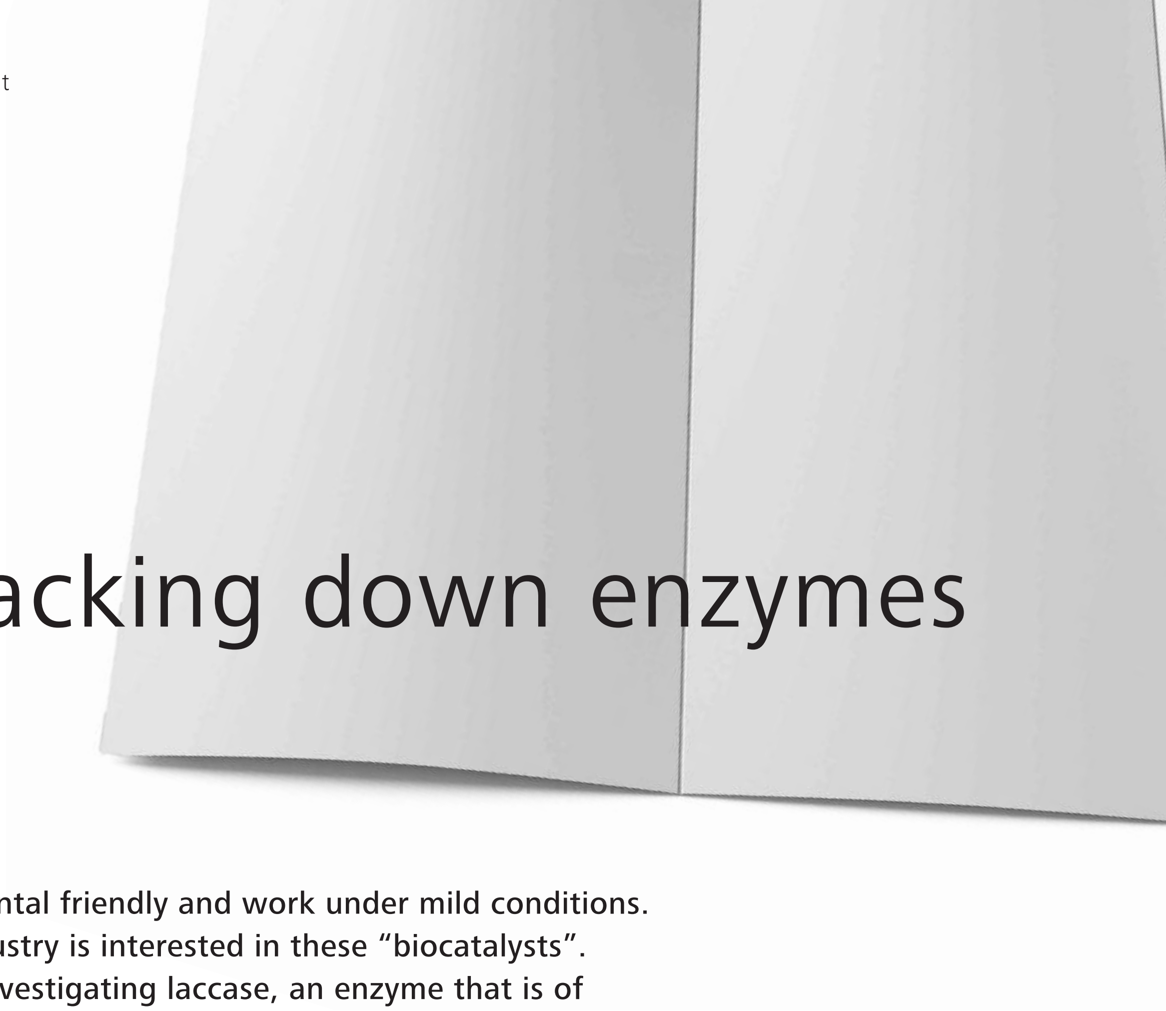

Tracking down enzymes

Enzymes are environmental friendly and work under mild conditions. It’s no wonder that industry is interested in these “biocatalysts”. Empa researchers are investigating laccase, an enzyme that is of particular interest for the textile and wood-processing industries. Here, interdisciplinary cooperation is essential.

TEXT: Nadja Kröner / PHOTOS: iStock, Empa

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Laccase is an enzyme which in nature acts as a catalyst for both the synthesis and decomposition of lignin, the main component of cells in plants. In industry it could prove to be quite useful as, for example, a biobleaching agent for the pulp used in the paper industry.

2
Reaction of the laccase enzyme with a colour-forming substance on an agar plate (blue-green colouring).

3
The filamentous fungi secrete the enzyme laccase into the culture medium.

Wood is a biomaterial. That’s why there’s a good opportunity for collaboration between Empa’s Wood Laboratory and Biomaterials Laboratory. Their common subject of research is laccase, an enzyme which is found in bacteria, fungi and higher plants and acts as a catalyst for both the synthesis and decomposition of lignin, the main component of woody cells. Because the enzyme works under mild conditions – meaning in aqueous solutions, at room temperature and under atmospheric pressure – and because it builds up no poisonous by-products, it is also useful for industrial applications.

One example is the treatment of pulp for the paper industry. The enzyme breaks down the lignin which turns paper brown and thus acts as a biobleaching agent. Until now, paper has been chemically bleached, but that process pollutes the environment. Laccases, instead, are biodegradable, for instance in wastewater treatment plants. They are already being used to bleach jeans because they can break down the indigo dye typical in that clothing. A further possible application would thus be the enzymatic processing of waste water in the textile industry.

Interest in new, efficient and environmentally friendly processes has grown enormously in industry. And while laccases can be put to use in many chemical-engineering processes, its widespread use is not possible at this time. The enzyme cannot yet be produced at prices which would allow its use on a large scale. In addition, the laccases available today are partially not active or stable enough to compete with chemical processes. There’s still a bit of development work ahead.

Wood research meets biomaterials research

For quite some time, the Wood Laboratory has been doing research on the wood-decomposing effects of certain fungi which are known as brown rot and white rot pathogens. Here the goal is, on the one hand, to find out what kind of damage the fungi cause and how various wood constituents can be decomposed. On the other hand, the researchers are also investigating how these properties of fungi can be used to change the material properties of wood. It’s been known for a long time that laccase plays a decisive role particularly in the de-

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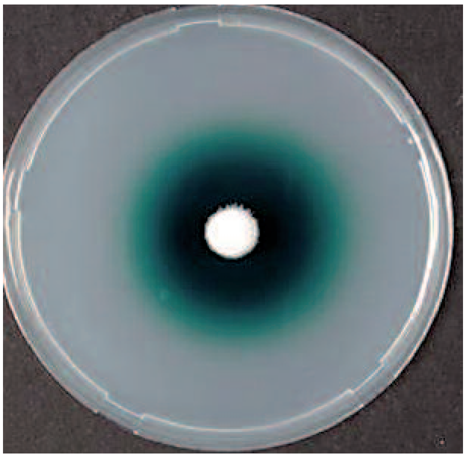

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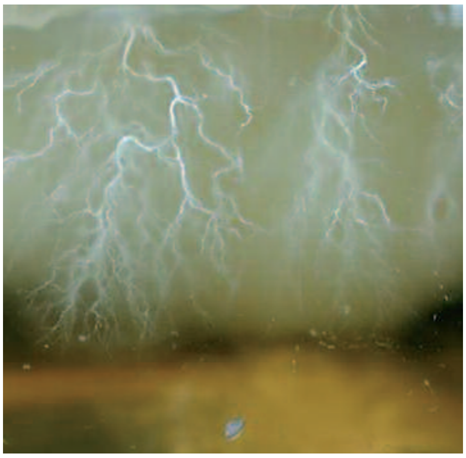

3

composition of lignin. This is where the enzyme specialists at the Biomaterials Laboratory came into the picture. In a common project, they proved that the build-up of laccases varies quite widely in white rot fungi, and even among various strains and under differing growth conditions. "Working with filamentous fungi is something rather exotic for us, and that's why collaboration with the experts from the Wood Laboratory makes so much sense", says Julian Ihssen from the Biomaterials Laboratory. In addition to laccases from fungi, other similar enzymes which occur in bacteria are being studied at Empa. Although bacterial laccases can basically be produced biotechnically more easily than those from fungi, there is still very little knowledge on these enzymes.

For technical applications, it's important that the properties of an enzyme are known in the greatest possible detail. That's because there's also a variation in the spectrum of molecules which can be transformed depending on the laccases originating from different fungi or bacteria. Further, the optimal conditions for the reaction such as temperature, pH value or solvents are different. Empa carried out related experiments with the help of miniaturised enzyme tests based on changes in colour. If it turns out that the properties of naturally occurring laccases are inadequate for industrial applications, there exist further possibilities to improve the enzyme in the laboratory through directed evolution. This technique, which is becoming increasingly important in biotechnology, has been established over the past two years in the Biomaterials Laboratory.

Every laccase has its optimal mediator

In order to accelerate reactions with laccases or to make these reactions possible at all, what are known as mediators are put to use. These are molecules that "mediate" between laccase and the substance to be decomposed. In other words, the laccase reacts with the mediator, which in turn reacts for example with lignin or dye, and in this way it is retransformed into its original state, which means once again ready for the laccase. In this way even large amounts of substances or those which are hard to access can be efficiently decomposed. "The search for the right mediator for the right laccase and for the right application is complex. Sometimes it's just a matter of luck", according to Empa wood expert Mark Schubert.

Empa is already recording its first successes. A very high yielding laccase producer, the white rot pathogen *Heterobasidion annosum*, was identified with the help of a newly developed screening method and was used to produce laccases. Furthermore, they have been successful in using genetic-engineering methods to produce, purify and characterise an until now unknown thermostable bacterial laccase in *E. coli*.

Interested industrial partners have been identified in the areas of fine chemicals and wood processing, and further research in two projects financed by the Swiss Commission for Technology and Innovation CTI has been taking place since the end of 2010. The industrial application of laccases should not be far away. //