



# Industrial Applications of Marine Enzymes

Innovative screening and expression platforms to discover and use the functional protein diversity from the sea

[www.inmare-h2020.eu](http://www.inmare-h2020.eu)

The INMARE project brought together facilities, biotechnology tools, genetic resources and scientific experts from more than 20 academic and industrial partners across 12 countries, to mine for and use newly-discovered marine microbial enzymes and metabolites for the targeted production of fine chemicals, environmental clean-up technologies and anti-cancer drugs.

The main objectives of INMARE were to:

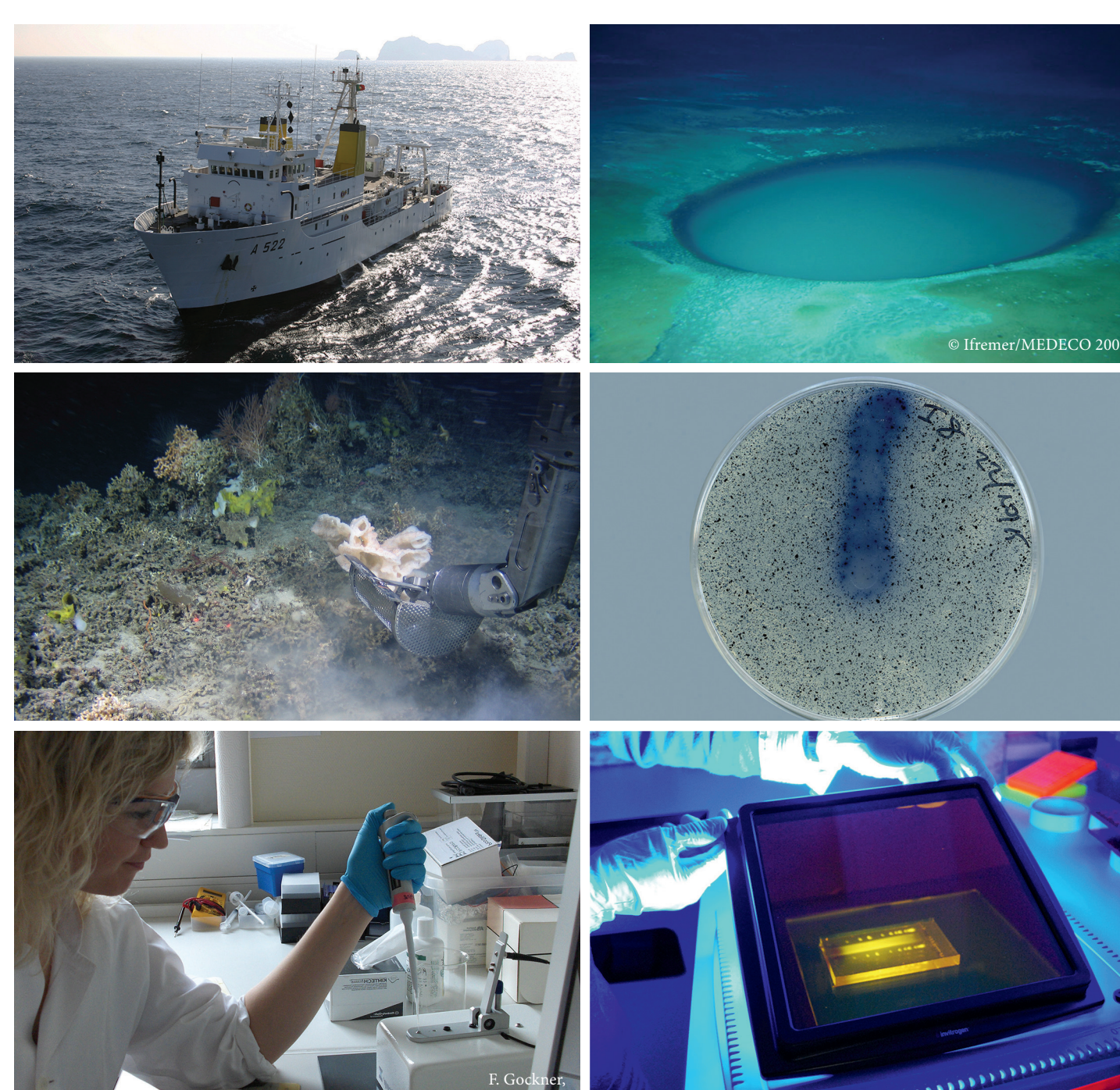
1. Streamline and significantly shorten the pipelines of marine enzyme and bioactive compound discovery for industrial applications;
2. Develop marine enzyme collections with a high proportion of promising enzyme-allrounders;
3. Identify new lead products and deliver prototypes for new biocatalytic processes based on marine microbial enzyme resources for targeted production of fine chemicals, drugs and materials for use in environmental clean-up applications.

## Marine enzymes for industrial applications

With a growing global population our societies are facing important challenges in food and energy security, sustainable economic development, maintaining the health of ageing populations and protecting the environment from over-exploitation, degradation and pollution. While terrestrial resources are being depleted, our seas and oceans offer a sustainable source of materials, food and energy, as well as potential for the development of new drugs and biotechnological applications.

Marine bio-resources hold particular promise for innovation in industrial biotechnology - a growing sector developing "greener" bio-based alternatives to current chemical production processes that use environmentally damaging bulk organic solvents and energy-demanding processes.

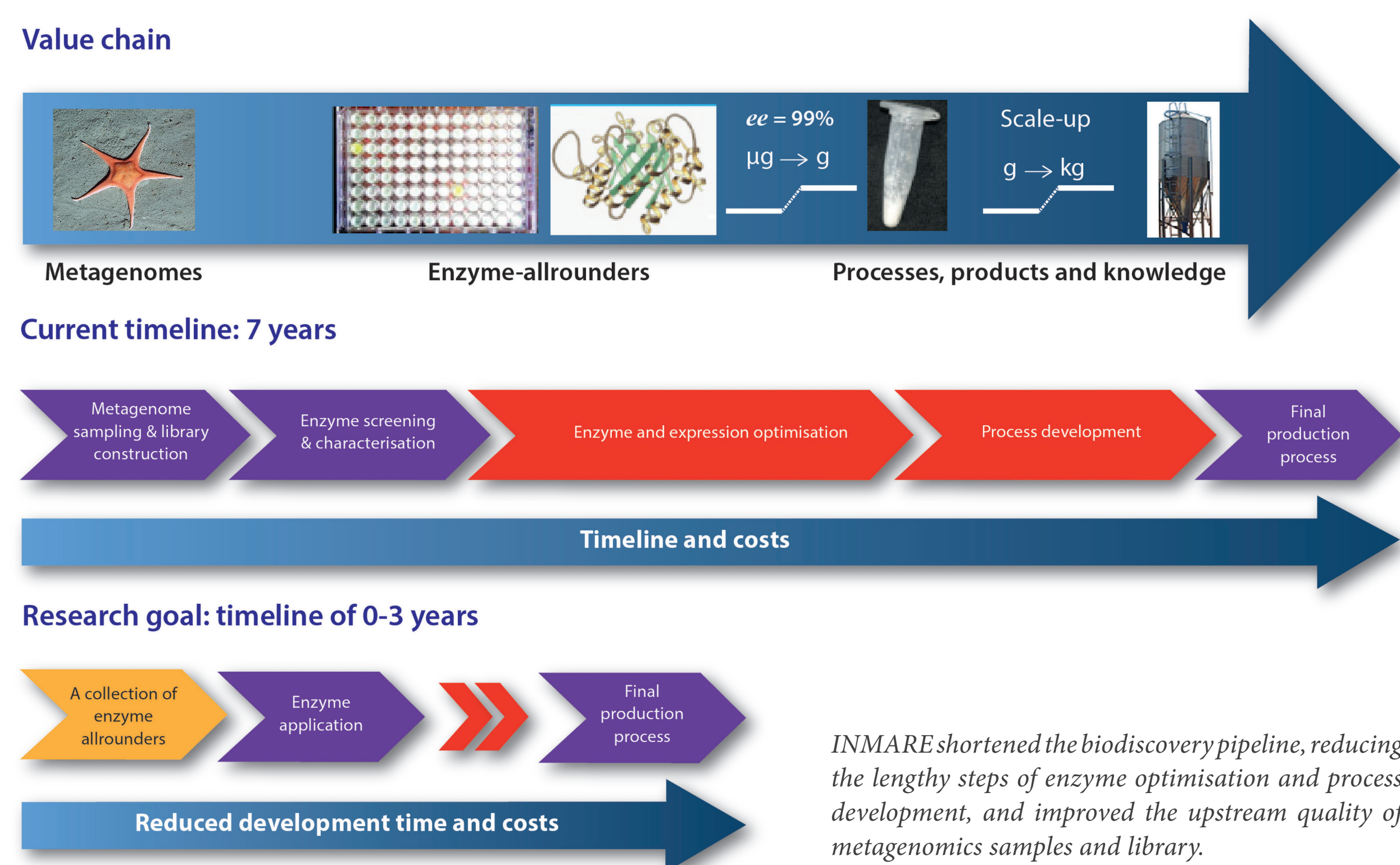
Most industrial biotechnology processes are derived from micro-organisms. It is anticipated that new processes and applications will emerge from research on as-yet unknown microbial biodiversity: "microbial dark matter". An area that remains largely untapped as a source of microbiological organisms is our ocean: the marine environment could provide new resources from which to produce safer, cheaper and greener products and industrial processes.



Life began in the ocean and 3.5 billion years of evolution has given rise to a wealth of untapped genes, enzymes and natural products that could have industrial application. Marine microbes are of special interest because they dominate our planet in terms of cell numbers, numbers of species, total biomass and the range of environmental conditions in which they can grow.

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 conditions could provide a source of enzymes uniquely able to perform in industrial settings characterised by harsh physical and chemical conditions.

INMARE partners have assessed enzymatic potential using genetic resources available from previous research efforts, but have also constructed novel gene libraries by collecting samples from new environments across about thirty hotspots of marine microbial biodiversity in the search for relevant enzymes and bioactive compounds.



## Streamlining the enzyme discovery pipeline

Despite their potential for industrial applications, to date very few (marine) microbial enzymes have actually made it to the commercial market. While approaches such as metagenomics have provided new avenues for developing novel applications from marine microbes, many challenges remain. One of the major bottlenecks is the laborious, costly and unreliable enzyme optimisation process required to make enzymes more stable and perform better in industrial processes.

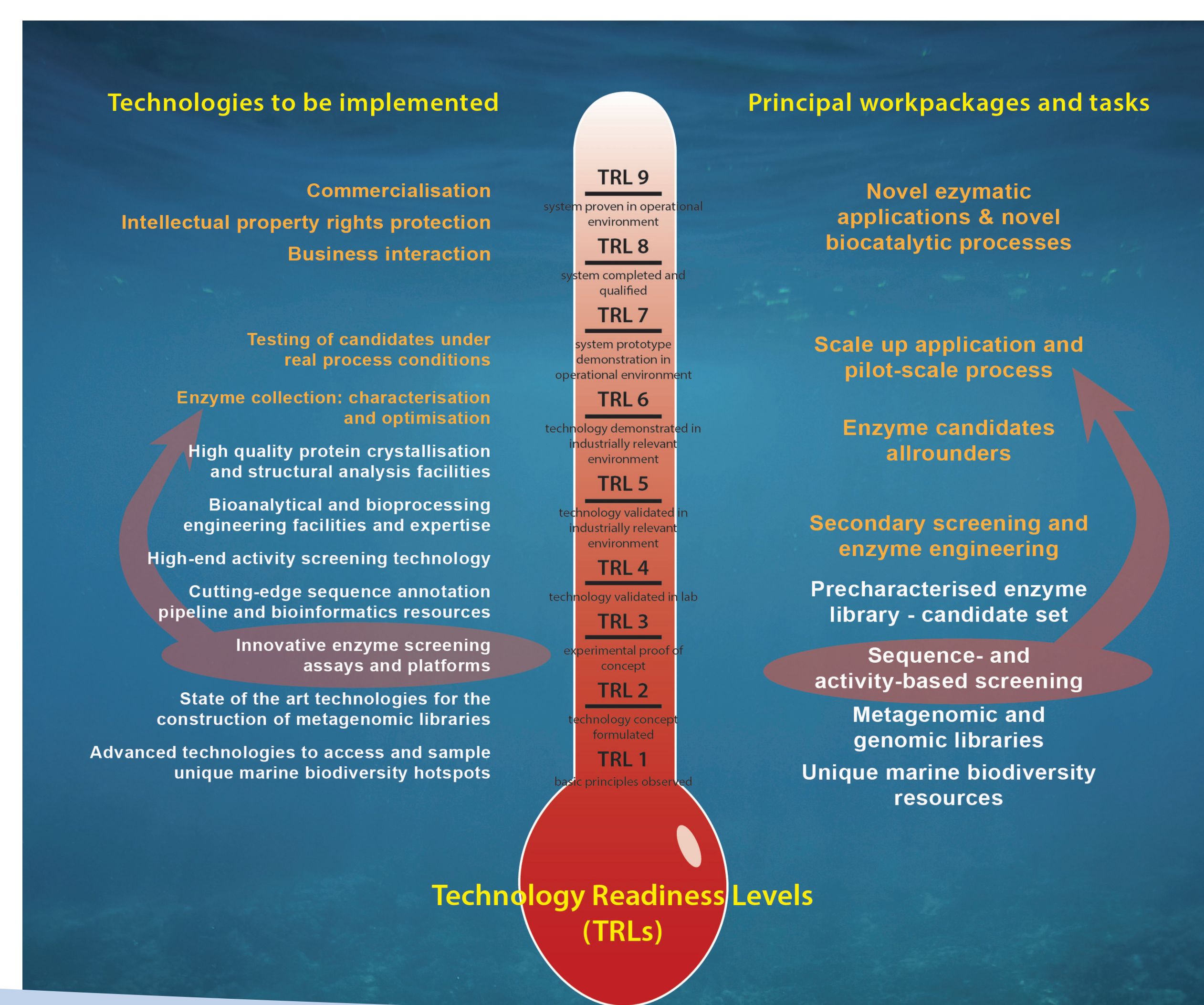
To circumvent this problem, the INMARE project focused more attention on the early stages of the biodiscovery pipeline towards finding better natural enzyme variants - so called **enzyme-allrounders** - that perform a range of desirable functions under a set of realistic industrial conditions.

INMARE aimed to streamline and shorten the biodiscovery pipeline, addressing all steps from sampling marine biodiversity hotspots, through to enzyme and bioactive compound discovery and development of prototypes. This involved state-of-the-art approaches and technologies covering various Technology Readiness Levels (TRL). Emphasis was placed on TRL 1-6, with the development and implementation of innovative approaches to bypass or significantly shorten the cumbersome enzyme and expression optimisation stage. Within the lifetime of INMARE, new enzymatic applications corresponding to levels TRL 7-9 have been brought to the market.

## Key INMARE outputs

To achieve the project objectives, INMARE scientists have performed the following key activities:

- Sampled known and novel marine biodiversity hotspots resulting in a curated catalogue of samples, genomic & metagenomic libraries;
- Developed state-of-the-art in vitro and in vivo screening platforms allowing earlier identification of relevant enzymes and bioactives;
- Constructed novel sequence analysis pipelines which target industrially relevant enzymes;
- Streamlined gene identification in positive clone hits and in sequencing data;
- Established multiple and complementary in vitro expression platforms facilitating production of enzymes of interest;
- Identified markers of enzyme promiscuity, thus accelerating identification of enzyme-allrounders;
- Shortened the enzyme optimisation and processing steps by testing enzyme candidates under application conditions at the early discovery stage;
- Discovered versatile biocatalysts and developed and designed small-scale reactors allowing assessment of biocatalytic performance of previously unannotated enzymes;
- Delivered one of the most extensive, curated collections of enzymes worldwide, some of which are already performing better than current commercial products;
- Advanced discussions regarding access and utilisation of genetic resources in research & development resulting in recommendations to support Due Diligence and ABS Compliance under EUR 511/2014.



## The INMARE partnership

Bangor University, UK (Coordinator)  
University of Hamburg, Germany  
Heinrich Heine University of Düsseldorf, Germany  
Consiglio Nazionale delle Ricerche, Italy  
Agencia Estatal Consejo Superior de Investigaciones Científicas, Spain  
Bayer Technology Services GmbH, Germany  
Novozymes A/S, Denmark  
National University of Ireland, Cork, Ireland  
Institute of Biochemistry, Vilnius University, Lithuania  
Jacobs University, Germany  
Pharma Mar S.A., Spain  
Technical University of Crete, Greece

University of Bologna, Italy  
Associação do Instituto Superior Técnico para a Investigação e Desenvolvimento, Portugal  
evoxx GmbH, Germany  
INOFEA Ltd, Switzerland  
University of Applied Sciences and Arts, Switzerland  
London School of Economics and Political Science, UK  
Cluster Industrial Biotechnology CLIB2021, Germany  
University of Toronto, Canada  
Seascope Belgium, BE  
NORCE Norwegian Research Centre, Norway  
University of Milan, Italy

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