



X-Zyme – a novel beamhouse technology

Christopher Henzel, Marc Hombeck, Thomas Schneider,
Christopher Tysoe, Lanxess Deutschland GmbH;
and
Niels Henrik Soerensen, Novozymes A/S

Enzymes are indispensable and their applications rapidly growing—from food and drink and laundry detergents to newer industries such as renewable fuel production. Enzymes contribute to sustainability by reducing the environmental impact of industrial and consumer processes.

In a partnership for the tanning industry, Novozymes and Lanxess are combining patented biotechnology with chemical and global processing know-how. The two companies are introducing a novel beamhouse process that delivers high-quality pelts and significantly improved effluents. Genetic engineering, high-throughput robotic screening and cutting-edge three-dimensional molecular modelling were deployed to help develop the new X-Zyme process, which comprises Peltec X-Zyme S for soaking and Peltec X-Zyme U for unhairing.

The properties of enzymes

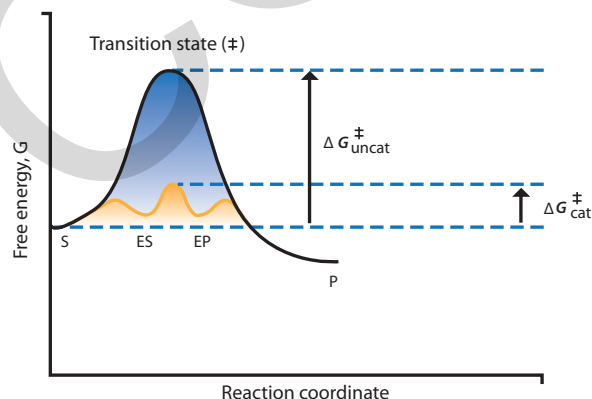
Enzymes are biological catalysts in the form of proteins. They catalyse chemical reactions in the cells of living organisms and are abundant all around us. Without enzymes, life as we know it would not exist. Biotechnology makes it possible to mass produce an enzyme in amounts that can be successfully applied to industrial processes.

Enzymes typically, but not exclusively, work under mild conditions. This enables successful processing at moderate temperatures and pH levels. As catalysts, enzymes lower the activation energy of chemical reactions (GCAT) compared to the non-catalysed reactions (GUNCAT), which also results in higher reaction rates as indicated in *Panel 1*.

Enzymes are highly specific both in the processes they catalyse and the substrates they work on. This has generally been depicted by a key-lock system where a given enzyme requires an exactly fitting substrate in order to react in its specific manner. Substrates



Panel 1: View of free energy transition states comparing catalyzed with non-catalysed reactions.



that do not fit within the specifically shaped active side of the enzyme will not react.

Enzymes are generally categorised in six different classes based on their reaction profiles. Of these six classes, hydrolases are best represented in industrial processing today. These enzymes split different substrates such as proteins, carbohydrates or fats while consuming water, hence the name “hydrolase”. In today’s leather processing, this type of enzyme is applied as proteases (protein splitting) and lipases (fat splitting).

The main problems reported by the tanning industry today are side reactions due to non-specific enzyme activity that is not ideally suited to leather. This problem usually relates to crude mixtures and/or natural source enzyme products that can still be found on the market.

State-of-the-art microbially produced enzymes are of high purity compared to natural ones. Their specificity for the task is at an optimum and key for success; their application is scientifically based and ensures vastly superior control.



Development of the X-Zyme products

Essentially, beamhouse operations can be regarded as a purification process for the removal of non-collagenous substances. The collagen fibres are opened up while keeping the collagen as intact as possible.

Enzymes in the beamhouse process target glucose amino glycans (GAGs) by degrading dermatan sulfate proteoglycan (DSP) and removing keratin. Other non-collagenous substances are also removed including natural fats or lipids, water soluble proteins and glycans.

SHORTER SOAKING PROCESSES

The first steps in a traditional beamhouse process include both dirt and main soaking. This is designed to attain full rehydration and to wash out water-soluble substances, such as salt, glycans such as hyaluronic acid (HA), and soluble proteins such as blood and albumins. This is in preparation for the liming and unhairing processes.

However, DSP remaining around the collagen fibrils reduces the effectiveness of soaking and hinders fibres from being opened at this stage. Targeting the removal of DSP was the primary goal for development of the first enzyme in the X-Zyme process. Opening fibres at the earliest possible stage speeds up soaking and this leads to a shorter and more efficient process.

CLEANER PELTS

The next traditional beamhouse step is liming or unhairing, where lime, sulfide and auxiliaries or sharpening agents are applied. The basic aim of this step is to remove keratin, swell the hide and open up the fibres. As fibre opening has already occurred in an effective soaking step, keratin removal becomes the primary goal of this process. The development of a hair removing enzyme was therefore the target for the second enzyme in the X-Zyme process, with the objective of providing better keratin removal and cleaner pelts.

The development programme was to first screen for a non-protein degrading enzyme. This enzyme would need to be effective in removing the GAGs from the protein backbone of DSP. The aim was to ensure opening of the collagen fibrils and enhancing the soaking process that removes GAGs. The starting point was carbohydrases, that are carbohydrate degrading enzymes.

The second enzyme had to be a protease, as keratin is a protein. The selected protease was optimised to provide the highest level of activity against keratin and, at the same time, with the lowest activity against collagen. Knowing the keratin and its amino acid composition, it was possible to narrow the range of proteases to those with specificity for unique amino acid combinations within the keratin protein.

THE SCREENING PROCESS

The selected enzymes and their variants that were derived from molecular manipulation were evaluated in a first attempt by high-throughput screening. The resulting enzyme libraries were already well characterised and so it was possible to make the first screening decisions by computer. Accordingly, the selection of suitable candidates to a limited number was possible at a very early stage of the development phase.

The next phase was more complex as the screening assays included pieces of hides. In the beginning this was performed at a 50-100ml volume level where the consumption of enzyme protein still was relatively limited. Proprietary methods and tools were developed to enable the monitoring and measurement of the

effects. This allowed the extrapolation of findings for predicting application results on a larger scale. This stage was repeated a number of times for both enzyme proteins. This reduced the number of enzyme candidates before introduction on a larger scale.

The selection of the right enzymes was only 25% of the work. A detailed application process and techniques for controlling the enzymes to gain the optimal benefit from them had to be established. In addition, a production process for the generation of these enzymes in large quantities had to be developed.

However, on the conclusion of the project two new enzyme products were produced, and a novel application process was developed.

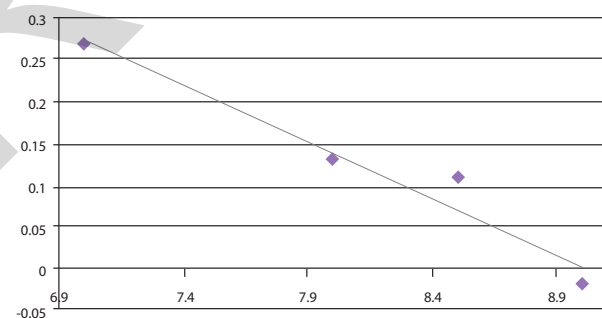
THE RESULTS FROM THE INVESTIGATIONS

Two new enzymes that are used in combination in the soaking and unhairing processes are being introduced to the tanning industry:

THE FIRST ENZYME

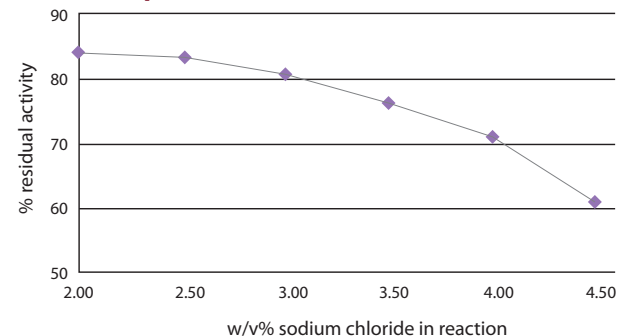
This is used during soaking and is responsible for fibre opening. Its action is pH-dependent. Known as Peltec X-Zyme S, the best results were found between neutral pH and a pH of 8.5 and best performance was around pH 7 as illustrated in *Panel 2*:

Panel 2: pH-dependency of the GAG activity of Peltec X-Zyme S.



Actions that might be applied in traditional beamhouse processing such as increasing the pH during soak directly counteracts the activity of this soaking enzyme. It is also necessary to control the salt level in the float and to keep a lower Baumé level as shown in *Panel 3*.

Panel 3: Salt dependency of the GAG activity of Peltec X-Zyme S.



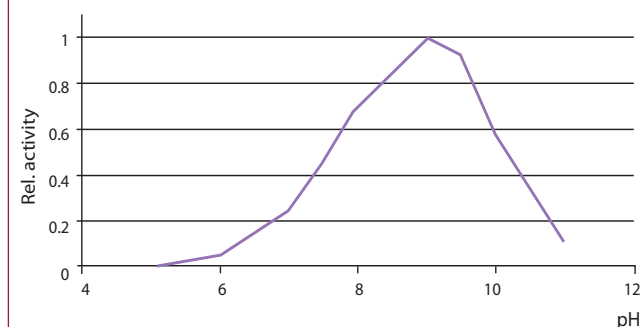
This is not surprising as enzymes are proteins and proteins are precipitated by the addition of salt.



THE SECOND ENZYME

This enzyme has a relatively sharp pH activity optimum, which makes it easy to turn on and off. Known as Peltec X-Zyme U, this enzyme is added at pH 9.2 and when required the activity can be effectively switched off by increasing the pH to above 10.5 as shown in *Panel 4*:

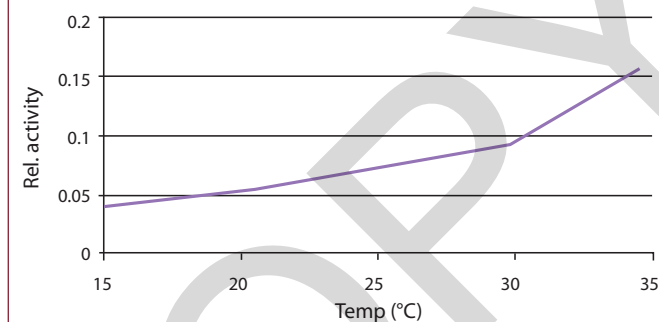
Panel 4: pH dependency of Peltec X-Zyme U.



The processing temperature is always important when working with chemistry and this is also true for enzyme reactions. This is illustrated in *Panel 5*:

As shown in *Panel 5*, the change from 25°C to 30°C in the float increases the enzyme activity by 30%.

Panel 5: Temperature dependency of Peltec X-Zyme U.



The traditional beamhouse process

The first two steps of a traditional beamhouse process usually comprise soaking and liming/unhairing processes. Soaking primarily aims to rehydrate wet salted hides and remove certain unwanted substances and contaminants from both fresh and salted hides. Blood, dung and non-structural interfibrillary proteins, such as HA, are common in both cases, and salt from salted hides.

CHALLENGES WITHIN THE SOAKING PROCESS

A proper soaking can only be attained efficiently by washing out water-soluble proteins and glycans, especially HA, located between the fibrils. Specifically, HA has a blocking effect on the interfibrillary space. Therefore, the removal of HA allows the penetration of water and subsequent process chemicals to ensure a thorough soaking result.

In the second beamhouse step—liming/unhairing—DSP is

degraded to ensure a proper opening of the fibres. Furthermore, the hair is removed, and this ideally results in clean and hairless pelts.

The removal of HA and further water soluble proteoglycans in a traditional main soak is a time consuming process. It simply uses surfactants alone and/or with proteolytic enzymes.

In this event both of these auxiliaries contribute to the soaking process, but there are potential downsides. Surfactants, due to their inherent emulsifying properties, will always increase the chemical oxygen demand (COD) of the effluent. Proteolytic enzymes, in turn, may attack the collagen and thus damage the delicate grain.

However, Peltec X-Zyme S does not contain proteolytic enzymes and so cannot harm the collagen even if an overdose is accidentally added. Compared to a standard traditional soaking process both the speed of the soaking process as well as the quality of the pelts and ultimate leather produced is improved, whilst lowering the COD of the effluent.

CHALLENGES WITHIN THE UNHAIRING PROCESS

There are generally two main routes for unhairing hides: hair-burning or hair-saving:

- The traditional hair-burning process results in the hair being dissolved and released in the effluent together with relatively large quantities of sulfides, being necessary to completely degrade/solubilise the keratin. Significant amounts of lime are also added to the float, another serious and increasingly costly waste-disposal issue. Conventional beamhouse processes also depend on high swelling to ensure fibre splitting. Excessive swelling is detrimental to leather quality and ultimately has a negative effect on area yield. Although this process is still commonplace, tanners are increasingly seeking alternatives to avoid wastewater legislations, ensure a sustainable future, and improve leather quality and yield.
- The hair-saving process focuses on an improvement in the effluent. Basically, lime is used to immunise the hair to prevent it being attacked by sulfides. A milder sulfur-reducing process is applied to loosen the hair roots and, with drumming, mechanically remove the hair. This hair can be filtered off and used for different applications, thus lowering the negative impact on the effluent. This process significantly improves the effluent, but controlling pelt cleanliness becomes more of a challenge.

The X-Zyme beamhouse process

The X-Zyme process is a robust one, allowing a 24-hour production process. It produces a clean and high quality pelt, it significantly improves the effluent, and offers the potential for area yield increase.

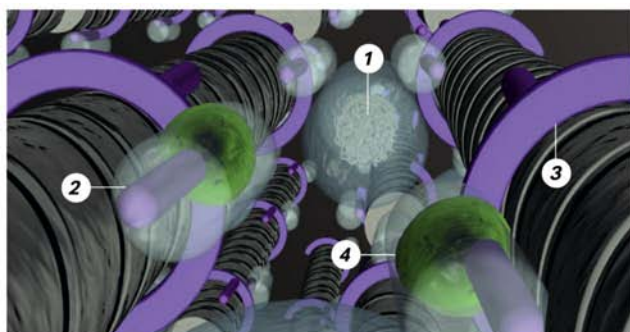
In the first stage of this process, the first enzyme, Peltec X-Zyme S, is applied directly after the dirt soak where it degrades DSP by cleaving the GAGs from the protein backbone. It is important to note that, in contrast to traditional soaking enzymes (that can harm the collagen), the target is not the protein backbone hence there is no effect on the collagen. The target is the connection of the glycan and the protein.

As a result the GAGs are easily washed out giving more space between the fibrils. As HA, which is also located between the fibrils, is locked in place by DSP, its degradation frees HA so it can be easily washed out. This is indicated in *Panel 6*.

This new step not only achieves a fast and thorough



Panel 6: Schematic view of the interfibrillary compartments and the degradation of DSP.



1. Hyaluronic acid 2. GAG 3. Protein backbone 4. Peltec® X-Zyme S



rehydration of the hide, but it also achieves an opening up of the fibres in the soaking stage.

The second stage of this process the second enzyme, Peltec X-Zyme U is used in hair-saving or semi hair-saving processes. After addition to the float, it degrades the pre-keratin in the basal membrane of the epidermis as shown in *Panel 7*. The hair root is thus loosened; it remains more flexible and less brittle, which to a large extent avoids broken hair and hair roots in the pelt. At the same time, the epidermis is prepared for complete removal.

As discussed previously, the control of the enzyme is significant and a prerequisite for the process. Due to its pH dependency as illustrated in *Panel 4*, the enzyme activity can be completely stopped by an increase of the pH above 10.5.

Once a significant loosening of the hair has taken place, the addition of lime stops the enzyme activity and at the same time immunises the hair. This is then followed by a conventional hair-saving/semi hair-saving process with a significantly reduced amount of sulfide and lime. The result is a very relaxed, let-out pelt with complete removal of hair and an improved effluent

The advantages of the X-Zyme process

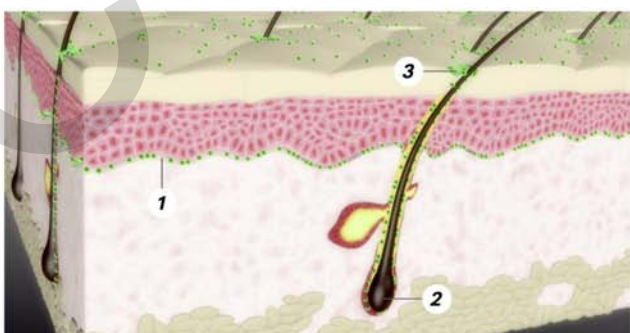
The process provides an improved soaking action, and combines the best of features of the hair-burn and hair-saving processes in the unhairing stage. This results in the following advantages:

- Improved quality of pelt; DSP is degraded in the soaking step, thus allows reduced lime and sulfide offers in the unhairing step. Premature and excessive swelling which leads to a constriction of the hair follicles trapping hair roots and scud is avoided as the swelling is more moderate. Combined with a

more complete removal of hair in the unhairing step a very relaxed and clean pelt is produced. The lower swelling also means a lower risk of belly draw and also less pronounced neck wrinkles. Both of these properties result in a leather of very high quality with good dyeing properties.

- There is a potential gain of area yield. The pelts are more even, more relaxed and more “let out” compared to conventionally produced pelts, because of the reduced swelling in the liming process. This offers the potential for an increase of area yield in crust leathers.
- Significantly lower amounts of chemicals such as surfactants, organic sulfur compounds and amines are required. The effluent profile is thus improved including sulfide-content values.
- As the hair can be filtered from the process, combined with the reduced consumption of lime, there is a significant reduction of sludge. Similarly the nitrogen content of the effluent and the oxygen demand is reduced compared to hair-burning systems.
- This is a robust process. The technology provides a safe and robust process that yields good results in terms of the final product and environmental profile. It is suitable for different production conditions, raw materials and leather articles. Both enzymes used in the process can be easily controlled and are readily adapted to the requirements of the raw material without risking damage to the collagen. A 24-hour soaking/liming process is now possible without any problems concerning the quality of the final leather. When compared to the conventional soaking/liming/unhairing techniques, this new processing offers an ideal alternative for the tanning industry today. ☺

Panel 7: Schematic view of the removal of hair and hair roots by Peltec X-Zyme U.



1. Epidermes basal membrane 2. Hair root 3. Peltec® X-Zyme S

