

## **BIOECO ANNEX 2 : Scoping document for PhD certification**

### *Link 1: Economics and ethics*

Designing and regulating markets of the bioeconomy is at the core of *Link 1*. New technologies will only develop if an efficient match between supply and demand in biobased products can be achieved through well-designed markets, while the social and environmental dimensions of the exchanged goods call for strong public policies (and regulations). Research will be oriented towards the development of various models and quantitative tools. Interactions with other modules will be systematic and developed at 6 distinct levels:

1) Economics of the chain: Reducing the uncertainty regarding the competitiveness of the proposed technologies, in the context of biorefineries producing various products final from various feedstock is a major challenge. Tools linking biological models and economic cost models will be developed (with *Links 4 & 5*). We will also analyze the final value of bio-based products integrating competition with standard products, and any additional value related to their specific characteristics. Finally, we will also investigate the issue of sharing the value within the chain.

2) Market impacts: Agricultural and food markets will be strongly impacted by the development of bio-based products. Whatever the feedstock used (*Link 2*) to produce bio-based products, competition issues concerning biomass usage will exist. However, biorefinery development is likely to provoke increased interdependence of the different food industries and greatly improve the use of various by-products. Tools linking different food industries in the economy are necessary rather than developing sectorial analysis of specific industries. These models, which need to incorporate environmental impacts (*Link 6*) depend on the competition for resources through the change in land or co-product use.

3) Public policies: Public policies affect the viability/development of the bioeconomy. Key questions relate to their design, the interactions between the various policies as well as their economic desirability. Models are needed able to predict the numerous, difficult to assess and often contradictory impacts of large projects on the economy including the territorial impact and the consequences of emerging specialization linked to feedstock availability, the existence of processing units, and end-user industries and consumers.

4) Long-term drivers: Possible trajectories and the various drivers (transition, global economic growth, evolution of agricultural systems, changes in food habits, demography) influencing the transition towards a bio-based economy will be characterized combining model-based quantitative approaches, especially through integrated assessment models, with conceptual analysis and scenario approaches at the global, sectoral and local levels. Strong collaborations will be developed with *links 5 & 6* to integrate environmental issues and multidisciplinary models with economic methodologies.

5) Understanding the role of innovation and R&D: The rise of a bio-based economy relies on correctly timed R&D innovation. The scope of innovation extends beyond the engineering protocols and includes societal and organizational management innovation. TSE economics expertise in the analysis of strategic interactions between industrial R&D actors will be used to provide assessments of crucial stakes: scope and role of intellectual property rights, role of norms and rules, competition regulation. At a macro scale, the interactions between economic dynamics, bio-transition process and R&D trends will be studied.

6) Ethics: The bio-based economy implies a new approach to life via the possibility of constructing *de novo* features for microorganisms. It strongly questions the definition of life and has

led to new ethical issues since the border between living and artificial machinery could modify the philosophical and social approach to both technical objects and living beings. An ethics committee will be set up to monitor this aspect.

#### *Link 2: Resources and fractionation*

This module will determine, in a sustainable agriculture context, the best available renewable resources for further industrial application and the production requirements facilitating efficient prior to transformation using enzymes or micro-organisms (*link 3*). To achieve this in a cost effective and sustainable manner, quantitative data on the characteristics and composition of the renewable resources, their long-term production requirements and their evolution during pre-treatments is needed. To attain such a database the three following topics will be addressed.

1) Biomass characterization: Biomass for biotransformation should meet criteria concerning its composition and structure. To obtain this information with details of time-dependent spatial molecular distribution enabling the bio-transformability to be predicted and exploited, effort is essential in developing novel analytical methodology (Raman, fluorometric, etc.) as well as more classical methods to access the quantitative distribution of target molecules (*link to links 3 & 4*). This data should be consolidated with confirmatory microscopic analyses enabling such distribution to be understood and modelled.

2) Bio-resources production and pretreatment: Sustainable exploitation of bio-resources is highly dependent upon maintaining soil fertility which requires an important mastering of the organic carbon content. Understanding how best to optimize these conflicting objectives is a key factor in the long-term capacity to sustain an important biorefinery activity. Agricultural methods and plant varieties requiring minimum introns (water, fertilizer, etc.) need to be identified and shown to be compatible with multi-criteria optimization of land use including agro-ecological approaches to reconstitute carbon to soil and environmental assessment (see *link 6*). Taking into account the composition of the biomass, the soil sustainability and the end-product target molecules (*link 3*) innovative and coherent development of agriculture will be proposed. Devoted cultures (like microalgae or green plant) and by-products valorization will be evaluated as part of an overall closed-loop solution. Biomass pretreatment strategies will be optimized to facilitate biotransformation in the most economical manner, incorporating improved mass transfer, to increase recovery of target molecule concentration or to remove potential inhibitors, while maintaining the basic biomass feedstock in a suitable form for further transformation, in accordance with green chemistry principles.

3) Database: Decision-making tools incorporating how best to treat specific plant and waste matter within defined industrial objectives will be developed incorporating the structural composition data and the process-dependent changes, compatible with life cycle analysis.

#### *Link 3: Development of natural and synthetic biotransformation*

Advances in systems and synthetic biology, biophysics, bioinformatics and biomathematics are providing unprecedented opportunities to accelerate the delivery of novel natural or synthetic, fine-tuned biocatalysts.

1) Exploring natural and man-made diversity for novel enzymes: Enzymes are widely used in industry as natural biological catalysts able to significantly accelerate biotransformation reactions. However, only a small proportion of the known enzymes are currently exploited, leaving an immense

reservoir of potential catalysts, which can be further enlarged by protein engineering technology used to generate new-to-nature enzymes. Modern biology offers a technological pipeline to accelerate enzyme discovery and optimization while meeting regulatory, environmental and process specifications. In collaboration with other modules, enzymes able to convert agro-resources, synthetic material or wastes, will be optimized for novel enzyme-based transformations as well as elemental components for innovative synthetic cell factory design. Generic high throughput methodologies combining activity- and sequence-based functional screening of genomes, metagenomes and metatranscriptomes will be implemented using various hosts, microfluidic screening and automated procedures, as well as state of the art analytical procedures to facilitate knowledge-based design of new catalytic elements. *In vitro* directed molecular evolution as well as rational or semi-rational design will be used to customize enzyme physico-chemical properties or reaction specificities more adapted to industrial applications. Breakthrough developments in biophysics are anticipated, which will rationalize novel computational protein design to better integrate protein flexibility, entropy, electrostatics and protein solvation.

2) Metabolic engineering and microbial systems: Genetic engineering breakthroughs revolutionised the manner in which micro-organisms could be pragmatically optimised to favour improved performance for biotechnology. New advances in systems and synthetic biology, will give rise to much more complex synthetic microorganisms, opening new routes to cost effective fuels, chemicals, materials and bio-products. However, development timelines are often long, notably due to the absence of connection between strain design/optimisation and the specific operational constraints linked to their exploitation in industrial fermentation strategies. To overcome this bottleneck, BIOECO will be built to efficiently integrate bioprocess, industrial, societal and economic specifications for accelerated design of high performance, robust platform strains able to maintain their performance in the extreme conditions of industrial fermentation. This will require a multidisciplinary approach integrating predictive mathematical modelling (*Link 5*) of the functional cell behaviour to design *de novo* living systems, system-level analysis of process-induced variations through increasing TRLs and synthetic biology methodologies to design original synthetic pathways using biological elements (*Link 3.1*) coherent with the previous criteria. Development will follow the “Design, Build, Test, Learn” cycles in order to rapidly build safer, more efficient and more robust microbes through a machine learning approach, employing some of the high throughput strain construction methods available to speed up strain development. Chassis organisms as well as natural or synthetic consortia will be selected and optimised based on their intrinsic metabolic characteristics to favour specific targeted domains of industrial biotechnology.

#### *Link 4: Innovative Process Engineering*

Innovative processes for industrial biotechnology and biorefining with reduction of both economic and environmental costs will be developed using creative solutions for design, control and scale-up, based on process engineering and advanced modelling.

1) Driving the new microbial potentialities for producing valuable compounds: An integrated approach to design and implement biocatalyst(s) in accordance with process constraints will be established. The development of robust bioprocesses need to tackle issues such as (i) variable accessibility of nutrients in inhomogeneous (spatio-temporal variations) environments encountered in large scale reactors or due to the complexity and variability of the substrate matrix (high solids, gas...), (ii) robustness of the biocatalyst towards fluctuations of environment, (iii) robustness of the biocatalyst towards toxicity of the targeted products or co-products (iv) ease and predictability of the biocatalyst as regards its bioengineering (for engineered strains). As yet the relatively poorly exploited

microbe/consortia improvements/controlled mixed culture need to be explored to exploit some of the complex polymeric feedstocks. New challenges in control strategies for such multi-component populations are to be met to exploit combinatory metabolic potentialities. Transdisciplinary research is required for description and modelling of the biotransformation combining microbial kinetics with fluid dynamics and thermodynamics (single cell analysis and local scale fluid dynamics, new numerical modelling approaches for optimal design).

2) Integrating biotransformation, separation and biorefining: New concepts will be developed for the separation of bioproducts or biocatalysts leading to optimal integration of separation processes within the global product chain, through the optimization and application of integrated or hybrid process principles, thereby diminishing global energy consumption and process costs. A key challenge is the recovery of bioproducts at a realistic concentration and purification level from the complex mixture coming from the bioreactor or directly from the renewable resources. Innovative *in situ* process technology is needed (solvent and supercritical fluid extraction, adsorption, chromatography, membrane separation...) involving similar physico-mechanical variables as during the transformation process to attain intensified production characteristics and cost-effective harvesting and purification. Optimal exploitation of feedstock needs each transformation to be part of a cascade in which the residue is further transformed until a final step of energy production, leading to the production of CO<sub>2</sub>, thereby closing the loop with photosynthesis. A special attention has to be given to nutrient recovery, for which combination of technologies could lead to organic fertilizers, closing the loop with soil quality and agricultural yields.

3) Scale-up from laboratory to industrial scale: Population mass balance modelling and computational fluid dynamics are capable of analysing the inherent heterogeneity phenomena associated with industrial-scale production units and proposing optimal design criteria. Another scale-up challenge is to integrate separation efficiency (*Link 4.2*) renewable resources (*Link 2*) and the biocatalyst (*Link 3*) in an integrated large-scale process design. Similar criteria apply also to the downstream aspects, and throughout the entire process, dynamic control strategies to control process trajectories are essential to retain both robustness and economic viability. Economic evaluation and the social acceptance of some new resources and process lines will be met via the development of a new demonstration platform (DEIFI-C, under construction).

#### *Link 5: Mathematical modelling and computing*

Among the major shifts which are shaping our future, the increasingly easy and extensive access to an ever growing abundance of analytical data on living systems is ineluctably giving modelling and computing a crucial importance. In order to understand biological processes, from molecules to populations, and exploit them to build a rationalized bioeconomy, simulation, prediction and optimization will be required to harness information and transform it into knowledge and efficient reliable operational processes. This module will facilitate the exchange of suitable mathematical and computational methodology and offer a low resistance pathway to interdisciplinary work.

1) Molecular modelling and design: Computational methods enabling multi-scale modelling and molecular design lead to accelerated understanding, and thus important economic gains, to the process of designing, optimizing or screening new molecules. New advanced modelling/design tools will be assembled to better represent the environment, molecular flexibility and complexity, including more accurate energy functions (*Link 3*). Ultimately, this will lead to accurate and rational molecular modelling and design tools, clearing the path to industrial enzyme design and reliable high-throughput screening tools for small molecules to reinforce the synthetic biology approach.

2) High-throughput sequence analysis: High-throughput sequencing at the genome and transcript level offers an unprecedented amount of data that needs to be efficiently analysed. High-throughput computational (meta)genome/transcriptome tools based on efficient algorithms and data-structures, allowing for efficient interpretation, screening of genes coding for unknown enzymes and better understanding of gene interactions and regulation (*Link 3*) will be developed.

3) Modelling metabolic and gene networks: To simulate and optimize the metabolism of microorganisms, the interactions between metabolites and enzymes, RNA, genes and DNA will be modelled. A multi-scale metabolic modelling platform which can scale from few reactions up to pan-genomic scale will be established. We will use probabilistic models to reconstruct gene regulation networks and their interaction with metabolism using high-throughput omics data, machine learning and statistics. This will allow *in silico* predictions of the metabolic characteristics of newly engineered micro-organism to be identified before initiating the experimental input, eliminating at source vital design weaknesses.

4) Systemic approach for multi-scale multi-objective modelling for sustainability: Generic models for bioprocess design require a spatial and temporal multi-scale approach, from a micro-organism and its metabolic capabilities to the supply chain. This also covers the dynamics of biological processes (energy, thermodynamics) as well as a quantification of non-sustainable or environmental adverse impacts. In an industrial perspective, such models will also be helpful to retro-design biological systems on the basis of an economic feasibility study. They should also facilitate life-cycle analysis, simultaneously on several criteria, whether economic (*Links 1 & 4*) or sustainability related (*Link 6*). We will tackle two crucial bottlenecks of the extreme computational costs of the optimization of complex systems and the analysis of multi-scale (stiff) problems.

#### *Link 6: Environmental assessment and eco-design*

Environmental damage and benefit will be assessed from new technology to be implemented in agriculture and industry and from new business models, in order to support the eco-design of systems at all production scales (*Link 4*), value chains and activity sectors. Sustainability requires holistic, system-oriented research and predictive methods for environmental protection, economic development and social well-being (*Link 1*). The multidimensional and multicriteria systems to be investigated range from the unit process, production unit to value chain including raw material production, activity sector and life cycle, from present to long term forecast, with a generic character or with a concrete territorial dimension. Environmental assessment methods like Life Cycle Assessment (attributional, consequential, operational, social), Carbon and Water footprint, will be used for evaluating environmental impacts (natural resource depletion, human health and ecosystem quality). Thermodynamic methods, *i.e.* eMergy and eXergy analysis (rooted in Thermoecology), will help at structural investigation of systems, and evaluation of ecosystem capital and services use.

Multidisciplinary modelling platforms will allow new process developments (outcomes from *Links 2 to 5*) to be coupled with multicriteria evaluation models. These approaches will be applied at all levels, from unit process to biorefinery (suprastructure models), for new processes design as well as for revamping existing processes. At larger scales, value chains eco-design should require (besides the process level approaches) the system dynamic modelling of bio-physical flows (*e.g.* dynamic Material Flow Analysis) with environmental performance evaluation and structural analysis (thermodynamic methods). New activity sectors and new business strategies potentially emerging from the technological

development will be prospected and evaluated (consequential LCA) for their relevance and real scale feasibility, in strong interaction with *Link 1*.

New evaluation methods rooted in existing ones will be developed to fill gaps and better integration of new environmental issues, *e.g.* carbon sustainable sequestration and land use, preservation of ecosystem services, renewable resources (renewability versus consumption), local risks on ecosystems and human health, social acceptability, nutrient (N, P, K, C) closed loop recycling and water resource degradation. The temporal scale will be included in the newly developed methods in order to capture the environmental impacts, the influence of practices in studied value chains, carbon sequestration/emission dynamics, agriculture dynamics and interconnection with related production units, on climate change deployment, on human health, eutrophication and other local impacts occurrence in time.

Another major aspect is the elaboration of a system value derived from multicriteria evaluations. A set of relevant sustainability indicators adapted at different scales will be developed to support decision making at scientific level (for eco-design proposes) but also for industrial players, practitioners and stakeholders (macro-indicators), as well as for societal communication purposes. Mathematical methods will be implemented for a reasoned choice of environmental indicators to be considered in eco-design (*Links 4 & 5*). The main outputs will be operational methods and tools (original models, proof-of-concept software, and sustainability indicators) for multicriteria evaluation and eco-design of bioeconomy scenarios. Besides the generic developments, a multi-dimensional meta-model will be initiated based on biophysical flows involved in selected bioeconomy scenarios (resources-transformation paths-products-wastes), coupled with evaluation of sustainability indicators, including the territorial dimension with effective roots in our region.