Bone-degrading enzymes found in the free-living bacteria on the seabed

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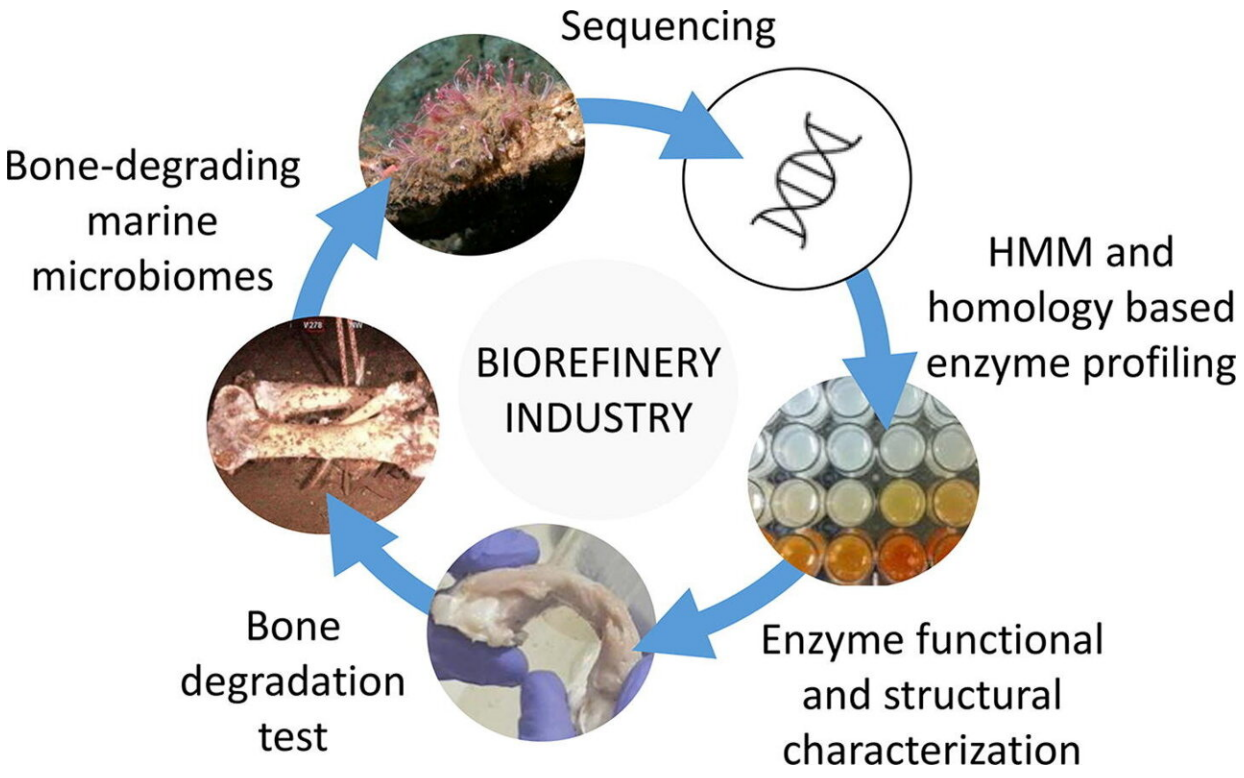

Graphical abstract. Credit: DOI: 10.1016/j.csbj.2021.11.027

Why is placing pieces of bone on the seabed so important?

Well, the scientists have hypothesized that the pieces of bone attract ocean organisms that have the right enzymes to break down bone mass.

It turns out that the scientists were right. NORCE researchers in Bergen, Norway, and their international colleagues, are now presenting the exciting results from their enzyme hunt in the *Computational and Structural Biotechnology Journal*.

In a sustainable circular bioeconomy, new and better products of high value—made from protein-rich residual raw material from cattle and poultry—are something the industry would like to offer.

There are already some commercially available enzymes for new processes that can break down bone mass. However, the industry is constantly on the lookout for new, natural enzymes that can perform the job of degrading bones even better.

This hunt may be about to yield results.

The scientists' findings show a unique brew of enzymes

"We have succeeded in putting together a unique enzyme cocktail, one which breaks down bones more efficiently than individual enzymes. We have already noticed that our findings are arousing interest in the industry," says NORCE scientist Antonio Garcia-Moyano, co-author of the new study.

The study is led by Spain's largest public research institution called the CSIC Institute of Catalysis, and the renowned German marine research institute called GEOMAR Helmholtz Centre for Ocean Research Kiel is also contributing.

The road to an effective brew of enzymes has not been straightforward.

It takes much more than just lowering bones down to a depth of 60–100 meters in Byfjorden, just outside Bergen, leaving the bones there in pots for several months, and hoping that valuable enzymes suddenly turn up.

If it had been that easy, anyone could have "hauled in a catch." On the contrary, considerable knowledge, creativity and patience are needed to succeed in the hunt for these enzymes.

Garcia-Moyano and his colleagues possess all of these qualities.

Microbial enzymes are the key

Since 2017, scientists have been working on the ambitious NORCE-led project called ProBone, together with international partners, to find enzymes that are involved in bone degradation.

When the work started, NORCE's Garcia-Moyano and project manager Gro Bjerga had a theory that both free-living bacteria in the ocean, and bacteria living inside animals that are attracted by shiny bones on the seabed, contain enzymes that have degrading effects on bone mass.

They eventually found enzymes in the free-living bacteria that could degrade bones.

A good starting point

Before we go any further, let us briefly explain what enzymes are, and what they do.

Enzymes are tiny chemical compounds that are used, among other things, in the processing of biomass, such as residual raw materials from cattle and poultry, fish or wood, and help to create completely new

products.

Enzymes with the right properties play a key developmental role in many industries and are relevant regarding the green shift.

In practice, enzymes act as catalysts, they participate in chemical processes by speeding them up, without being consumed themselves.

This is an ingenious ability, but one which also has a few small problems. For example, many enzymes are very sensitive to heat, which means that they risk losing their properties at a given temperature.

"There is a great need to discover both better and new enzymes for the industry. And find the right combinations, just like in a cocktail. This new study provides us with a good starting point where we see that the enzyme cocktail has a greater effect than single enzymes when it comes to bone degradation," says Garcia-Moyano.

Advanced utilization of bone residues

But why is it so important to establish that a mixture of enzymes is more effective at breaking down bones, you might be wondering?

Previously, the utilization of bones has often taken place without any advanced chemical process. This was often done by boiling bone residues to extract gelatin, grinding them into bone meal, or also using them as an ingredient in fertilizers.

There are now several examples of industrial operations in Norway that are based on enzymatic processes involving both fish and meat.

Norilia, a subsidiary of the food manufacturer Nortura, is one of the actors behind Bioco. There is a new, modern biotechnology facility at

Hærland that specializes in the enzymatic hydrolysis of bone residues from chicken and turkey.

"We chose enzymatic hydrolysis because it is a controlled and gentle process that allows us to produce sought-after ingredients for high-value markets. We currently sell the ingredients for pet food in Norway and export some of it as protein supplements for human consumption. We are also working on developing a sports nutrition concept," says Director of Business Development at Norilia, Heidi Alvestrand.

When it comes to improved processes regarding the scientists' enzyme cocktail, new possibilities are on the horizon.

"We hope new enzymes can contribute to even better utilization of residual raw materials from the industry, through the development of differentiated ingredients adapted to different applications," says Heidi Alvestrand.

The industry can extract more value out of the raw materials

Going forward, Antonio Garcia-Moyano and his colleagues at NORCE will identify more enzymes and better combinations of cocktails that break down bones better and faster.

He explains that one usually looks for a single enzyme that can do the job in a particular process. The approach of enzyme cocktails represents a more future-oriented and more innovative way of looking at solutions.

"More high-quality products can be made from an enzymatic process involving a functioning cocktail. The industry can simply get more value out of the residual raw materials, and this is an ideal way of making the

meat industry more sustainable, for example. You use the whole animal, increase the value of the products and support the circular bioeconomy," says Garcia-Moyano.

Removing bottlenecks in marine biotechnology

The goal of the ProBone project has been to find enzymes that actually break down bones, and also to bring about new knowledge and innovation by using technology and developing new methods to solve previous bottlenecks in marine biotechnology.

This work will now continue in new projects. One example is in a dedicated Centre for Research-based Innovation (SFI Industrial Biotechnology) where NORCE is among the research partners. One of the sub-projects deals specifically with enzymes.

"Among other things, we will continue to work on optimizing the enzyme cocktail in relation to industrial conditions. This will entail things such as finding out which enzymes are optimal for bone degradation when placed together, and which also tolerate high temperatures the best," says Garcia-Moyano.

More information: Laura Fernandez-Lopez et al, The bone-degrading enzyme machinery: From multi-component understanding to the treatment of residues from the meat industry, *Computational and Structural Biotechnology Journal* (2021). DOI: 10.1016/j.csbj.2021.11.027

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