PROJECT FINAL REPORT

Grant Agreement number: 282945

Project acronym: BIO-MIMETIC

Project title: New Bio-inspired processes and products from renewable feedstocks

Funding Scheme: EU FP7 ENV.2011.3.1.9-1 Eco-Innovation

Period covered: from 1 July 2012 to 30 June 2015

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Final publishable Summary Report

Executive Summary:

The BIO-MIMETIC project was funded by the European Union's Seventh Framework Program (agreement nr 282945) with the aim to set-up a novel pre-industrial scale enzymatic bio-polymerization process for the development of marketable bio-inspired & bio-based products.

Coherently, BIO-MIMETIC generated a new class of bio-inspired, lignin-derived polymers via extraction of natural compounds from renewable resources, through a process developed by the company CIMV (a project participant). Such biological transformation involves environmentally friendly enzyme-based processes delivering a new class of completely renewable polymers.

BIO-MIMETIC's objectives were to:

- Design & develop processes for the transformation of biomass feedstock via catalytic routes based on enzymes;
- Develop & validate the performance of "bio-mimicked" phenolic polymers in the formulation of household products, such as detergents, new bio-based cosmetics, emulsions for beauty care use as well as within the textile sector;
- Quantify the environmental benefits & economic prospects, using Life Cycle Analysis (LCA) & Life Cycle Costing (LCC) of such bio-processes & bio-based products results, taking into account the whole value chain from biomass transformation up to integration into end-user products.

BIO-MIMETIC's Key Technical Outcomes:

- Utilised different biomass sources to obtain reproducible & purified batches of lignin & lignin oligomers. Lignin, which is an interesting, abundant & renewable source of aromatic groups, has been successively transformed into new bio-polymers & bio-based products by environmentally-friendly enzymatic processes developed within the project, involving enzymes such as laccases, oxidases & lipoxygenases.
- Explored opportunity to bind lignin & its derivatives with chitin Nanofibrils which are natural polymer obtained from crustacean waste by a simple & green process.
- Studied the use of these new bio-polymers in household & beauty care consumer bio-products e.g. Automatic Dishwashing, a Compact Hard Surface Cleaner, & anti-aging/anti-inflammatory Skin Creams & Beauty Masks.
- In addition a life cycle assessment has been carried out to evaluate the environmental sustainability of both the new bio-polymers & the consumer products.
- Finally, the project has also carried out a stakeholder's analysis to enable the valorisation, the maximum dissemination & exploitation of the knowledge & the results created within the BIO-MIMETIC project. To this end, the BIO-MIMETIC partners have produced a large number of publications, newsletters, & press releases, & organized events. At the same time they have participated in many external events to increase the dissemination of the BIO-MIMETIC project results. An Exploitation plan to define the exploitation route & the management procedures related to relevant IPR was created within the project & has been realized.

BIO-MIMETIC provided solutions that are environmentally friendly as well as competitive from an economic point of view, namely:

- **Showing proof-of-concept for the production via predominantly enzymatic routes of highly weight-efficient functional bio-polymers based on lignin feedstock**
- **In a cradle-to grave product context these new bio-polymers will allow the reduction of CO_2 footprint over the life cycle**, thanks to the replacement of fossil feedstock with bio-based feedstock for the polymer production;
- **Eliminating toxic/harmful solvents** in its processes compared to synthetic alternatives, leading to a safer & cleaner production pathway. This has been assessed via the ecotoxicity impacts in the Life Cycle Analysis;
- **Re-using waste from the fisheries industry**, by the use of chitin & chitin nanofibrils in conjunction with bio-lignin & bio-polymers for various new product applications

Summary Description of Project Context

Procter & Gamble coordinated a project named BLUE4GLUE. The BLUE4GLUE project partners investigated biological processes in the field of blue biotechnology as a basis for developing new bio-inspired adhesives and release materials for adhesives. One of the most studied processes was the water-resistant adhesive mechanism used by marine organisms (mussels, barnacles). The hardening of insect shells (sclerotization) is another natural process which closely resembles the setting of the mussel glue.

The BIO-MIMETIC project was aimed to generate a new class of bio-inspired polymers via extraction of natural compounds from renewable resources. These polymers with novel properties were produced through a new biological transformation route, involving environmentally friendly enzymatic processes.

At this aim, BIO-MIMETIC project is based on the approach used by marine organisms, such as mussels, to anchor themselves on underwater surfaces by secreting adhesive bio-polymers, known as Marine Adhesives. The mechanism that nature uses consists of enzymatic processes by a class of enzymes called polyphenol oxidases (PPO). PPOs are able to convert precursors into more reactive compounds, which subsequently undergo a variety of non-enzymatic reactions. The reactions lead to further processes such as the polymerization of monomers, the conjugation of polymers or the formation of cross-linked structures. The natural polymers, used by mussels, are however peptides with repeating sequences of hydroxylic functionalities. These structures have the capacity to compete successfully with water for their structural composition is made of hydrophobic and hydrophilic side chains. The hydrophilic side chains allow strong electrostatic interaction with surfaces. In conclusion, these glue proteins act as electrostatic cross-linkers between the negative charged polysaccharides chains, allowing the produced gel to firm quickly on any kind of dry or wet surfaces. Inspired by these processes, the Bio-Mimetic project, coordinated by Procter & Gamble, UK, is focused on the development and validation of new bio-based pre-industrial processes and polymers. The project, in fact, aims at transforming plant raw material in biological glue without the use of detrimental chemical substances but by the conjugation of polymers and enzymatic processes producing cross-linked structure. In order, to reach the project objectives the consortium is formed of research partners with experience in enzymatic transformation and bio-based synthetic polymers (IFAM and UNITOV), an SME expert in production of enzymes (Dyadic), in biomass transformation (CIMV), in computational modelling of bio-chemical processes (CULGI) and in Technology Transfer (CTECH/PNO). P&G and MAVI have tested the innovative bio-derived polymers structures in their products, to assess the potential for new green, environmentally friendly and competitive products. P&G and UNIMAN have carried out out LCA and LCC assessments over the value chain, to validate environmental and cost benefits of the BIO-MIMETIC innovations.

Coordinator and project partners

The Project involved 10 partners, a large industry as coordinator (and also its service company as partner), 3 public research organizations and 5 SMEs:

Coordinators:
PGUK – Dr. Anju Brooker as Project Coordinator along with Procter & Gamble Technical Centres Limited (United Kingdom)
Partners
P&G BIC – Procter & Gamble Services Company N.V.(Belgium); IFAM - Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V. (Germany); UNITOV – Tor Vergata University (Italy); MAVI – MAVI SUD s.r.l. (Italy); DNL – Dyadic Netherlands (Netherlands); CULGI – Culgi B.V. (Netherlands); CIMV – Compagnie Industrielle de la Matière Végétale (France); CTECH – Ciaotech srl (Italy); UNIMAN: University of Manchester (United Kingdom).

Description of the main S&T results/foregrounds

In the BIO-MIMETIC project, Lignin and its oligomers (Fig. 1) have been used to design household and beauty care products.

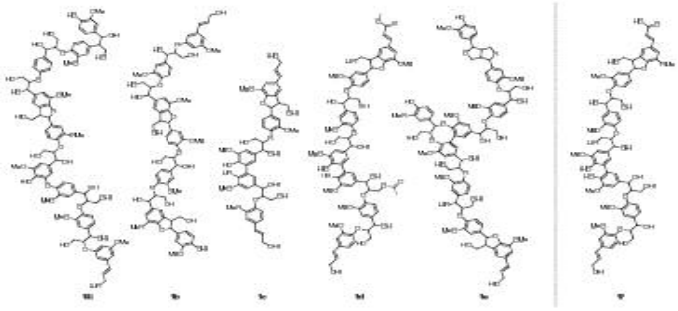
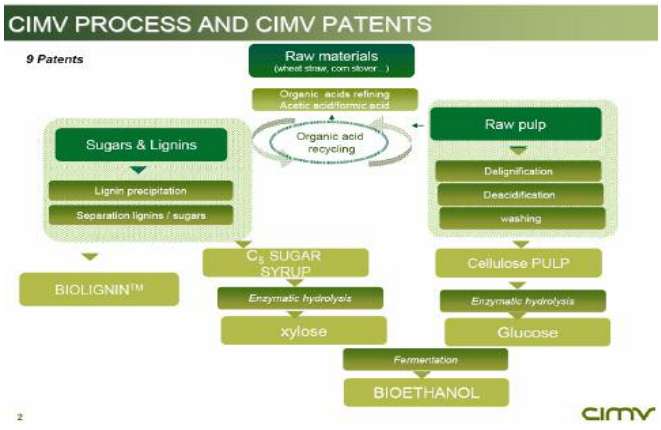


Fig. 1 Example structures of Ligning oligomers.

The research project has leveraged the expertise of CIMV (Dr. Bouchard Benjelloun, director R&D, www.cimv.fr) to obtain bio-lignin from different renewable resources, such as forest residual, wheat straw, sugar cane, corn straw and rice straw (Fig. 2). This has been possible via the patented organosolv process developed by CIMV (Fig. 3 and Fig. 4).

Fig. 2

The biolignin produced by CIMV has subsequently been refined and functionalised by chemical and enzymatic approaches to produce a range of functionalized oligomers by Prof Claudia Crestini and Dr. Heiko Lange from University of Tor Vergata, Rome, Italy (www.uniroma2.it). The strategies reported in the talk of prof. Crestini at the final BIO-MIMETIC work-shop in Latina entitled "Lignin Biorefinery: Advanced and Challenges chemoenzymatic Functionalized Lignin-based Oligomers" have shown that it is possible to obtain different molecular weight fractions of lignin-oligomers. Furthermore, a new specific functionalization process has been developed based on the coupling of lignins with specifically activated polymers. The fundamental procedure to synthetize functionalized lignins is based on opening reactions of epoxy moieties. Such tailored lignins may be of potential use in detergents for hard surfaces and in cosmetic applications. Alternative approaches have been developed based on the use of enzymes. More specifically polyphenoloxidases, laccases and lipoxygenases have been used to modify lignin oligomers.



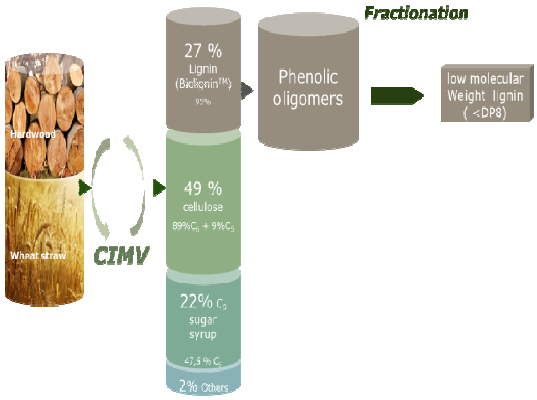


Fig. 3 Organo-solv process used by CIM

Fig. 4 Lignin Extraction Process

The different enzymes used to produce and functionalize the biolignin oligomers, have been realized and produced by the Dyadic Netherlands BV team led by Dr. Martijn Koetsier (www.dyadic.nl). The Dyadic enzyme library is composed ofmore than 100 functional single homologous enzymes. Dyadic developed 14 new enzymes in the project among them a special laccase. Laccases, in fact, are able to catalyze the oxidation of lignin phenols. However, it has been shown that by using the Dyadic Cl methodology, it is possible to screen single target enzymes (Cl-library) from a defined enzyme mixture, and thereby producing relatively pure single enzyme or dedicated enzyme combinations. It is also worth highlighting that the enzymes developed in the project could be valuable tools in the valorization of lignin biomass, also outside the scope of the BIO-MIMETIC project, such as the production of paper pulp, textile, food or bio energy.

An important outcome of the BIO-MIMETIC project has been the realization of a new software able to predict the surface affinity of the novel molecules. Dr Jan-Willem Handgraaf from Culgi B.V. (www.culgi.com) and Dr Peter Schiffels, Dr. Micahel Hoffmann and Dr. Klaus Rischka from the Fraunhofer Institut IFAM (www.ifam.fraunhofer.de) have partnered in the realization of the modelling program. Coarse-grained molecular models have been developed that can be used in dissipative particle dynamics (DPD) simulations. The coarse-grained models and simulations were in turn verified by all-atom molecular dynamics simulations (Fig. 5 and 6). Following this approach, the Culgi team in collaboration with Fraunhofer IFAM have realized a new simulation model which may be used in an R&D environment to address the interaction of active agents derived from bio-based feed stocks such as lignin with surfaces such as cotton, glass and plastic.

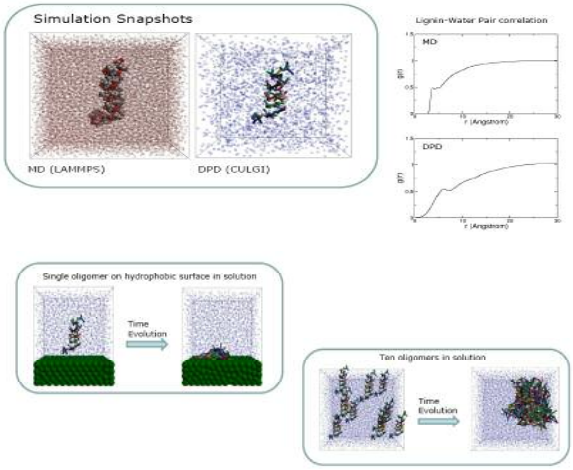


Fig. 5 Example for the verification of coarse grained simulations by all-atom molecular dynamics simulations.

Fig.6 Example for a dissipative particle dynamics (DPD) simulation.

IFAM and Culgi have identified the detailed molecular mechanisms of the interaction of lignin-based compounds with polymers and different surfaces.

Dr. Mauro Vaccaro and Dr. Stefano Scialla from P&G has evaluated a broad range of consumer related benefits delivered through the incorporation of functionalised lignin molecules in consumer good products with promising results on new surfaces modifications properties and effective delivery of benefit agents to surfaces.

Prof. Pierfrancesco Morganti from MAVI, an Italian SME, has utilised the above mentioned lignin molecules to produce green cosmetics and non-woven beauty masks. Block copolymeric micro/nanoparticles of chitin-lignin and chitin- hyaluronic acid were produced and embedded into cosmetic gels and tissues. The final products were tested for their human safety and effectiveness in the Department of Experimental Dermatology at the 2nd University of Naples, (Italy), whilst size and stability of the micro/nano particles and the elasticity and strength of non-woven tissues were controlled at the Engineering Department of La Sapienza University, Rome, Italy. The obtained nanoparticles of Chitin and Lignin were loaded with a range of different active ingredients; these particles have shown interesting medical properties.

The activity testing has shown that the beauty masks are capable of reducing the skin face wrinkling, slowing down the formation of black spots. The study of the anti-aging and anti-inflammatory activities has shown a high effectiveness without providing any toxic effects. The innovation achieved by MAVI is related to the possibility of using chitin nanofibril-lignin as both active ingredient and carrier to be embedded into micro/nano emulsions and entrapped into natural fibers to produce innovative cosmetic emulsions and non-woven tissues having a high effectiveness and safeness.

A life cycle assessment (LCA) and life cycle costing (LCC) analysis have also been conducted on the extraction of lignin (and co-products) from wheat straw and corn stover using CIMV's organosolv process, on the production of functional bio-polymers, as well as on four finished product applications. This work was led by Prof. Adisa Azapagic and Dr.

Laurence Stamford from University of Manchester and Dr Diederik Schowanek from P&G BIC.

P&G has established models and a systemic set of LCA procedures for compiling and examining the inputs and outputs of material and energy together with the associated environmental impacts, directly attributable to the functioning of every product or service system throughout its life cycle (Fig. 7 – example for a washing product).

In addition, LCA also involves cradle-to-grave analysis of every production systems and provides comprehensive evaluations of all upstream and downstream energy inputs and multimedia environmental emissions. Prof. Adisa Azapagic and Dr. Laurence Stamford of the University of Manchester (www.manchester.ac.uk) have shown how to use the freely available CCaLC software (www.ccalc.org.uk) for LCC. CCalc provides a simple user interface and access to a large amount of data licensed from commercial databases. CCaLC allows users to map the entire life cycle of their products, including raw material acquisition, processing steps, waste management, storage, use and transport giving the possibility to SMEs also to conduct their own LCA without need to outsource. An important output of the BIO-MIMETIC project is the addition of LCA and LCC case studies to CCaLC analysing various parts of the BIO-MIMETIC value chain (from raw material extraction to end-use products), so that SMEs can investigate the area themselves.

Fig.7 LCA

LCC case studies in CCaLC can also be used to validate the economic impacts of the bio-based copolymers by comparing the newly developed technology vs. existing synthetic polymers based on a similar function in a real product context. The BIO-MIMETIC project has also been focused on the valorisation and the achievement of the maximum dissemination and exploitation of the knowledge and the results created within the BIO-MIMETIC project. This work has included:

- ✓ actions for broad dissemination and exploitation of the project results.
- ✓ setting-up an exploitation plan that defined the management procedures related to relevant IPR created within the project, both internally within the project consortium and externally (protection of knowledge).

The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

The transition to an integrated Bioeconomy is the way to secure a future worth living to the succeeding generations. If, renewable raw materials of higher quality will sustainably be produced, food security, biodegradable and/or compostable goods, and a healthy environment will continue to be assured. A main theme also addressed by the EXPO 2015 held this year in Milan (Italy).

The development of innovative production systems and an optimized use of agricultural and industrial by-products will be necessary to address this challenge because of the increasing demands of natural resources from a growing population at global level. New products can be created from biomass to replace those based on fossil fuels resulting for example in a reduction of greenhouse gas emissions (GHGs). In addition, the use of enzymatic reactions as alternative to currently-used organic solvents to produce bio-based products may help industrial production to meet the challenges imposed by the climate change. Downstream processing which uses bio-catalysis to replace chemical processing can save, in fact, billion tons of CO_2 emissions across aside range of industrial sectors. This will enable scientists to discover, design and construct artificial micro-organisms, new biomaterials and green industrial processes to be used in the production of renewable chemicals, bio fuels, renewable specialty and fine chemicals, food ingredients and health-care products.

Bioeconomy and sustainable industry are based on the use of advances in biological science and nanotechnologies to create economical activity and public benefit. However, a growing world population requires increased health services and more resources such as food, animal feed, fibers for clothing and housing, and sources of a green energy and bio-chemicals for manufacturing. By 2050, in fact, the world population is estimated to reach about 9 billion, compounding the need for food and goods! Therefore, the industrial and environmental nanobiotechnology can contribute to make production processes more resource efficient and environmental friendly developing more sustainable bio-based products and processes, as for the Bio-Mimetic project, by which different and innovative household products and skin care cosmetics have been realized by biological technologies, without the use of petrol-derived chemicals.

Dissemination Activities:
During the first period of the project the main activities of dissemination have been focused on the realization of the strategy. The strategy foreseen:

- ✓ The Realization of the communication and information material such as:

- **Project logo**: The project identity is linked with a graphically coherent and consistent representation of the BIO-MIMETIC logo on project results and documentation.

For the BIO-MIMETIC project a graphical logo has been realized with the main intention to remember the name of the project in one hand and the main project goal (to develop New bio-inspired processes and products from renewable feedstocks) on the other.

Dissemination Materials: the design and the contents to share with the public have been agreed amongst the partners. As it is possible to see in the deliverable D 8.4 "First Informational Brochure" the most important objectives and benefits are reported as well a description of the project consortium and the partners' role within the project. The second and third brochure, D8.13 "Informational Brochure with second year activities" and D8.14 "Brochure with final results" contained updates on the results reached during the second and third year of the project. (Please, see attachment 1, 2, 3).

As part of the dissemination tools, at month 24, re-usable illustrations were realized demonstrating key (public) project results and submitted in the deliverable "D 8.5 Re-usable illustrations", at month 36 "D8.15 Video and updated re-usable illustrations" were also realized. (Please, see attachment 4).

Finally, at month 36 a Policy brief, D8.7 has been realized (Please, see attachment 5.). This represents a document which outlines the rationale for producing and communicating environmental policy-related results of the BIO-MIMETIC project.

Project Web Site, available at the address http://www.biomimetic-eu-project.eu/.

Both a public and reserved areas of the website have been realized. The public area of the website allows access to other sections of the portal, containing the key information of the project, in particular:

- Home Page (clearly communicates the site's purpose, and shows all major contents available on the Web Site.)
- Project Objectives (The section includes a description of the project's specific objectives as well as the benefits expected for the Environment)
- Public Results (this contains the public results of the project including the dissemination material such as brochures, reusable illustrations, the video and published articles) to be downloaded
- Partners (this section hosts the information of each partner of the BIO-MIMETIC project.)
- News & Events (this section contains an archive of interesting events on bio-inspired processes and products from renewable feedstocks in order to create a network all through Europe with other events and organizations.)
- Interesting links (in this section, links to other EU projects and web-platform are included in order to create a network with other EU projects.)
- Contact (section where basic information can be required).
- Private area is accessible only to BIO-MIMETIC partners, to share documents and information. It can be accessed through the login area at the bottom of the page. After the log-in the page is automatically redirected to BIO-MIMETIC page on Innovation Place web-platform (the web platform where the private area of BIO-MIMETIC site is hosted).

- ✓ Planning of the Dissemination activities:

As reported below the first months of the project have been focused more on dissemination strategy and on the realization of the dissemination tools. Nevertheless many dissemination activities were planned (and afterwards carried out) such as participation to conferences, publications, press release etc., as reported in the following.

Use and dissemination of foreground

As part of the dissemination strategy agreed among the partners, various dissemination activities have been carried out by all the partners, including participation at conferences, publications, press releases, and newsletters. During the project timeframe the following activities have been realized:

- The partners took part in or organized 38 Global Events
- 12 Articles and publications have been realized both at scientific and industrial level (all the articles are available at the address http://www.biomimetic-eu-project.eu/project-results/)
- 26 other dissemination activities have been realized
- during the events about 1500 brochures have been distributed to the audience
- Links with other 5 projects and 2 programmes have been established.

A final workshop, seminar and SME training were also realized on the 25th of June in Italy. The event focused on sustainability. The BIO- MIMETIC partners wanted to teach the lessons learnt from the BIO-MIMETIC project to other companies in order to replicate the successful story: how to increase the sustainability of industrial processes while maintaining or increasing the competitiveness of enterprises.

The aim of the workshop, seminar and SMEs training was to demonstrate to all, but to the companies in particular, that it is possible to develop novel eco-efficient environmental technologies whose use can substantially contribute, directly or indirectly, to the reduction of materials and resource use, energy consumption, polluting emissions, etc., by using bio-mimetic technologies capable of deriving from nature's way of functioning, the design principles that lead to eco-efficient processes. The SMEs training focused on CCaLC, a LCA tool recently developed from UNIMAN for SMEs. CCaLC is free of charge and enables calculations of the carbon and water footprints as well as other life cycle impacts usually considered in LCA. In addition, it enables estimations of life cycle costs and valued added along whole supply chains. It comprises extensive databases (over 5000 items) and case-studies (over 60), providing SMEs with base data for sustainability assessment of their processes and products. BIO-MIMETIC has been added as an additional set of case studies in the tool, as explained during the training.