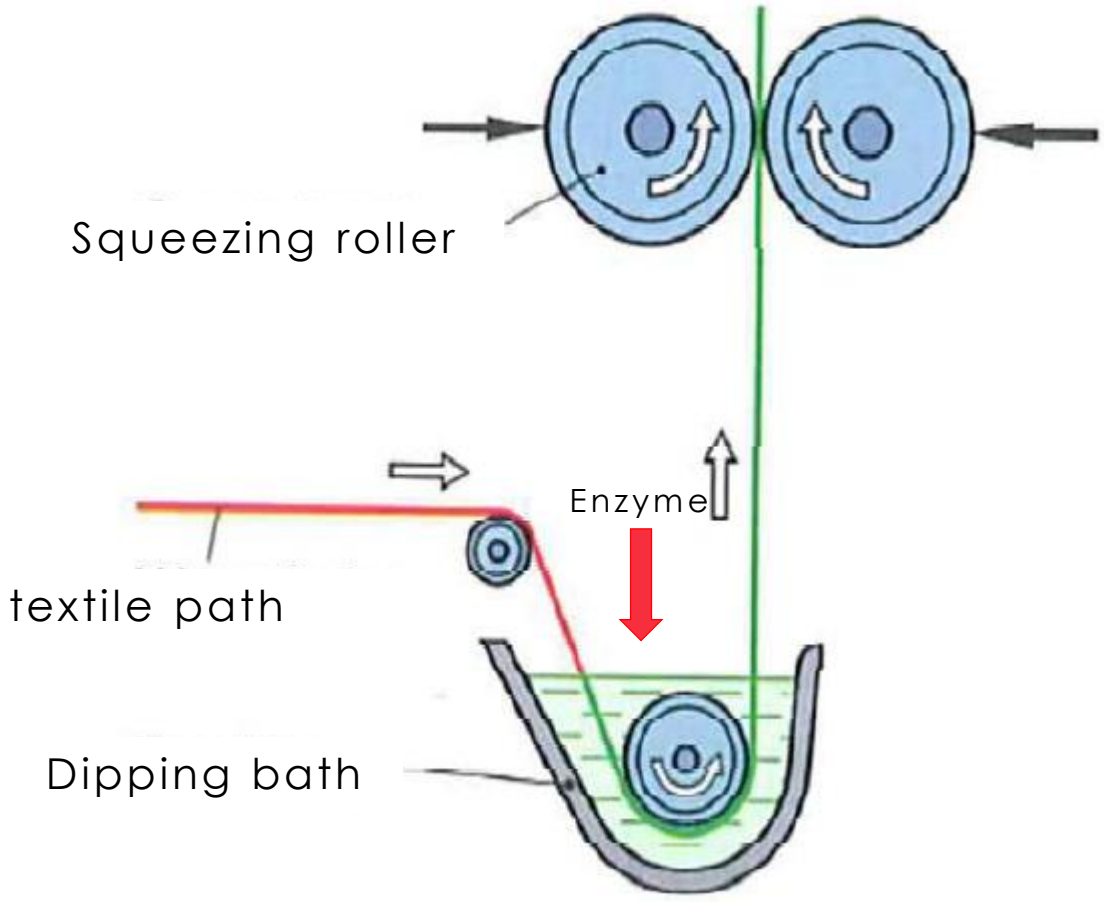
- Removal of the above-mentioned additives/preparation materials
- Water-based process, low temperature, fewer water discharge, fewer energy consumption
- Please refer to the qualitative/quantitative analysis already sent from Schoeller Textiles to all partners

Potential approach

Priority 1: Trying enzymes on a foulard for a very first step

Removed by enzyme desizing would be acrylic acid, ester, etc.
Future procedures:

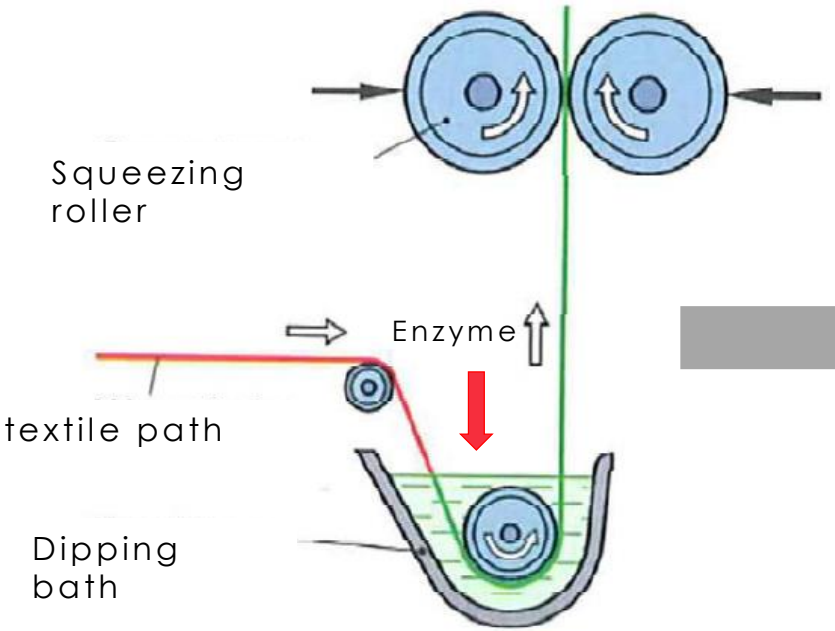
- Padding
- Wet storage about 24 hours
- Wash on Benninger
- Drying on a stenter



Possible approach: Feed the enzymes into the water jet machines during weaving, so you can pretreat the goods directly during weaving

Process steps

Priority 1: Trying enzymes on a foulard for a very first step



Foulard-Padding

Textile roll retention time <12 hours

Dying machine directly on jet or Benninger machine

Live example from current situation in the production u

Priority 1: Trying enzymes on a foulard for a very first step

Raw materials

Determination of substances soluble in petroleum ether

Sample	Determination of the particles on the surface	
	Qualitatively [%]	Quantitavely [%]
8X03G	4.2	Mineral oils, fatty acid ester, -amid, Silicon
8X04G	6.0	Mineral oils, Silicon, fatty acid ester, -amid
8X05G	2.8	Mineral oils, Silicon, small amounts of fatty acid ester
8X06G	2.6	Mineral oils, Silicon, small amounts of fatty acid ester
8X07G	2.9	Mineral oils, fatty acid ester, -amid, Silicon
8X08G	2.6	Mineral oils, Silicon, small amounts of fatty acid ester

Sizing material is PVA

Pre-treatment
LMR+fixation
BEN+fixation and one without fixation
BEN+fixation
BEN+fixation
BEN+fixation
BEN+fixation

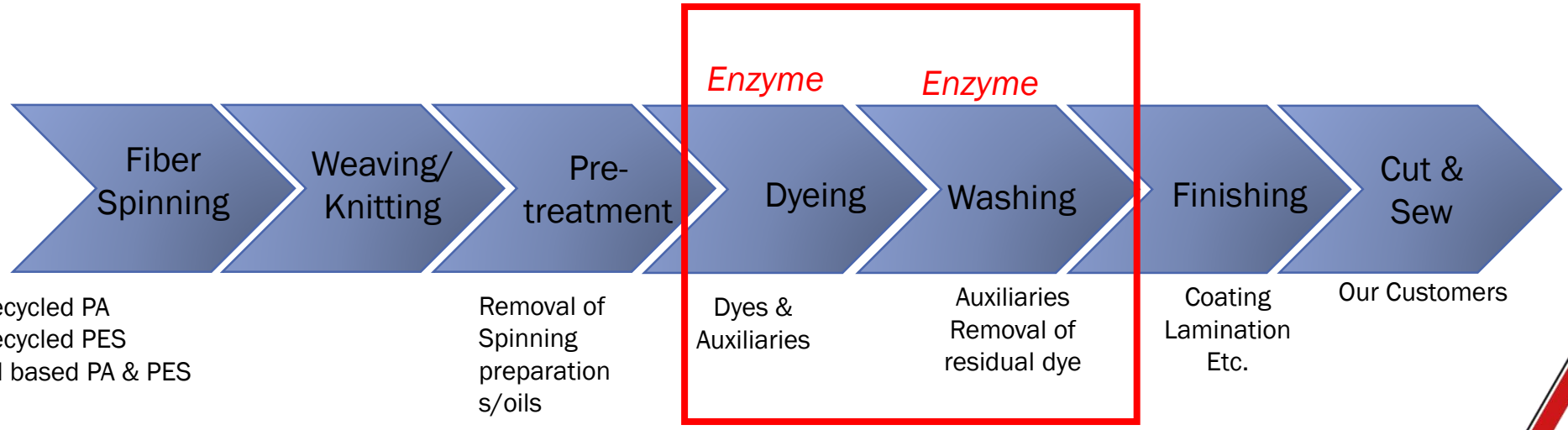
Pre-treated materials

A. substances soluble in petroleum ether-qualitative determination
B. Quantitative determination

Sample	Determination of the particles on the surface	
	Qualitatively [%]	Quantitavely [%]
8X03G	0.2	fatty acid ethoxylates, -amid, small amounts of Silicon
8X04G	5.4	Mineral oils, Silicon, fatty acid ester, -amid
8X05G	2.1	Mineral oils, silicon, fatty acid ester
8X06G	2.2	Mineral oils, silicon, -amid, fatty acid ester
8X07G	2.6	Mineral oils, fatty acid ester, Silicon
8X08G	1.6	Mineral oils, Silicon, fatty acid ester

Priority definition

Priority 2: Saving water, energy and reducing carbon footprint



Problem:

- Textile industry is one of the most polluting industries for water and energy consumption
- Extensive rinsing process during the dyeing/fixing of the textile materials
- Dyeing of the textile materials needs a lot of water
- Prior to dyeing procedure, removal of sizing products (Acrylic acids)

10% off global greenhouse gas emissions are caused by clothing and footwear production. Textile dyeing, and finishing is responsible for over 20% of global water pollution. Europeans use nearly 26 kilos of textiles and discard about 11 kilos of them every year.

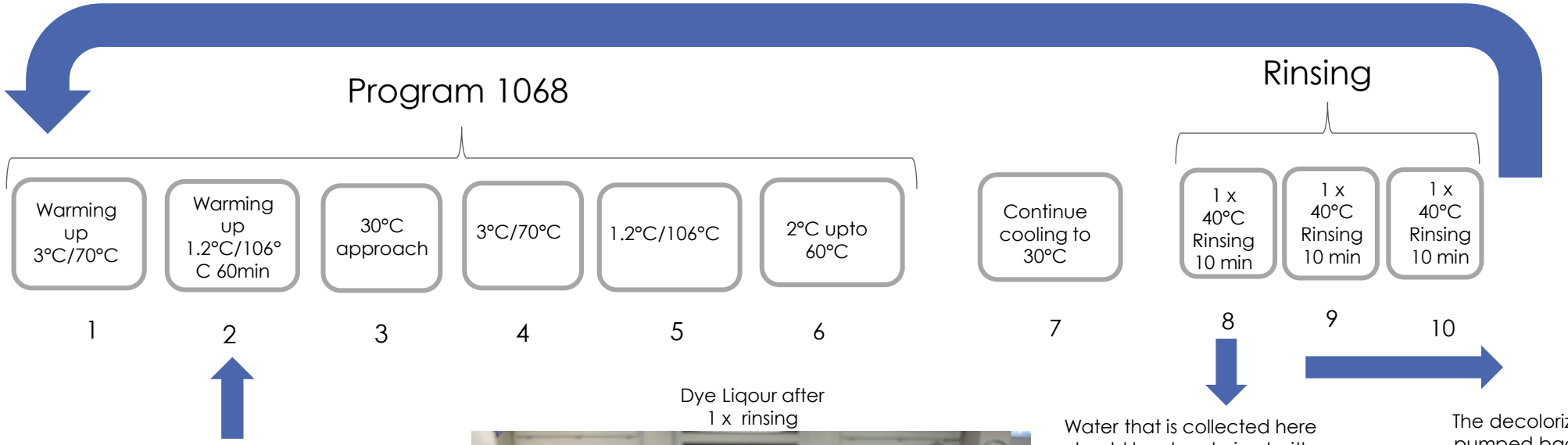
(Ambitious) Goal by the newly-developed enzymes :

- Reduce water/energy consumption and reduce the carbon footprint
  - Reducing the rinsing steps/duration
  - Optimize the dyeing process
- Discoloration and neutralization of the used processed water and circulate it in the system again

Potential approach

Priority 2: removing dye molecules from the dye bath

Discoloration and neutralisation of the water
to pump it back to the system



Adding chemicals (Periwet WDP New, Biavin BPA, Sarabid 14, Ammoniumsulfate 33% and dormic acid 85%)

Dye Liqour after 1 x rinsing

Water that is collected here should be decolorized with the help of enzymes

The decolorized water can be pumped back into the system for the next coloration

INOFEA
empowering enzymes

Dye Liqour

Dye Liquor before rewind

140l/lfm water consumption in the Schoeller production

Challenges with this potential approach

Priority 2: removing dye molecules from the dye bath

Problematic of this approach:

- Each fiber has ist own dye chemistry
- Different colors with complicated combinations
- We have different salts and polymers/leveling agents in the dye cocktail
- Even if one enzyme is applicable for one formulation, there is no promise that it can be widely applied to other ones

Dyeing department STX, Sevelen