INMARE SCIENCE POLICY BRIEF

Transforming Industrial Enzymes Biodiscovery: The INMARE Legacy

What is INMARE?

INMARE is a collaborative research & innovation project funded by the EU Horizon 2020 programme that ran from 2015 to 2019. INMARE stands for 'Industrial Applications of Marine Enzymes' and its central aim was to streamline the biodiscovery pathway for the identification of novel marine enzymes with industrial applications.

INMARE had three specific objectives:

- to develop a smoothly functioning consortium combining research-excellent academic partners with industrial market players;
- to shorten & streamline the industrial enzyme pipeline by increasing the value of enzyme collections; and
- to identify new lead products and prototypes during the projects lifetime.

Why was INMARE needed?

According to the 2018 update to the EU Bioeconomy Strategy[1], the bioeconomy accounts for 8% of the EU's workforce and bio-based industries could create up to 1 million green jobs by 2030 especially in rural and coastal areas. Marine biotechnology is one area of the bioeconomy with significant potential for growth. Under the EU's Blue Growth strategy[2], marine biotechnology has been identified as an important enabler of sustainable economic growth, with the potential to contribute to various fields such as industrial processes, biopharmaceuticals and nutraceuticals, food and feed production and bio-chemicals. A 2015 market report[3] predicted a global market for marine biotechnology derived products and processes of US$4. 8billion by 2020, rising to US$6.4 billion by 2025. Global challenges such as limiting natural resources (food and energy), pollution and human health, have ignited the quest for more sustainable and cleaner industrial processes and bio-based products. This, in turn, is driving the demand for new metabolites, small molecules and industrially relevant enzymes.

Why enzymes?

Enzymes are nature's catalysts. They provide clean and energy efficient natural catalysts for chemical reactions in a variety of industries. Using enzymes makes industrial processes faster, cleaner, safer and more energy and resource efficient. Traditionally enzymes have been used by a diversity of industrial sectors including the food, agricultural, cosmetic, and pharmaceutical industries, to name but a few.

An urgent need to provide environmentally sensitive and sustainable alternatives to toxic industrial processes means that demand for industrial enzymes will continue to grow. Europe is a market leader in this field, with 64% of the world's leading enzyme companies located in Europe. Thus the industrial enzyme sector represents significant potential for Europe in terms of escalating global leadership in the area of biobased products and processes.

"We need new enzymes because we need more sustainable solutions. Being in a project like INMARE where we worked together with academic partners is important for filling some of the knowledge gaps we have in the enzyme biodiscovery industry."

Dr Charlotte Blom, Department Manager, Novozymes

1. https://ec.europa.eu/research/bioeconomy/pdf/ec_bioeconomy_strategy_2018.pdf#view=fit&pagemode=none
2. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:52012DC0494
3. Smithers Rapra (2015) The Future of Marine Biotechnology for Industrial Applications to 2025

Why enzymes from the ocean?

The marine environment hosts some of the most challenging conditions on Earth, from high pressures in the deepest parts of the ocean, to temperatures of more than 300°C at hydrothermal vents, and extreme chemical conditions in hypersaline brine pools and at cold seeps. The metabolic diversity of microorganisms adapted to survive in these harsh conditions suggests that they may harbour enzymes uniquely able to perform in industrial settings, characterised by similarly harsh physical and chemical conditions.

For this reason, increasingly, attention is shifting towards the ocean as a sustainable source of materials, food and energy, as well as resources with potential for the development of new drugs and biotechnological applications. Because enzymes offer a greener, safer and cheaper alternative to chemical (production) processes, marine microbial resources hold promise for innovation in industrial biotechnology.

"The marine microbiome hosts more microbial species, biomass, biomolecules and genomic potential than any environment on our planet. Focusing our search here has contributed to the success of INMARE"

Professor Peter Golyshin, INMARE Coordinator and Professor of Environmental Genomics, Bangor University

So what was the challenge for INMARE?

"*..the potential to unveil novel interesting enzymes from marine sources remains very high. However, this potential does not automatically guarantee novel commercial products.*[4]"

In spite of their potential for industrial applications, to date very few microbial enzymes have actually made it to the commercial market. This is largely because the enzyme optimisation process, which seeks to make enzymes more stable and perform better in the harsh environment of industrial processes, is laborious, costly and has a low success rate, thereby forming an important bottleneck in industrial applications.

How did INMARE address this challenge?

To tackle this problem, INMARE scientists focused at the early stages of the biodiscovery pipeline towards finding better natural enzyme variants – so called 'promiscuous enzymes', i.e. enzymes that can catalyse more than one substrate, making them extremely versatile and useful in different industrial processes.

INMARE isolated samples from a wide range of marine environments, ranging from shallow coastal inlets to the deep sea, and from an array of organisms, such as macroalgae, sponges and marine invertebrates. INMARE scientists have successfully sampled and processed materials from unique microbial biodiversity hotspots.

"Enzymes are key for cleaner and greener chemistry and so we always need new enzymes which can be applied in industrial processes. INMARE means that we now have new enzymes to work on, but we also benefited from working with specialists in the field of enzyme discovery".

Dr Simon Esser, Scientist, evoxx technologies

4. Topic Description - Enhancing the industrial exploitation potential of marine-derived enzymes BG-04-2014

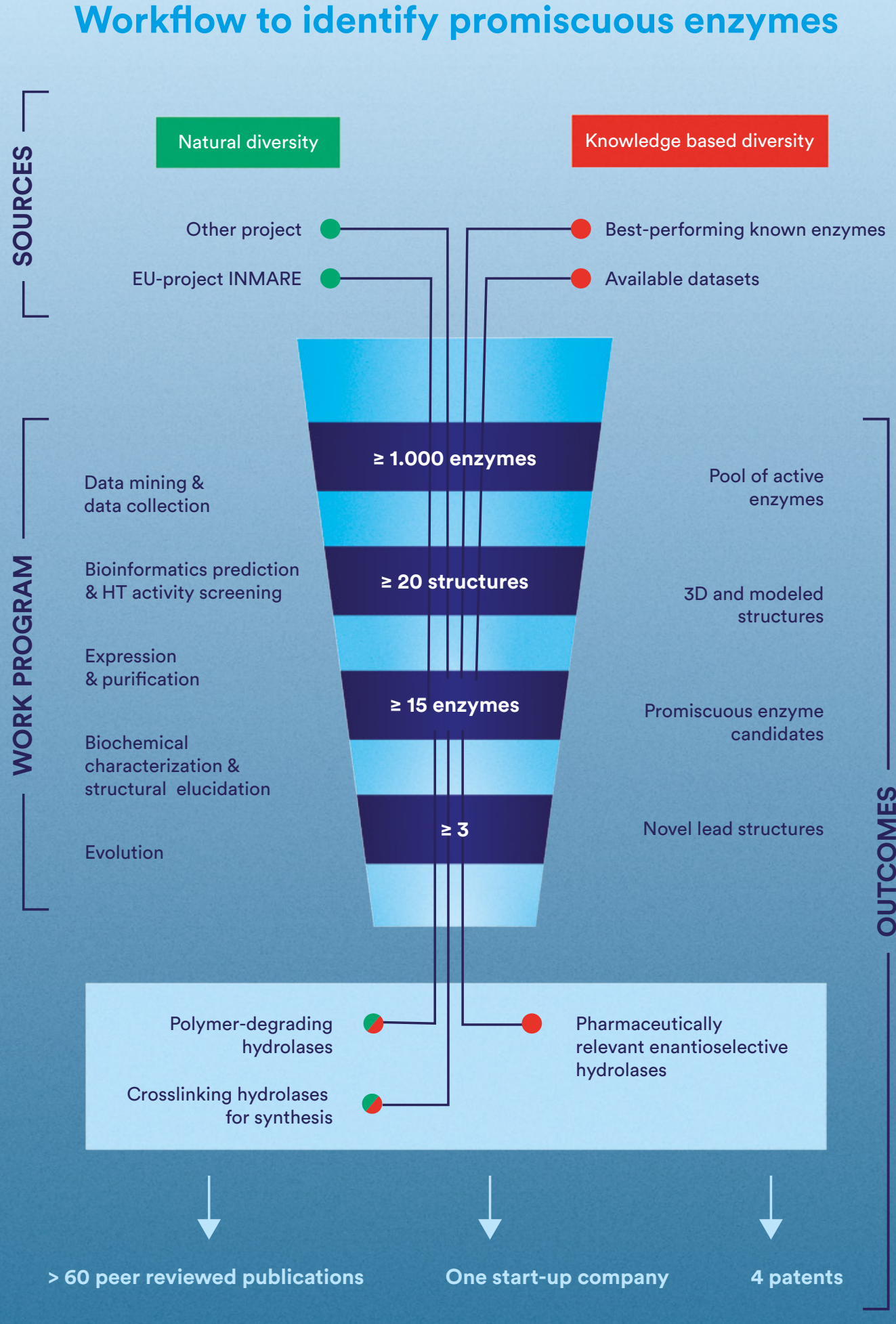


Figure 1 Graphical representation of the INMARE workflow and main outcomes. Adapted from original by Karl-Erich Jaeger with updates from Manuel Ferrer.

What has INMARE achieved?

At the end of its four-year journey, INMARE has produced one of the world's largest collections of enzymes, some of which are already performing better than current commercial products. This valuable resource is already providing solutions in industrial enzyme biotechnology.

INMARE has developed novel technologies in data mining, bioinformatics prediction, high-throughput screening and expression platforms. These technologies, together with new knowledge on biochemical characterization, structural elucidation and biocatalysis will accelerate enzyme and bioactives discovery, making current discovery pipelines more resource-efficient and increasing success rates. These advances, together with INMARE's vast reservoir of curated biological samples will also support future research.

INMARE's legal research work has also advanced discussions with regard to research on genetic resources and associated access and benefit-sharing regulations[5], resulting in a recommendation to guide the development of principles of due diligence for scientists, universities, technology transfer managers and businesses.[6]

Before the INMARE project, it could take from five to seven years to find new industrially useful enzymes. INMARE has succeeded in reducing this timeframe to less one year in some instances. Within the project's four year lifespan, INMARE's has produced four patents and a start-up company focusing on high-throughput enzyme activity recording has been established.

Finally, INMARE's new insights and knowledge outputs, discoveries and advances have been captured in over 60 peer reviewed papers and 11 book chapters**.** These are all available on the INMARE website http://www.inmare-h2020.eu/.

What challenges remain; what are the next steps?

INMARE is an exemplar of how European collaborations can fast-track innovations in industrially relevant research and development. It has demonstrated the crucial role of the European Union's Horizon Research and Innovation Programmes in driving innovation. It is important to continue INMARE's work and build on what has been achieved, in particular focusing on areas identified by INMARE partners as remaining challenges in the biodiscovery pipeline.

FUTURE RESEARCH PRIORITIES IN ENZYME BIODISCOVERY

1. Implementation of a biocatalytic process requires scale-up and process development with robust enzymes capable of withstanding high temperatures and, in particular, high concentrations of organic solvents. There is a need to identify key parameters allowing a proper scale up from mL-scale reactors to L- and cubic-m scales. This work is regarded as less 'interesting' scientifically and, as such, does not receive high levels of financial support.

2. Biocatalytic models using industrially relevant substrates to compare potential biocatalysts from different research groups must be developed.

3. Development of high throughput analytical tools are required to efficiently identify the most promising biocatalyst(s) and to provide essential kinetic data.

4. INMARE results should be used to define enzyme chassis that can provide suitable starting points from which to engineer enzymes with high enantioselectivity or substrate promiscuity.

5. Methods pipelines should be developed to identify enzymes with useful properties from metagenomic DNA sequences.

6. IT solutions and database approaches are needed to manage large and very diverse data, to make them available and manageable for project partners with differing needs.

5. http://ec.europa.eu/environment/nature/biodiversity/international/abs/index_en.htm
6. Thambisetty, Sivaramjani, Due Diligence and ABS Compliance under EUR 511/2014 (January 25, 2019). LSE Law - Policy Briefing Paper No. 33, 2019. Available at SSRN: https://ssrn.com/abstract=3323389

Some general challenges that remain include:

- **Ambiguity around Access and Benefit Sharing (ABS) Regulations** – Both academic scientists and industry need legal clarity about the relationship between the origin of a biological sample or derivative and applicable ABS regulations. Large mixed partner-type consortia, conducting both basic and applied research on common samples in different institutes, in different countries can make for a very confusing ABS scenario. Given also the wealth of ex-situ biological resources currently in collections around Europe, many of which cannot be used due to this lack of legal clarity, there is a real need for more guidance for academics (who often represent the point of entry of samples into the biodiscovery value chain), better ABS tools and transparent user-friendly information about applicable national ABS obligations. Industry will not enter into any work process on samples or derivatives for which there is no legal clarity.

- **Scale up from lab to industrial scale** – There remain outstanding technical challenges to facilitate the scale-up of bioprocesses from lab scale, generated though research actions, to a scale needed for industry. Whilst this may be a critical issue for industry, it is often not sufficiently interesting scientifically for academic partners to work in this area, given pressures to publish and do novel research with limited funding sources. This can lead to academics pursuing other avenues of research and so an important bottle-neck in the pipeline remains. It is important that innovation funding calls identify real-life challenges so that critical issues can be addressed.

The INMARE project was co-created to involve industrial partners from the outset. This ensured that INMARE's work was market-orientated and that the industrial partners were able to directly benefit from and apply the scientific findings of the project. This was key to INMARE's success and will accelerate the appearance of a novel products on the market. Project partners should always be chosen based on their potential to advance the projects aim, and all roles clearly defined during preparation of the proposal. This helps manage expectations as the project progresses, and avoids non-functioning consortia where partners are included to achieve the required make-up of public sector science actors and commercial enterprises.

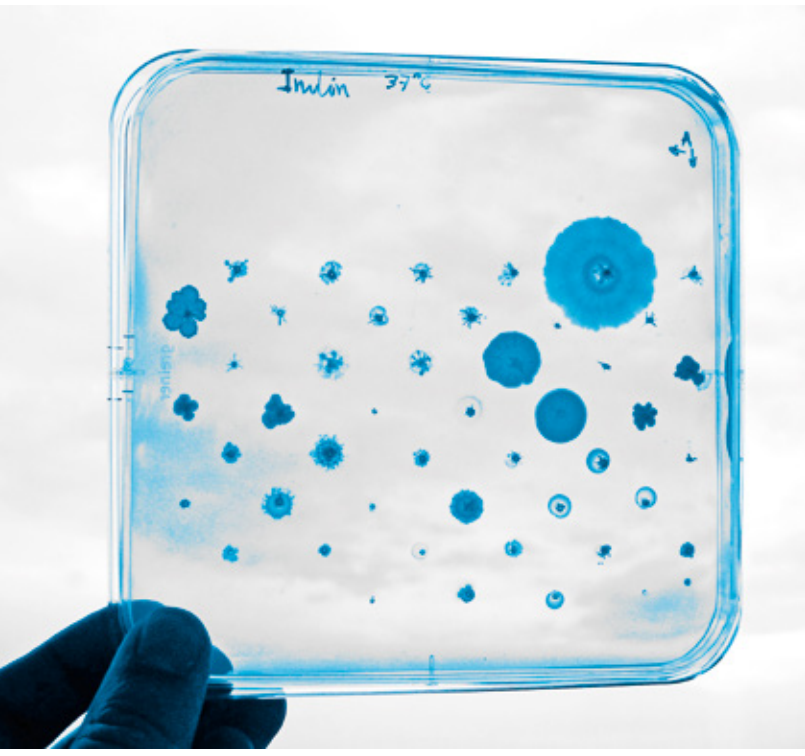
'It is important for R&D projects such as INMARE to create awareness of potential business opportunities early in the project. Having a dedicated forum to discuss these opportunities, throughout the lifetime of the project, maximises the potential for commercialisation, both at the end of the project and beyond.'

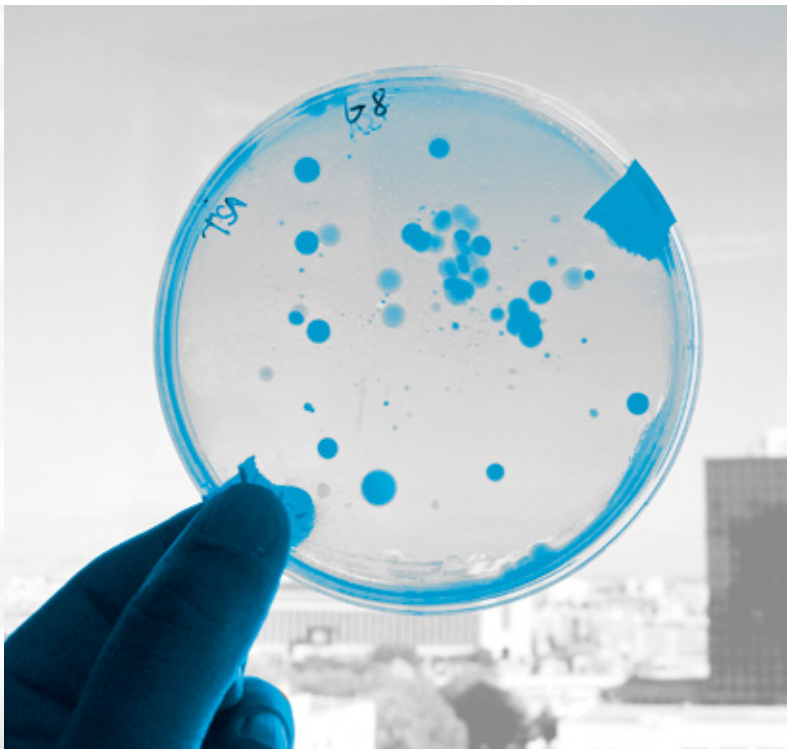
Dr Sarah Refai, Project Manager, CLIB - Cluster Industrielle Biotechnologie

In September 2016 the European Marine Biotechnology ERA-NET[7] (ERA-MBT) published a marine biotechnology strategic research and innovation roadmap (Hurst *et al.*, 2016). The roadmap set out a European marine biotechnology research and innovation agenda to 2030. Recognising that research and innovation in marine biotechnology spanned scientific, technological, economic and societal challenges, the roadmap identified five thematic areas; exploration of the marine environment; biomass production and processing; product innovation and differentiation; policy support and stimulation; and the provision of enabling technologies and infrastructure. INMARE's outputs, its scientific and technological advances, products and prototypes contribute to advancing all five of these key areas. INMARE's work will revolutionised industrial enzyme biodiscovery in Europe, and beyond. As a publicly funded project, it has clearly demonstrated value creation within its lifetime, but its work will continue to support growth in Europe's bioeconomy long after the project finishes.

"Through producing one of the largest enzyme collections worldwide, INMARE has provided solutions for the future manufacturing of bio-based commodities, and background knowledge that may open new research avenues."

Dr Manuel Ferrer, Research Scientist, Institute of Catalysis, Spanish National Research Council (CSIC)





7. Funded under the FP7 ERA-NET scheme, ERA-MarineBiotech is a consortium of national funding bodies seeking complementarities between national activities to pool resources to undertake joint funding of transnational projects in the area of Marine Biotechnology. http://www.marinebiotech.eu/marine-biotechnology-era-net

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