# FuturEnzyme WP6 – Development and supply of best enzyme prototypes

FuturEnzyme WP6: Overview Start date: September 1<sup>st</sup>, 2022 (M16) - End date: May 31<sup>st</sup>, 2025 (M48)



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₽₹	T1.1 T1.2	Iechnical, administrative and financial management (1-48)   Confidentiality legal documents Inter-Consortia for establishing synergies (1-48)			
	T2.1	Compile the on-demand manufacturers' needs and specifications (1-6)			
4	T2.2	Pre-selecting candidate sequences thr ugh extensive homology search (1-48)			
3 (1	T2.3	Mc if buildup for massive and smart search of enzymes fitting manufacturers' needs (1-42)			
5	T2.4	Iterative and decision-making hierarch cal procedure for speed up enzyme discovery (3-48)			
	T3.1	Exploitation of the FuturEnzyme bio-resource collections (1-24)			
Νm	T3.2	Sampling extreme environments for generating new microbial bio-rescurces (6-30)			
	T3.3	Next Genera tion Sequencing for generating sequences of target enzy nes (1-36)			
	T4.1	Ultra-fast and efficient ger e cloning and synthesis: downstream enzyme production (2-30)			
	T4.2	Sampling extreme env conments for generating new microbial bio-resources (2) 30)			
4 ₹	T4.3	Product on of enzymes from their natural hosts (2-30)			
~	T4.4	Enzyme c aracterisation for selecting those with manufacturers' s lecifications (2-36)			
	T4.5	Decision-making strategy for selecting lead enzyn e candidates (6-36)			
11	T4.6	Design of multi-enzyme blen is to process complex ingredient mixtures (12-40)			
	T5.1	Disruptive engineering computational tools (3-42)			
2 S	T5.2	Developing disruptive PluriZymes vith multipurpose activities (6-42)			
	T5.3	Other advanced and classical mutation methods (4-42)			
1	T5.4	Empowering enzymes by immobilization-guided supramolecular engineering (6-40)			
	T6.1	Fermination and DSP of best 18 project enzymes (16-42)			
M 0	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)			
1	16.4	Safety, risk and environmental impact assessments of enzyme supply (20-48)	_		
	T7.1	Pre-industrial validations: formulation of real-life and solution-oriented detergents (20-48)			
2.	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)			
14	T7.4	LCA assessments: detergent, textile and cosmetic products (1-48)			
	T8.1	Dissemination and Communication (1-48)			
	T8.2	FuturEnzyme Website and strong on-line meeting platform (1-48)			
1.1	T8.3	Dissemination and Communication: toolkit and public relations (PR) (1-48)			
W 80	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)			
9 e	T9.1	Ethic requirements (1-48)			
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Work package number <sup>9</sup>	WP6	Lead beneficiary <sup>10</sup>	16 - EUCODIS
Work package title	Development	and supply of best enzyme pro	ototypes
Start month	16	End month	48

#### Objectives

As different downstream users will require different enzyme functions, WP6 aims with high priority to develop and scale up fermentation and DSP methods, formulation and quality assessments, of the WP5-best 18 engineered enzymes (2 x 9 enzyme classes; including artificial biomimetic proteases). After confirming their performance, they will be supplied to downstream application tests in WP7, which focuses on the implementation of real-life products integrating these enzymes or products derived from them. The amount of the enzymes to be generated, and thus the fermentation scale, will be fixed depending on the amount needed for the real-life tests needed in WP7. Although WP6 and WP7 will run independent from each other, feedback-loops will be established.

• In the first loop the application test partners will give feedback about the performance of all 18 pre-selected enzymes obtained at small/medium scale on the basis of which the 9 best enzymes (1 x 9 classes) will be up-scaled (≥200 L), and formulated so that they can be manufactured as verified enzymes, taking care of the formulation development (stability, liquid, dried, preparation of multi-enzyme blends, immobilization based on silica - an abundant and safe natural resource, etc.) and safety and risk assessments; and

• In the second, the application test partners will give feedback about the performance of 9 lead enzyme formulations, and WP6 partners will then supply improved formulations to be again transferred to WP7.

Finally, based on enzyme supply costs of best formulations to be integrated in best real-life products to be produced in WP7, the economic feasibility/sustainability of the enzyme supply process will be evaluated in the context of a circular economy.

### Description of work and role of partners

WP6 - Development and supply of best enzyme prototypes [Months: 16-48] EUCODIS, CSIC, IST ID, ITB, CLIB, INOFEA AG, Bio\_Ch

Task 6.1 Fermentation and DSP of best 18 project enzymes M16-M42 Lead partner – BIO\_CH Participants: CSIC, IST-ID, EUC, INOFEA

Task 6.2 Upscaling (non-GMP) fermentation and DSP of 9 best enzymes M20-M48 Lead partner – EUC Participants: CSIC, BIO\_CH, INOFEA

Task 6.3 Development of optimised formulations of 9 lead enzyme candidates M20-M48 Lead partner – EUC Participants: CSIC, INOFEA

Task 6.4 Safety, risk and environmental impact assessments of enzyme supply M20-M48 Lead partner – EUC Participants: CSIC, CLIB, INOFEA, ITB

Participation per Partner			
Partner number and short name	WP6 effort		
1 - CSIC	6.00		
6 - IST ID	23.00		
8 - ITB	3.00		
10 - CLIB	3.00		
11 - INOFEA AG	20.00		
12 - Bio_Ch	15.90		
16 - EUCODIS	27.00		
Total	97.90		

### List of deliverables

Deliverable Number <sup>14</sup>	Deliverable Title	Lead beneficiary	Type <sup>15</sup>	Dissemination level <sup>16</sup>	Due Date (in months) <sup>17</sup>
D6.1	Best 18 Pre-Lead Enzyme Materials obtained at gram scale for real-life tests	12 - Bio_Ch	Other	Confidential, only for members of the consortium (including the Commission Services)	32
D6.2	Report on fermentation, DSP and activity verification for 18 Pre- Lead enzymes	6 - IST ID	Report	Confidential, only for members of the consortium (including the Commission Services)	32
D6.3	Best 9 Lead Enzyme Materials obtained at multi-gram/kg scale for real-life tests	16 - EUCODIS	Other	Confidential, only for members of the consortium (including the Commission Services)	36
D6.4	Report on fermentation, optimization and verification for 9 Lead Enzyme Materials	16 - EUCODIS	Report	Confidential, only for members of the consortium (including the Commission Services)	46
D6.5	Safety, risk, and environmental evaluation sheet for 9 enzymes with pre-market value, available	16 - EUCODIS	Report	Confidential, only for members of the consortium (including the Commission Services)	48

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Schedule of relevant Milestones

Milestone number <sup>18</sup>	Milestone title	Lead beneficiary	Due Date (in months)	Means of verification	Associação do Insti para a Investigação	
MS19	First set of best enzymes at gram scale	12 - Bio_Ch	20	Report available – this milestone will attest the realisation of the first production baches at gram scale for best enzymes to be used for tests prior to pre- industrial validations.	CLI	
MS20	First set of lead enzymes at multi-gram scale	16 - EUCODIS	22	Report available – this milestone will attest the realisation of the production baches at gram scale for lead enzymes to be used for pre- industrial validations.	Conse Italbio	
MS21	LCA report for supplying the first set of enzymes	16 - EUCODIS	32	Report available - this milestone will attest the completion of the LCA for the production of first set of enzymes produced at large.		





# WP6: Development and supply of best enzyme prototypes

We need to define:

- How do we receive lead candidates from WP4 and WP5 partners?
  - Examples:
    - Sequence information
    - Orgin information
    - Starting fermentation conditions
    - Available activity tests (for QC)





- What are the targets for each candidate handed over for strain and process development?
  - Examples:
    - Yield (e.g. amount, activity required for commercial viability)
    - Preferred expression system (bacterial, yeast, fungal, GRAS status etc.)
    - Quality parameters (e.g. purity, activity, stability, food-grade, endotoxins, heavy metals, microbiology, TAMC, TYMC, GMO-free/DNA-free, etc.)
    - Preferred formulation (liquid, freeze-dried, spray-dried, immobilized (and if so: requirements for particles?))

Idea: Implementation of a "Lead Candidate Process" (possibly online?)

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## WP6: Development and supply of best enzyme prototypes

Idea: Implementation of "Lead Candidate Process" (possibly online?)

For example:

- 1. Step: WP4/WP5 partners enter information about candidate online
- 2. Step: A notification is generated to WP7 industrial partners to select candidate, add e.g. amount, quality etc. required for further application tests
- 3. Step: A notification is generated for WP6 partners
- 4. Step: WP6 partners decide after discussion, who will produce the enzyme
- 5. Step: Production
- 6. Step: Delivery note of the lead candidate sample to the WP7 partner

Benefits: no questions "what do I have to do now...?, whole process is well-documented, important information collected in database for later (not only buried in long reports or spread by mouth...)





FuturEnzyme Technologies of the FUTURe for low-cost ENZYMEs for environment-friendly products

FUTURE ENZYME

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