

KETmaritime Case Study 3 - Marine Industrial Biotechnology

A pioneering scientific centre in northern Portugal has led a major two-year project studying advanced manufacturing technologies with the potential to revolutionise the Atlantic maritime sector. The International Iberian Nanotechnology Laboratory (INL) located in Braga is the lead partner in the €1million 'KETmaritime' project backed by the European Regional Development Fund. Project coordinator Ana Vila said the INL is now releasing details of a third case study focusing on 'Marine Industrial Biotechnology'. She said the pan-European project identifies how advances in this field will benefit industrial processing for the production of chemicals, materials and fuels.

Key findings from the KETmaritime project are now being revealed following two years of intensive international work involving a world-class consortium of partners across Spain, Portugal, France, Ireland and the UK. Its mission is focused on identifying KET (Key Enabling Technologies) to meet the future needs and demands of the Atlantic maritime industry.

Project partners include French multidisciplinary research laboratory CIMAP (CEA group), Portuguese maritime economy association Fórum Oceano and Spanish technology centre IDONIAL. Ireland's national centre for marine and renewable energy MaREI, UK marine cluster organisation Marine South East and Spanish non-profit research association AIMEN.

A total of five detailed case studies have been produced under the €1million initiative. Topics include 'Advanced Manufacturing Shipbuilding Applications', 'Nanotechnology Marine Applications', 'Marine Industrial Biotechnology', 'Photonic Marine Applications' and 'MEMS (Micro Electromechanical Systems) Marine Applications'.

Industrial Biotechnology - cleaner manufacturing processes

The third case study led Spanish technology centre IDONIAL in collaboration with Marine South East in the UK focuses on 'Marine Industrial Biotechnology' (access <http://ketmaritime.eu/media/> to view the full report). In addition, the International Iberian Nanotechnology Laboratory (INL) provided key input. The INL was created by the Portuguese and Spanish governments as a world-wide hub for the deployment of nanotechnology addressing society's grand challenges.

The European Commission defines 'industrial biotechnology' - also known as white biotechnology - as the application of biotechnology for industrial processing and production of chemicals, materials and fuels. The definition includes the use of microorganisms and enzymes to generate new products or to improve the sustainability of manufacturing processes. It is being used to great effect to replace polluting technologies with cleaner alternatives, while increasing process efficiency.

Biotechnology is commonly split into several other areas including 'red biotechnology' for biopharmaceutical processes including the development of new drugs, vaccines or antibiotics. 'Green Biotechnology' involves agriculture processes such as the generation of new plant varieties, biofertilizers and pesticides. Lastly, 'blue Biotechnology' is used within the marine sector to explore and exploit marine resources to develop industrial products and processes.

While the study of biotechnology is split into sectors, it remains highly interchangeable with large synergy for instance between 'industrial' and 'marine' biotechnology.

Applying 'Industrial Biotechnology' to the marine environment

Following analysis of the EU's strategy on industrial and marine biotechnology researchers driving the KETmaritime project identified a number of key applications involving a high level of joint development.

This includes areas such as 'Novel Enzymes and Micro-Organisms'. The development of novel bioprocesses demands new enzymes and micro-organisms with high performance qualities, which are able to withstand challenging environments such as high temperature and pressure. The marine environment offers an excellent source for these kinds of enzymes and micro-organisms, which have adapted to live in extreme conditions.

Secondly, 'Marine Biomaterials and Biopolymers'. The marine environment is again the source of a variety of components such as proteins and peptides (collagen, gelatine), polysaccharides (alginate, carrageenan, agar, chitin, chitosan), fatty acids (omega-3, DHA, EPA), vitamins and minerals. The main sources of marine biomaterials are marine algae, marine invertebrates, sponges, molluscs, echinoderms and crustaceans.

Lastly, 'Bioenergy' involving biofuels derived from the marine environment are being used as a potential source of sustainable energy contributing to future global demands. Marine algae is the most relevant source of bioenergy within the marine environment and marine biofuels production.

Marine enzymes and Micro-organisms - natural catalysts boosting chemical reactions

Enzymes are natural catalysts that can be used to improve chemical reactions. Enzymes can be isolated and purified from a wide range of microorganisms, animals and plants. However, the increasing use of enzymes in industrial processes demands for novel enzymes with improved properties - for instance resistance to pressure and temperature.

The marine area offers huge potential in terms of discovering new novel enzymes, due to its complete contrast to the terrestrial environment, involving high salinity, high pressure, low temperature and special lighting conditions. Industrial

processes can greatly benefit for the use of marine organisms, or extremophiles, adapted to live in these extreme conditions.

Examples include 'Halophilic' enzymes with PH and salt tolerance. One area of biotechnological interest related to these organisms involves the production of biopolymers with halophiles. Additionally, the microbial production of ethanol from carbohydrates (biofuels) is mainly dependent on the process of saccharification requiring enzymes to hydrolyze carbohydrates before fermentation. Another possible application of this kind of enzyme involves the environmental remediation domain with halophilic enzymes used to significantly improve environmental pollution which occurs in saline conditions.

Other marine derived enzymes include 'Thermophiles' which offer hyper-thermostability. Within industrial processes at high temperatures they present many advantages related to the increase of substrate solubility, reduction of viscosity and increased reaction rates. However, most enzymes degrade with high temperatures. Therefore, the use of enzymes of marine origin that are resistant to high temperatures could offer significant improvements to those processes.

Furthermore 'Psychrophile' enzymes provide cold adaptivity which is of great potential within biotechnological processes. For example, their use could reduce heating steps helping to reduce energy consumption and increase the thermal protection of reactants and products. Certain marine-derived enzymes also demonstrate chemical or stereochemical properties, such as substrate specificity and enantioselectivity, which can be exploited in organic synthesis processes.

One of the key challenges across marine biotechnology involves the exploration of the marine environment in the search for novel materials and organisms. While the seas and oceans hold major potential there are technical challenges related to the difficulties and costs of accessing areas outside coastal zones, including deep-water exploration. Technological developments including Remotely Operated Vehicles (ROVs), Autonomous Under-water Vehicles (AUVs) and remote systems for in-situ analysis are greatly improving access to the marine space.

In addition to the challenge of exploring seas and oceans, more than 99pc of identified organisms cannot be cultured in laboratories. This is where genomic technologies can provide information about the uncultured microbial world and help direct the discovery of novel enzymes. Furthermore, traditional and novel taxonomic approaches, including molecular based methods or chemical and biochemical analysis, with the creation of repositories or biobanks for marine materials, can support the identification of marine species.

Marine enzymes - Fields of activity and production industry

There are many fields of activity which could benefit from the application of marine derived enzymes. Within biorefineries for instance the use of extremophiles and thermostable enzymes can help to overcome biocatalyst limitations in current lignocellulosic biomass conversion (cellulases, pectinases, proteases, amylases, etc.). Additionally, high resistant lipases could improve the conversion of feedstock oils into biodiesel.

For many years the marine environment has also been seen as a promising source of catalysts for food applications. Efforts are currently focussed on the recovery and processing of food wastes to convert them into valuable by-products. Meanwhile, marine biomarkers can be used for pollution monitoring. Bioremediation technologies are based on the use of natural organisms that metabolically transform toxic products into less dangerous substances, which naturally lends itself to marine-derived enzymes with stereochemical properties which can significantly affect these processes.

There are several research and development projects geared towards the discovery of new and improved marine-source enzymes. For example, the INMARE Project (Industrial Applications of Marine Enzymes) is a collaborative effort founded by the European Commission under the Horizon 2020 Programme. The project has been designed to streamline pathways of discovery and industrial applications of new marine enzymes and bioactives for targeted production of fine chemicals, drugs and environmental clean-up applications.

Companies across Europe have developed enzymes from marine sources which are now commercially available. They include 'ArticZymes' from Biotec Pharmacon, Norway. The company develops and markets recombinant enzymes derived from cold-water marine species. BASF in Germany has developed 'Fuelzyme®' alpha-amylase, a marine-source enzyme which can be used for starch liquefaction in the production of biofuels. Novozymes in Denmark has invested in the study of cold-activity enzymes across arctic regions and manufactures a wide range of enzymes for multiple industrial applications. In addition, Zymetech in Iceland specialises in research, purification and utilization of cold adapted enzymes from deep sea cod fish.

Marine Bio-based polymers

Bio-based Polymers refers to polymers that are totally or partially derived from biomass. They can be produced from different kinds of biomass feedstock. Bio-based 'biodegradable' biopolymers are of particular interest to the maritime sector. Apart from the potential use of marine biomass as feedstock to produce these polymers, their biodegradability could help partially reduce the problem of marine litter - considered by the European Commission as a main current threat to the environment.

The most important bio-based biodegradable biopolymers are PHAs (Polyhydroxyalkanoates) and PLAs (Polylactic acid). PHA polymers are thermoplastic and can be transformed by means of injection-moulding to produce films and sheet, fibers, laminates, nonwoven fabrics and adhesives. PHA and its copolymers can also be used as biomedical implant materials. PLA production involves the generation of lactic acid and sugars. Once purified, the lactic monomer is subjected to chemical

polymerisation in order to be converted in PLA. PLA is also a thermoplastic polymer that can replace traditional polymers such as PET, PS, and PC for packaging applications.

Despite the opportunities offered by biopolymers several technical barriers remain largely related to improving efficiency and reducing costs. This has led to increased research to expand knowledge on production pathways and search for new biomass feedstocks from areas such as the maritime environment. For example, marine bacteria can be used to naturally produce PHAs, offering some potential advantages. Sterilized seawater can be used as a culture medium, eliminating the need for a synthetic medium, leading to savings on fresh water. Additionally, high seawater salinity inhibits contamination with other bacteria that lack salt-water resistance.

Naturally occurring biopolymers

Marine biomasses such as seaweeds are an excellent source of commercially important biomaterials. Polysaccharides such as agar, alginate, fucoïdan and carrageenan are obtained from algae. Agar, carrageenan and alginate are currently used as gelation and thickening agents in different food, pharmaceutical, and biotechnological applications.

Other naturally occurring biopolymers present in marine biomasses include 'chitin' and 'chitosan'. Both of which are currently commercially produced through chemical extraction processes involving marine sources such as crab, shrimp, and prawn wastes.

Chitosan shows interesting characteristics such as biodegradability, biocompatibility, antimicrobial activity, non-toxicity, chemical inertness, high mechanical strength, good film-forming properties and low cost. These features make this polymer suitable for a wide variety of applications including waste-water treatment, agricultural materials, food and feed additives, biomedical and pharmaceutical materials, wound-healing materials, blood anticoagulant, antithrombogenic and haemostatic materials, cosmetic ingredients, textile, paper, film and sponge sheet materials, as well as analytical reagents.

The exploitation of fish and shellfish biowastes to produce chitin and chitosan presents a large business opportunity for the EU market. The global chitin market is expected to reach \$2900m by 2027. The healthcare segment, in particular, is projected to grow at the highest rate, with waste and water treatment applications also offering significant growth opportunities.

Companies already heavily involved in the production of marine 'chitin' and 'chitosan' derivatives include Chitocan from Canada which produces high-quality medical-grade chitosan from shrimp shells. Advanced Biopolymers in Norway produces high-quality chitosan for medical (wound care), cosmetics and drug delivery applications. AgraTech - KYTOSAN USA produces chitosan-based products for industrial, consumer, pharmaceutical, and agricultural markets. In addition, BioLog Heppe in Germany produces a range of chitosan products with various qualities for applications in water and wastewater treatment, agriculture, paper and textile industry, cosmetics and pharmacy.

Marine bio-based polymers current challenges

The bio-based plastics sector is developing rapidly. However, bio-based plastics remain too expensive to compete with conventional fossil plastics. The price is mainly driven by the cost of feedstocks and processing steps. Therefore, the greatest challenge involves reducing production costs along the whole value chain.

Feedstock selection is crucial for increasing process efficiency. First-generation feedstocks like sugar, cane or corn compete with the production of food. Therefore, second and third generation feedstocks such as algae, marine biomass or waste streams are needed in order to increase process implementation. Additionally, the use of mixed bacterial cultures or novel microorganisms can increase process performance.

Novel bio-polymers can be processed with standard processing equipment if their properties are similar to those of the fossil counterparts. However, replacing a conventional plastic with a bio-plastic could require a re-definition of the manufacturing process, especially in what relates to polymer additives. Therefore, new additive chemistry must be developed in order to improve the performance and properties of bio-based polymers.

Other aspects of the value chain must also be addressed such as the development of logistics for biomass feedstocks, new manufacturing routes that could increase yields, new microbial strains/enzymes, and efficient downstream processing methods for recovery of bio-based products.

Microalgae industry moving to bioplastics generation

The global bioplastics and biopolymers market is projected to surpass \$5Billion by 2021. In terms of application, the 'packaging' sector is projected to account for the highest market share. Demand is being driven by a widespread move across many countries to prohibit or limit the use of conventional plastics. However, in order to realise the growth forecast, the market faces challenges to decrease production costs and improve infrastructures.

A number of major players are currently using marine feedstock as a source for biopolymers manufacturing. They include BASF which is working with several collaborators to develop new algae-base products and processes. One such project involving Solazyme involves the development of a surfactant derived from microalgae oil for use in home and personal care

applications. BASF has also commercialised marine-based products such as Omega-3 Powders extracted from seafood, oils from fish, krill and algae.

Other companies are specifically focusing on producing bioplastics from algae. SOLAPLAST® is using bioplastic technology to blend aquatic feedstocks with commercial polymers to reduce cost and dependence on fossil-fuel and food-based feedstocks.

Further developments have seen in the Netherlands with Studio Klarenbeek & Dros working alongside French firm Atelier Luma to develop algae-based biopolymers to compete with traditional plastics. The material can be applied on an industrial scale and processed like traditional plastic. It has proven to be suitable for injection moulding with 3D printing processes being the main focus for the polymer. In Italy, Algamoil and Teregroup are also working on the development of 100pc biodegradable plastic made with algae in the form of filaments perfectly compatible with 3D printers.

Wider works has seen Biopolymer in Sweden producing MAP (Mussel Adhesive Protein), the molecule that binds mussels to different kinds of structures. MAP can form strong bonds to human tissue and close surgical cuts. No stitches are needed and risk for inflammation and scars are dramatically reduced. On metal surfaces MAP can bind strongly and crosslink to a nano-thick surface layer. By enhancing the redox function this layer will give the surface a very efficient corrosion protection and can be used as an anti-corrosion primer.

Marine-Based Biofuels

Aquatic biomasses such as macro and micro algae and photosynthetic cyanobacteria have the potential to be used as biomass for biofuel production. Aquatic biomass presents major advantages over other kinds of biomass including greater yields per area of cultivation, non-competition with arable-land, use of sea water, wastewater or saline water for cultivation, use of CO₂ as carbon source or wastewater as nutrient input, and non-competition with food and feed applications.

The composition of algae biomass consists of carbohydrates, proteins, lipids and other substances - with lipids being the most important component for conversion into biofuels. Macroalgae, is largely cultivated offshore in open systems while microalgae can be cultivated onshore in either open ponds or closed systems.

Algal biomass feedstocks can also be generated by heterotrophic fermentation, where organic carbon is supplied to algae as a source for aerobic growth. However, the primary targets of the heterotrophic cultivation development are high-value food and feed applications. Many companies are already commercially producing heterotrophic algal oils for higher value product applications in the food, feed and nutraceutical markets.

Algae-based biofuels current challenges

There have been major advances in the production of biofuels from algae in recent years driving efficiency and productivity. However, the effective industrial implementation of algae as a source for biofuel generation still faces challenges related to all process steps, from feedstock cultivation to conversion and infrastructures.

In terms of feedstock, stronger development of ecological, genetic and biochemical aspects of different algal species is required to improve productivity and robustness of species. A deeper understanding of culture dynamics and stability is also needed to up-scale process performance and cost-effectively manage resources for biomass production. Additional research is required to maximize the recycling of nitrogen, phosphorus, carbon and other nutrients from residual materials, while advancing understanding of CO₂ utilization on an industrially relevant scale. Another area requiring attention involves the development of harvesting, dewatering and drying technologies at industrially relevant scales.

Within the conversion step further investigations will be made into the techno-economic impact of up-scaling, as well as fuel conversion to optimise fuel and nutrient recovery at industrially relevant scales, while minimising energy use, emissions and contaminants. Further innovation is also required to optimize co-product extraction and recovery, as well as quality and safety trials to meet applicable standards.

Challenges within the infrastructure process involve characterising algal biomass, intermediates, biofuel, and bioproducts under different storage and transport scenarios for contamination, weather impacts, stability, and end-product variability. Further advances will need to be made to better understand the integration of CO₂ waste emitting industries such as wastewater treatment plant co-locations with algal cultivation facilities.

Microalgae industry moving to biofuels generation

According to global market reports, the global algae biofuel market was valued at circa \$4.7billion in 2017 and is expected to grow at a CAGR (Compound Annual Growth Rate) of around 8pc between now and 2024. A series of companies are currently commercializing marine-based biofuels. They include Algenol in the USA which produces biofuels using proprietary algae through a two-step patented process. Fellow US firm Cellana uses marine microalgae to photosynthetically produce biofuel feedstocks along with Omega-3 oil and animal feed/food under the concept of integrated algae-based biorefinery.

Italian company Algamoil designs and constructs plants for the production of vegetable oils and biodiesel from algae with a patented and certified technology. Meanwhile Spanish outfit AlgaEnergy uses microalgae and cyanobacteria as feedstock, to



produce products for feed/food, agriculture and the cosmetics industry, as well as biofuels. Around a decade ago, ExxonMobil Corporation and Synthetic Genomics began collaborating on a joint algae biofuel research program with an objective to produce 10,000 barrels of algae biofuel per day by 2025.

A number of companies are also focusing efforts on the design and development of new algae cultivation systems (ponds and photobioreactors) for the final production of bioproducts such as biofuels. They include A4F AlgaFuel in Portugal dedicated to the research and development of bioengineering projects for the industrial production of microalgae based products and applications. In the Netherlands AlgaeLink is working in collaboration with KLM on the development of microalgae-based aviation fuel, and British Algoil in the UK provides a photobioreactor using LED lighting technology to provide algae with the optimal light conditions.

For more information on the KETmaritime project visit the website www.ketmaritime.eu phone +351 253 140 112 email Ana Vila on Ana.Vila@inl.int

Project Partners:



Notes to editors:



KETmaritime is a €1million project that aims to transfer Key Enabling Technologies (KETs) to the Maritime Industries of the Atlantic Area. Focused on building a cooperative network that stimulates innovation and competitiveness, the project embraces the challenge of increasing the capabilities, skills and knowledge on KETs in an integrated and cross-national approach. The consortium brings together seven partners from five countries that, under the framework of the project, work closely with industries and businesses. The ultimate goal is to bring to market new processes, products and services. Partners in the consortium include French multidisciplinary research laboratory CIMAP (CEA group), Portuguese maritime economy cluster Fórum Oceano and Spanish industrial design centre IDONIAL. Ireland's national centre for marine and renewable energy MaREI and UK marine cluster organisation Marine South East are delivering further support, alongside Spanish non-profit research association AIMEN. KETmaritime is a project funded by the Interreg Atlantic Area Program, supported by the European Regional Development Fund.