

EnzOx2 (Enzymatic Oxidations/Oxyfunctionalizations) Project on “New enzymatic oxidation/oxyfunctionalization technologies for added value bio-based products”

Call: H2020-BBI-PPP-2015-2-1-720297

Topic: BBI.R10-2015 (“Innovative efficient biorefinery technologies”)

Budget (EC Contribution): 5 M€ (3 M€)

Duration: 3 years (GA under negotiation)

*INFO-DAY de la JTI de Bioindustrias - Plan Anual de
Trabajo 2016 (AWP 2016) CDTI, Madrid, 5 Mayo 2016*

Biotechnology for Lignocellulosic Biomass


www.cib.csic.es

CIB group of **Biotechnology** for Lignocellulosic **Biomass**

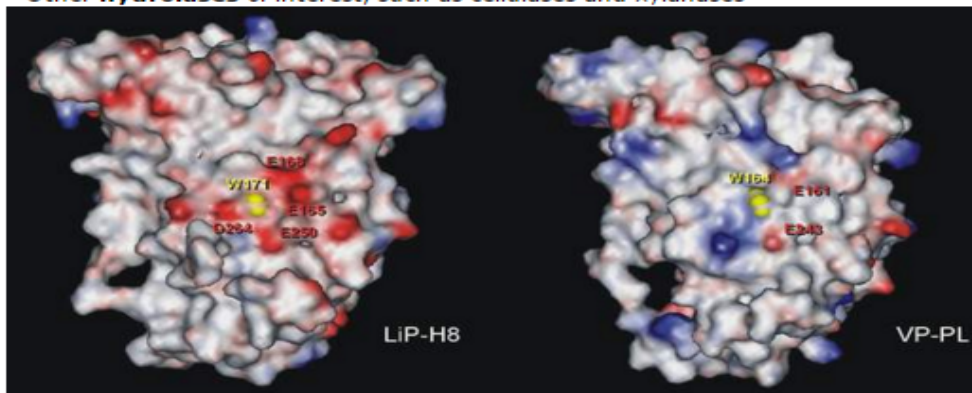
Home: **Departments:** **Environmental Biology:** Research groups: **Biotechnology for Lignocellulosic Biomass**

Our scientific **objectives** are related to the use of **microorganisms** (mainly filamentous fungi) and their enzymes in **industrial** processes to obtain fuels, materials and chemicals (White Biotechnology) from **renewable** plant resources. The final aim is to contribute to the sustainable development of our society and reduce the biosphere warming by a reduced consumption of fossil resources. These objectives are well in agreement with those of the new CIB Department of "Environmental Biology".

The work of the group has provided important contributions to the knowledge of the **enzymatic system** involved in the **degradation of lignin** (and other recalcitrant compounds) by **fungi**, which represents a key step for C recycling in land ecosystems and a central issue in the industrial use of plant biomass in agreement with the **Biorefinery concept** (for the integrated production of fuels, chemicals and other products). According to these results, the most recent studies combine basic and more applied aspects (this dual approach in the field of the enzymatic degradation of lignin has been discussed in a review of the group published in *Curr Opin Biotechnol*, see [Martínez et al. 2008](#)):

i) Basic projects on structure-function of key enzymes involved in lignocellulose biodegradation (to **improve** their catalytic properties) including:

- Ligninolytic hemeperoxidases, like **versatile peroxidase** (VP)
- Flavooxidases providing peroxide, like **aryl-alcohol oxidase** (AAO)
- Multicopper oxidases like **laccases** (and their redox mediators)
- **Esterases** with different substrate specificities
- Other **hydrolases** of interest, such as cellulases and xylanases



Lignin oxidation (by LiP or VP) is produced by electron transfer from a surface Trp

ii) More applied projects related to the use of **fungi** and their **enzymes**, **new** or improved by **rational design** or **directed evolution**, in industrial or environmental applications such as those related to the future **lignocellulose biorefineries** for:

- Production of second generation **bioethanol**
- Sustainable production of **materials** and **chemicals**

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Heads of laboratory:

[Martínez Ferrer, Ángel Tomás](#)
[Martínez Hernández, María Jesús](#)

Staff Scientists

[Camarero Fernández, Susana](#)
[Martínez Ferrer, Ángel Tomás](#)
[Martínez Hernández, María Jesús](#)
[Prieto Orzanco, Alicia María](#)

Junior PI

[Ruiz Dueñas, Francisco Javier](#)

Technical Staff

[De Eugenio Martínez, Laura Isabel](#)

Postdoctorals

[Linde López, María Dolores](#)
[Pérez Boada, Marta](#)
[Serrano Esteban, Ana](#)

Technician

[Rodríguez Ventura, Francisco](#)

Postgraduates

[Ayuso Fernández, Iván](#)
[Barriuso Maicas, Jorge](#)
[Carro Aramburu, Juan Rogelio](#)
[De Salas De la Cuadra, Felipe](#)
[Mendez Liter, Juan Antonio](#)

EU projects coordinated by the CIB Lignocellulose group

✓ **“Optimized oxidoreductases for medium and large-scale industrial bio transformations”** (INDOX; 2013-16; 16 partners with 8 companies, including **Anaxomics**, **Biopolis**, **Novozymes**, etc)

FP7 (KBBE)

✓ **“Novel and more robust peroxidases as fungal biocatalysts”** (PEROXICATS; 2011-13; 6 partners including 2 companies, **Novozymes** and **BIOS**; **CEPSA** collaboration)

FP7 (KBBE)

✓ **“White Biotechnology for added value products from renewable plant polymers: Design of tailor-made biocatalysts and new industrial bioprocesses”** (BIORENEW; 2006-10; 26 partners with 12 companies including **ENCE**, **Celesa**, **Novozymes**, etc)

FP6 (NMP)

✓ **“Fungal metalloenzymes oxidizing aromatic compounds of industrial interest”** (PELAS; 2000-04; 9 partners including 2 companies, **Beldem** and **Novozymes**)

✓ **“New environmentally-sound methods for pitch control in different paper pulp manufacturing processes”** (PITCH; 2000-05; 11 partners including 3 companies, **ENCE**, **UPM** and **Novozymes**)

FP5 (Life)

✓ **“Wood extractives in pulp and paper manufacture: Technical and environmental implications and biological removal”** (WEB; 1995-99; 7 partners including 2 companies, **ENCE** and **Parenco**)

FP4 (FAIR)

✓ **“Biological delignification in paper manufacture: Optimization of enzyme mixtures for treating cereal straw and other non-woody materials”** (1994-97; 8 partners including 1 company, **Saica**)

FP3 (AIR)



“Engineering improved activity and stability of oxidative enzymes to meet industrial application requirements.”

The **indox** Project is a collaborative research initiative funded by the European Commission 7th Framework Programme (FP7) that gathers together the expertise of sixteen participants from seven EU countries plus one partner from an EU candidate country. [MORE](#)

Last publications

[VIEW ALL](#)

Fernandez-Fueyo E, Ruiz-Dueñas FJ, López-Lucendo MF, Pérez-Boada M, Rencoret J, Gutiérrez A, Pisabarro AG, Ramírez L, Martínez AT (2016) A secretomic view of woody and nonwoody lignocellulose degradation by *Pleurotus ostreatus* *Biotechnology for Biofuels*, 9: 49

Background

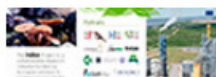
Pleurotus ostreatus is the second edible mushroom worldwide, and a model fungus for delignification applications, with (...)

[MORE](#)

Acebes S, Fernandez-Fueyo E, Monza E, Lucas F, Almendral D, Ruiz-Dueñas FJ, Lund



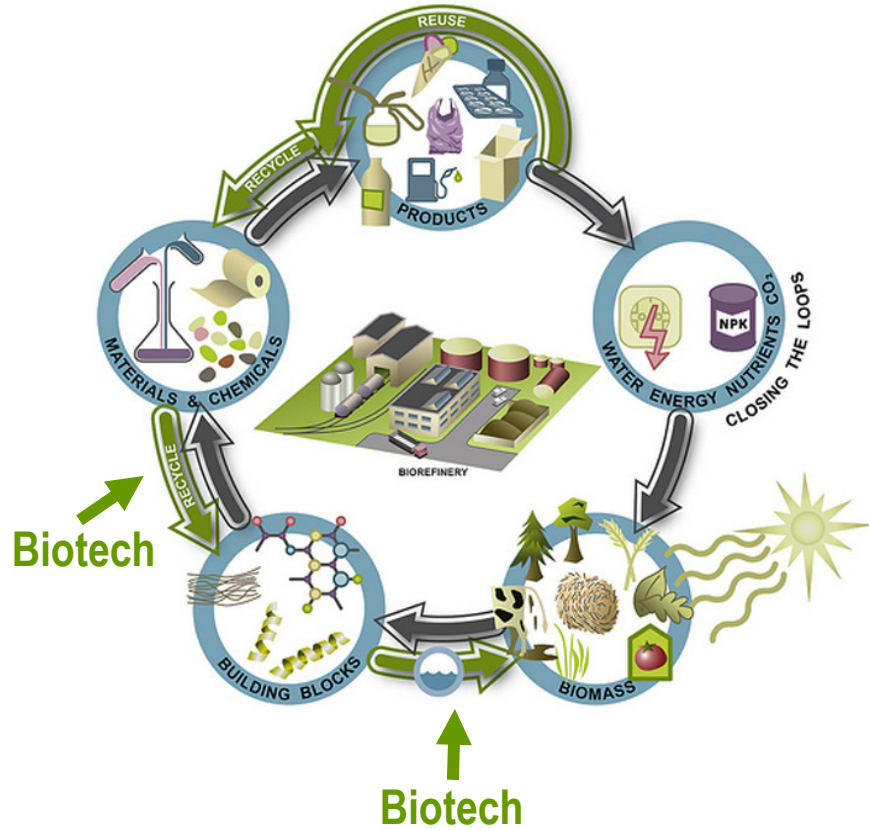
Click on the map locations or in logos to access information on the partners



Download our brochure about the project in pdf

CDTI, 5-May-16

CSIC is an Associated RTO in the BioBased Industries Consortium



Topic: “Innovative efficient biorefinery technologies” (BBI.R10-2015)

<http://ec.europa.eu/research/participants/portal/desktop/en/opportunities/h2020/topics/1150-bbi.r10-2015.html>

SPECIFIC CHALLENGE: The establishment of **competitive** integrated **biorefineries** that process biomass in a cascading approach, and that are able to continuously operate year-round is one of the key goals of the whole BBI Initiative

This requires not only the establishment of new business and cooperation models (e.g. for primary and secondary economic sector cooperation) but, very importantly, **research and development** investments in:

- (1) **improving technologies** pioneered by existing biorefineries in order to become cost-competitive with respect to fossil counterparts
- (2) developing a **new generation** of **breakthrough technologies** to form the basis for tomorrow’s biorefineries
- (3) the enabling the combination and processing of **different** kinds of input **biomass** leading to the establishment of larger biorefineries, benefiting from scale economy

At present, most existing biorefineries are designed to process only one kind of feedstock (or at most a family of similar feedstock) with constant properties throughout the year

As a result, significant value chains are not exploited or under-exploited because of seasonal variation or unavailability of adequate quantities of sufficiently uniform feedstock at any given time of the year

SCOPE: Proposals should develop innovative **biorefinery technologies** enabling the establishment of new efficient year-round operation biorefinery concepts and bio-based value chains through the conversion of different types of biomass feedstocks, including underutilised seasonal feedstocks

Proposals should aim to significantly increase the **efficiency, yield** and **cost-effectiveness** of technologies enabling the production of cost-competitive bio-based products in a cascading approach. Technologies might include:

- ✓ **Fractionation** and separation technologies to simplify the disintegration of biomass into its basic components
- ✓ Advanced technologies to **mildly extract** or separate components while preserving their functionalities and minimising the degradation of other components to enable their further valorisation
- ✓ **Bio-chemical** or **chemo-catalytic** technologies to convert bio-based components into **high added value products**

Proposals should fit in a cascading concept aimed at the integral use of the feedstock

Proposals should take into account feedstock availability and flexibility throughout the year, with the goal of producing bio-products (**chemicals, materials**) that can be **cost-competitive** with **fossil** counterparts and/or feature new or improved properties

A **life cycle** oriented approach should be carried out to evaluate the environmental and socio-economic performances of the new concepts in comparison with their current alternatives

Strong weight will be put on **industrial** leadership to fully exploit the developed products/processes.

*It is considered that proposals with a **total** eligible budget in the range of **EUR 2-5 million** would allow this specific challenge to be addressed appropriately (nonetheless, this does not preclude submission and selection of proposals with another budget)*

EXPECTED IMPACT:

A **20 % increase** of conversion efficiency (product/ton of biomass in input) over state of the art comparable technologies.

Integrated processes leading to products with **significant higher value** than the current application of the biomass.

A **20 % improvement in resource efficiency** (consumption of energy and water) over state of the art comparable technologies.

Year round operation due to smart combination, pre-treatment and storage of feedstock.

Achieving technological validation of **one or more** of the following products:

- ✓ **new building blocks** based on biomass of European origin functionalised chemicals and materials with lower environmental footprint and societal benefits valorisation of proteins from plant residues
- ✓ **bio-based materials** (e.g. such as specialty fibres, plastics, composites and packaging solutions)
- ✓ new 'consumer' products based on **bio-based chemicals** and materials
- ✓ new biodegradable, compostable or recyclable bio based products and materials for short life application.

EnzOx2 Partners: 4 RES, 2 HE, 4 SME, 2 IND

- 1a. **CIB** (Centro Investigaciones Biológicas, **CSIC, ES**): Angel T Martínez (**Coordinator**)
- 1b. **IRNAS** (Inst. Recursos Naturales y Agrobiología, **CSIC, ES**): Ana Gutiérrez
- 1c. **ICP** (Inst. Catálisis y Petroleoquímica, **CSIC, ES**): Miguel Alcalde
2. **TUD** (Technische Universität Dresden, **DE**): Martin Hofrichter
3. **BIOS** (JenaBios Biotechnologien Serviceleistungen GmbH, **DE**): Katrin Scheibner
4. **FIR** (Firmenich SA, **CH**): Andreas Taglieber
5. **AVA** (Ava-Biochem, **CH**): Gilbert Anderer
6. **NZ** (Novozymes A/S, **DK**): Henrik Lund (**Industrial Manager**)
7. **TU Delft** (Technische Universiteit Delft, **NL**): Frank Hollmann
8. **Chiracon** (Chiracon GmbH, **DE**): Ralph Zuhse
9. **CLEA** (CLEA Technologies B.V., **NL**): Jo-Anne Rasmussen
10. **AIMPLAS** (AIMPLAS Plastic Technology Center, **ES**): Amador García Sancho

The EnzOx2 Concept:

- ✓ Sustainable production of chemical **building blocks** and other **added value products** from **plant biomass** is required for a bio-based economy. Moreover, biomass biorefineries should benefit also from **greener** and **more efficient** bio-chemical (enzymatic) technologies.
- ✓ Unexplored **oxidation/oxyfunctionalization** reactions (of sugar and lipid compounds) catalyzed by **microbial oxidoreductases** will be optimized in **EnzOx2** to achieve 100% biochemical conversion of:
 - **5-hydroxymethylfurfural (HMF)** into **diformylfuran**, a platform chemical, and **2,5-furandicarboxylic acid (FDCA)**, a plastic building-block

5-hydroxymethylfurfural conversion by fungal aryl-alcohol oxidase and unspecific peroxygenase

Juan Carro¹, Patricia Ferreira², Leonor Rodríguez¹, Alicia Prieto¹, Ana Serrano¹, Beatriz Balcells¹, Ana Ardá¹, Jesús Jiménez-Barbero¹, Ana Gutiérrez³, René Ullrich⁴, Martin Hofrichter⁴ and Angel T. Martínez¹

1 Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid, Spain

2 Facultad de Ciencias and Instituto de Biocomputación y Física de Sistemas Complejos, Zaragoza, Spain

3 Instituto de Recursos Naturales y Agrobiología de Sevilla, Consejo Superior de Investigaciones Científicas, Seville, Spain

4 Department of Bio- and Environmental Sciences, International Institute of Zittau, Germany

Keywords

2,5-formylfurancarboxylic acid;
2,5-furandicarboxylic acid; 5-hydroxymethylfurfural; aryl-alcohol oxidase; unspecific peroxygenase

Correspondence

A. T. Martínez, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Ramiro de Maeztu 9, E-28040 Madrid, Spain
Fax: +34 915360432
Tel: +34 918373112 (extension 4407)
E-mail: ATMartinez@cib.csic.es
Website: www.cib.csic.es/lignina/lignina_en.html

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Oxidative conversion of 5-hydroxymethylfurfural (HMF) is of biotechnological interest for the production of renewable (lignocellulose-based) platform chemicals, such as 2,5-furandicarboxylic acid (FDCA). To the best of our knowledge, the ability of fungal aryl-alcohol oxidase (AAO) to oxidize HMF is reported here for the first time, resulting in almost complete conversion into 2,5-formylfurancarboxylic acid (FFCA) in a few hours. The reaction starts with alcohol oxidation, yielding 2,5-diformylfuran (DFF), which is rapidly converted into FFCA by carbonyl oxidation, most probably without leaving the enzyme active site. This agrees with the similar catalytic efficiencies of the enzyme with respect to oxidation of HMF and DFF, and its very low activity on 2,5-hydroxymethylfurancarboxylic acid (which was not detected by GC-MS). However, AAO was found to be unable to directly oxidize the carbonyl group in FFCA, and only modest amounts of FDCA are formed from HMF (most probably by chemical oxidation of FFCA by the H₂O₂ previously generated by AAO). As aldehyde oxidation by AAO proceeds via the corresponding *geminal* diols (aldehyde hydrates), the various carbonyl oxidation rates may be related to the low degree of hydration of FFCA compared with DFF. The conversion of HMF was completed by introducing a fungal unspecific heme peroxygenase that uses the H₂O₂ generated by AAO to transform FFCA into FDCA, albeit more slowly than the previous AAO reactions. By adding this peroxygenase when FFCA production by AAO has been completed, transformation of HMF into FDCA may be achieved in a reaction cascade in which O₂ is the only co-substrate required, and water is the only by-product formed.

The EnzOx2 Concept:

- ✓ Sustainable production of chemical **building blocks** and other **added value products** from **plant biomass** is required for a bio-based economy. Moreover, biomass biorefineries should benefit also from **greener** and **more efficient** bio-chemical (enzymatic) technologies.
- ✓ Unexplored **oxidation/oxygenation** reactions (of sugar and lipid compounds) catalyzed by **microbial oxidoreductases** will be optimized in **EnzOx2** to achieve 100% biochemical conversion of:
 - **5-hydroxymethylfurfural (HMF)** into **diformylfuran**, a platform chemical, and **2,5-furandicarboxylic acid (FDCA)**, a plastic building-block
 - highly (regio/stereo) selective **hydroxylation of plant lipids** (such as fatty acids, terpenes and steroids) for cost-effective production of **flavours and fragrances, APIs** and others.



Contents lists available at SciVerse ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Epoxidation of linear, branched and cyclic alkenes catalyzed by unspecific peroxygenase



Sebastian Peter*, Matthias Kinne, René Ullrich, Gernot Kayser, Martin Hofrichter

Department of Bio- and Environmental Sciences, International Graduate School of Zittau, Markt 23, 02763 Zittau, Germany

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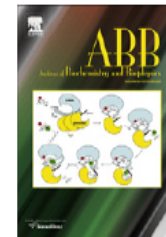
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Keywords:

Biocatalysis
Heme-thiolate
Oxygenation
Peroxygenase
Stereoselective

ABSTRACT

Unspecific peroxygenases (EC 1.11.2.1) represent a group of secreted heme-thiolate proteins that are capable of catalyzing the mono-oxygenation of diverse organic compounds, using only H_2O_2 as a co-substrate. Here we show that the peroxygenase secreted by the fungus *Agrocybe aegerita* catalyzed the oxidation of 20 different alkenes. Five branched alkenes, among them 2,3-dimethyl-2-butene and *cis*-2-butene, as well as propene and butadiene were epoxidized with complete regioselectivity. Longer linear alkenes with a terminal double bond (e.g. 1-octene) and cyclic alkenes (e.g. cyclohexene) were converted into the corresponding epoxides and allylic hydroxylation products; oxidation of the cyclic monoterpene limonene yielded three oxygenation products (two epoxides and an alcohol). In the case of 1-alkenes, the conversion occurred with moderate stereoselectivity, in which the preponderance for the (*S*)-enantiomer reached up to 72% *ee* for the epoxide product. The apparent Michaelis–Menten constant (K_m) for the epoxidation of the model substrate 2-methyl-2-butene was 5 mM, the turnover number (k_{cat}) $1.3 \times 10^3 \text{ s}^{-1}$ and the calculated catalytic efficiency, k_{cat}/K_m , was $2.5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. As epoxides represent chemical building blocks of high relevance, new enzymatic epoxidation pathways are of interest to complement existing chemical and biotechnological approaches. Stable and versatile peroxygenases as that of *A. aegerita* may form a promising biocatalytic platform for the development of such enzyme-based syntheses.



Regioselective oxygenation of fatty acids, fatty alcohols and other aliphatic compounds by a basidiomycete heme-thiolate peroxidase

Ana Gutiérrez^{a,*}, Esteban D. Babot^a, René Ullrich^b, Martin Hofrichter^b,
Angel T. Martínez^c, José C. del Río^a

^aInstituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, P.O. Box 1052, E-41080 Sevilla, Spain

^bUnit of Environmental Biotechnology, International Graduate School of Zittau, Markt 23, 02763 Zittau, Germany

^cCentro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, E-28040 Madrid, Spain

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Fatty acids
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Alkanes
Steroids

ABSTRACT

Reaction of fatty acids, fatty alcohols, alkanes, sterols, sterol esters and triglycerides with the so-called aromatic peroxygenase from *Agrocybe aegerita* was investigated using GC-MS. Regioselective hydroxylation of C₁₂-C₂₀ saturated/unsaturated fatty acids was observed at the ω-1 and ω-2 positions (except myristoleic acid only forming the ω-2 derivative). Minor hydroxylation at ω and ω-3 to ω-5 positions was also observed. Further oxidized products were detected, including keto, dihydroxylated, keto-hydroxy and dicarboxylic fatty acids. Fatty alcohols also yielded hydroxy or keto derivatives of the corresponding fatty acid. Finally, alkanes gave, in addition to alcohols at positions 2 or 3, dihydroxylated derivatives at both sides of the molecule; and sterols showed side-chain hydroxylation. No derivatives were found for fatty acids esterified with sterols or forming triglycerides, but methyl esters were ω-1 or ω-2 hydroxylated. Reactions using H₂¹⁸O₂ established that peroxide is the source of the oxygen introduced in aliphatic hydroxylations. These studies also indicated that oxidation of alcohols to carbonyl and carboxyl groups is produced by successive hydroxylations combined with one dehydration step. We conclude that the *A. aegerita* peroxygenase not only oxidizes aromatic compounds but also catalyzes the stepwise oxidation of aliphatic compounds by hydrogen peroxide, with different hydroxylated intermediates.

Expected impacts from **EnzOx2**:

- ✓ **On the BBI JU objectives:** **EnzOx2** will provide a competitive answer to the “specific challenge” of the BBI-JU-2015-R10 topic by providing a new technology being able to:
 - i) Improve existing biorefinery technologies “*to become cost competitive with respect to fossil counterparts*” by introducing more efficient and potentially green processes based on **oxidative biocatalysts**
 - ii) Develop “*a new generation of breakthrough technologies*” (**biocatalytic toolbox**) enabling production of new bio-based chemicals and building blocks, which are not accessible for the currently-available technologies, “*to form the basis for tomorrow’s biorefineries*”.

- ✓ **On the EU bio-based chemical sector:** The **EnzOx2** choice of oxidation reactions will enable to obtain important transformations for the bio-based industries that can be very difficult to manage with traditional synthesis tools, e.g.:
 - Production of sugar-derived building blocks
 - Production of lipid-derived added value chemicals

- ✓ **On the EU Biotechnology sector:** The project and the joint development of new oxidoreductases will have a strong impact on this sector due to the presence in the consortium of Novozymes, the world-leader company in enzyme production, together with other biotech SMEs.

Proposal Evaluation Form



EUROPEAN COMMISSION

Horizon 2020 - Research and Innovation Framework Programme

Evaluation Summary Report - Research and innovation actions/Innovation actions

Call: H2020-BBI-PPP-2015-2-1
Funding scheme: BBI-RIA
Proposal number: 720297
Proposal acronym: EnzOx2
Duration (months): 36
Proposal title: New enzymatic oxidation/oxyfunctionalization technologies for added value bio-based products
Activity: RANKING_BBI-2015-R10

N.	Proposer name	Country	Total Cost	%	Grant Requested	%
1	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	ES	1,814,000	36.28%	1,306,000	43.53%
2	TECHNISCHE UNIVERSITAET DRESDEN	DE	600,000	12.00%	450,000	15.00%
3	JENABIOS GMBH: UMWELTBIOTECHNOLOGIEN & SERVICELEISTUNGEN	DE	325,000	6.50%	244,000	8.13%
4	FIRMENICH SA	CH	118,750	2.38%	0	0.00%
5	AVA BIOCHEM BSL AG	CH	609,000	12.18%	0	0.00%
6	NOVOZYMES A/S	DK	200,000	4.00%	0	0.00%
7	TECHNISCHE UNIVERSITEIT DELFT	NL	533,000	10.66%	400,000	13.33%
8	CHIRACON GMBH	DE	333,000	6.66%	250,000	8.33%
9	CLEA TECHNOLOGIES BV	NL	267,250	5.34%	200,000	6.67%
10	AIMPLAS - ASOCIACION DE INVESTIGACION DE MATERIALES PLASTICOS Y CONEXAS	ES	200,000	4.00%	150,000	5.00%
Total:			5,000,000		3,000,000	

Abstract:

Sustainable production of chemical building blocks and other added value products from plant biomass is required for a bio-based economy. However, the biomass biorefineries should benefit not only from the use of renewable feedstocks but also from greener and more efficient bio-chemical technologies. Previous projects have shown the potential of oxidative enzymes in the production of some added value compounds from biomass components. Of special interest are still unexplored oxidation/oxyfunctionalization reactions (of sugar and lipid compounds) by microbial oxidoreductases, including new (self-sufficient) heme-thiolate peroxygenases. In this context, EnzOx2 plans to develop a 100% biochemical conversion of bio-based 5-hydroxymethylfurfural (HMF) into diformylfuran, a platform chemical, and 2,5-furandicarboxylic acid (FDCA), a plastic building-block. Oxidases (flavo and copper/radical) and peroxygenases will be used to perform the three-step oxidation of HMF to FDCA in a co-substrate and side-product free, one-pot conversion. On the other hand, highly (regio/stereo) selective hydroxylation of plant lipids (such as fatty acids, terpenes and steroids) by peroxygenases will be optimized for cost-effective production of flavours and fragrances (F&F), active pharmaceutical ingredients (APIs) and others. ENZOX2 aims to solve some main bottlenecks in these industrial processes by the use of biochemical tools (new/engineered enzymes and optimized biotransformations), to be later validated at the pilot/flagship scale. To attain this objective the consortium includes: i) two world leaders in industrial enzymes (Novozymes) and F&F (Firmenich); ii) two chemical SMEs producing HMF and chiral APIs (AVA-Biochem and Chiracon); iii) two specialized biotechnology SMEs (JenaBios and CLEA); iv) one technology centre in the Plastics sector (AIMPLAS); and v) three CSIC institutes and two universities (Dresden and Delft) with expertise in enzyme reactions and bioprocess implementation.

Criterion 1 - Excellence

Score: **5.00** (Threshold: 3/5.00 , Weight: -)

The following aspects will be taken into account, to the extent that the proposed work corresponds to the topic description in the work programme:

Clarity and pertinence of the objectives

Credibility of the proposed approach

Soundness of the concept including trans-disciplinary considerations, where relevant

Extent that proposed work is ambitious, has innovation potential, and is beyond the state of the art (e.g. ground-breaking objectives, novel concepts and approaches)

The objectives are very clear and pertinent to the call topic dealing with selection and optimisation of enzyme biocatalysts to overcome bottlenecks in the production of biochemicals from biomass feedstocks.

The proposed approach is technically detailed and demonstrates the credibility of the proposal. One of the targeted products, hydroxymethyl furfural (HMF), is a valuable platform chemical. This is very good.

The concept is very sound.

The work is ambitious. The state of the art is well reviewed and correctly established. The proposal aims to go beyond the state of the art insofar that it seeks to develop routes to molecules not efficiently attainable through current catalytic toolboxes. The expected innovations are relevant and important for those processes to become more resource and cost efficient and thus crucial to become successful.

Criterion 2 - Impact

Score: **4.50** (Threshold: 4/5.00 , Weight: -)

The following aspects will be taken into account:

The expected impacts listed in the BBI-JU annual Work Plan under the relevant topic

Enhancing innovation capacity and integration of new knowledge

Strengthening the competitiveness and growth of companies by developing innovations meeting the needs of European and global markets and, where relevant, by delivering such innovations to the market

Any other environmental and socially important impacts (not already covered above)

Effectiveness of the proposed measures to exploit and disseminate the project results (including IPR management), to communicate the project and to manage research data, where relevant

Extent to which the proposed consortium contribution will help maximising the impact of the action

The proposal aims to go beyond the majority of the expected impacts, this is credible and realistic. However, impact on the year-round operation is not adequately addressed. This is a shortcoming.

The capacity to innovate will be increased through the development of new enzyme technology.

The proposal will improve the competitiveness of the companies through the delivery of more efficient technologies that enable the production of attractive chemicals.

The environmental impact is adequately addressed by a life cycle analysis. A positive aspect is the comparison of the environmental performance of the chemicals developed using the proposed technologies with the state of the art.

The IPR management strategy is effective. The proposal builds on existing knowhow and patents, and strengthens further the IP position of leading or promising European universities, research institutes and small and large companies involved. This is positive.

The proposed exploitation of results is effective. The dissemination and communication measures are appropriate.

The in-kind contributions from industry reflect the commitment to the project.

Criterion 3 - Quality and efficiency of the implementation

Score: **4.00** (Threshold: 3/5.00 , Weight: -)

The following aspects will be taken into account:

Coherence and effectiveness of the work plan, including appropriateness of the allocation of tasks and resources

Complementarity of the participants within the consortium

Appropriateness of the management structures and procedures, including risk and innovation management

The work plan is very well organized and detailed.

Task and resource allocation is appropriate.

The expertise required to successfully conduct the project is available and the participants complement each other. This is good.

The management structure is appropriate. Roles and responsibilities are clearly defined. All partners are represented in the project committee and the scientific committee; however resources are only allocated to two partners in Work Package 7. This is a shortcoming.

The potential scientific risks are well evaluated and described in detail. The proposed risk mitigation measures are appropriate.

EnzOx2 GAP process: Actions and feedback

BBI JU H2020

Grant Agreement Preparation (GAP)

Actions & Feedback from the Coordinator/Consortium

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1. Financial Viability Check

Please note that the financial capacity must be checked for coordinators if the requested EU funding for the action is equal or superior to EUR 500,000. The financial viability will not be checked for coordinators that are public bodies, international organisations, financially guaranteed by a member state (or an associated country) or that are secondary or higher education establishments. However, any participant will be checked if there are justified grounds to doubt its financial capacity.

When requested via the electronic exchange system of the Participant Portal, please provide, **within 1 week**, basic financial data and supporting documents proving financial viability. In particular for the last financial year for which the accounts are closed, a copy of the following documents will be needed:

- Balance sheet;
- Profit and loss account;
- Statutory audit report on the 2 above financial statements (if available);
- Audit report certifying the accounts of the last available financial year (the one mandatory under national law, not a specific one).

Note: You can test your financial viability on the Participant Portal with the financial capacity [self-check tool](#). For further information, please consult section III.4.3 of the ['Guide on beneficiary registration, validation and financial viability check'](#).

Feedback from the coordinator:

We need an **EnzOx2** Consortium Agreement..

“New Enzymatic Oxidation/ Oxifunctionalization Technologies for Added Value Bio-Based Products”

(“EnzOx2”)

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CONSORTIUM AGREEMENT

THIS CONSORTIUM AGREEMENT is based upon REGULATION (EU) No 1290/2013 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 11 December 2013 laying down the rules for the participation and dissemination in “Horizon 2020 – the Framework Programme for Research and Innovation (2014-2020)”, the (hereinafter referred to as “the Rules”), COMMISSION DELEGATED REGULATION (EU) No 623/2014 of 14 February 2014, establishing a derogation from Regulation (EU) No 1290/2013 of the European Parliament and of the Council laying down the rules for participation and dissemination in ‘Horizon 2020’, the Framework Programme for Research and Innovation (2014-2020)’ with regard to the Bio-Based Industries Joint Undertaking and the European Commission Multi-beneficiary General Model Grant Agreement and its Annexes, and is made on [Date on which the Project shall start](#)), hereinafter referred to as the Effective Date.

