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BioTimes®

The quarterly bioindustrial magazine from Novozymes



novozymes 

Unlocking the magic of nature

Novozymes is the biotech-based world leader in enzymes and microorganisms. Using nature's own technologies, we continuously expand the frontiers of biological solutions to improve industrial performance everywhere.



Tanning enzymes help towards a better environment



An environmental comparison of chemical and enzyme-assisted soaking and unhairing/liming in a Chinese tannery indicates that, if all tanneries switched to the enzymatic processes, the carbon dioxide emissions saved would be equivalent to the annual load from 170,000 cars.

Enzymes have been used in the tanning industry for centuries because they are efficient at degrading protein and fat. In early times, the enzymes were derived from animal excrement, and later on from the pancreas of cattle. Nowadays, many of the enzymes are produced using microbial fermentation, and Novozymes is a major supplier of this type of enzyme.

One of the applications of microbial enzymes in the tanning industry is in soaking and unhairing processes. Enzymes can replace chemicals and shorten processing times, thereby reducing production costs. Other benefits can include increased area yield and improved quality of the final product.

As part of Novozymes' work with Life Cycle Assessment (LCA), the company collaborated with a Chinese tannery to assess the environmental implications of the application of enzymes in the soaking and unhairing of bovine hides.

True production data

The assessment is basically a comparison between two different ways of soaking and unhairing/liming bovine hides. An entirely chemical method was compared to

an enzymatic method with reduced chemical consumption.

Life Cycle Assessment has been used as an environmental analytical tool to evaluate the changes in enzyme and chemical consumption that occur when switching from the chemical method to the enzyme-assisted method. The information has been derived from a major Chinese tannery that applies chemical as well as enzyme-assisted production methods.

The study was based as far as possible on true production records and refers to an enzymatic hair-saving process - where the hairs on the hides are not dissolved by the chemicals but removed intact.

The enzymes Greasex® 50 L and NovoCor® SG were used by the tannery for soaking to degrade fat and proteins. They reduced the soaking time, which leads to electricity savings in turning the drum. There were also savings in the tenside and soda requirements for the process.

The unhairing enzyme NUE 0.6 MPX was used to degrade proteins. It reduced the sulphide requirements for the process but did not influence processing time or temperature. The lower level of sulphides

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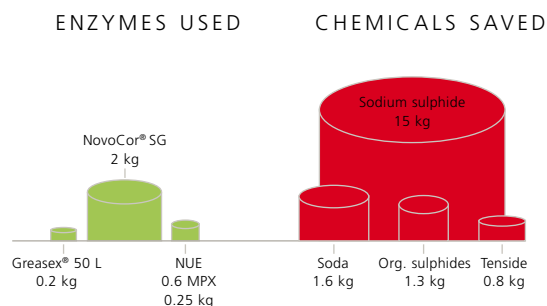


Fig. 1. A relatively small quantity of enzyme products (left) serves the same function in the tannery as a relatively large quantity of chemicals (right). The saved electricity is equivalent to using a 60 W light bulb for 35 hours. The volume of the columns provides an indication of quantity in terms of weight. All figures are per ton of hide.

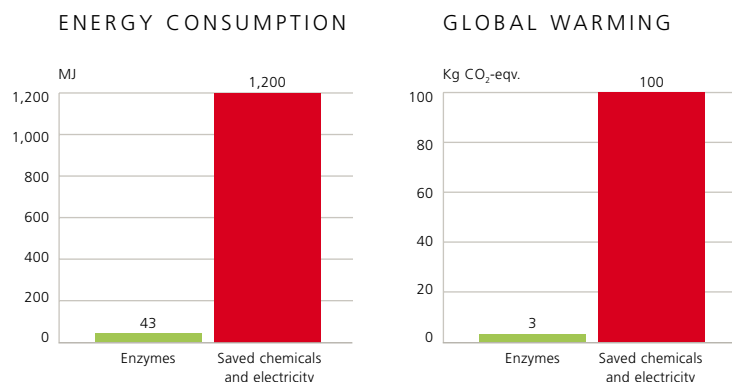


Fig. 2. Use of energy and contribution to global warming from enzymes and from saved chemicals and electricity when enzymes are introduced in soaking and unhairing/liming.



Principle of Life Cycle Assessment (LCA)

LCA is a holistic environmental assessment tool which addresses raw material uses and emissions in all processes in the product chain from raw material extraction through production to use and final disposal.

See www.howproductsimpact.net for a quick introduction to the LCA concept.

SOAKING			UNHAIRING/ LIMING
Soda saving	Tenside saving	Electricity saving	Sulphide saving
0.5%	0.5%	1%	98%

Table 1. Share of environmental improvements (global warming) when enzymes are introduced in the soaking and unhairing/liming processes.

required reduces the sulphide content of the wastewater and some manganese sulphide can be saved.

Savings in chemicals

Savings in chemicals as a result of the enzyme application are shown in Figure 1. As can be seen, a relatively small quantity of enzymes replaces quite a large quantity of chemicals.

The environmental impacts of producing and delivering the enzymes to the tannery on the one hand and savings in chemicals and electricity on the other hand have been evaluated and the results in terms of energy consumption and contribution to global warming are shown in Figure 2.

Figure 2 shows that a small 'investment' in energy and carbon dioxide emissions in enzyme production results in considerable savings when these enzyme products are used to replace chemicals and electricity in the soaking and unhairing/liming processes. A small amount of enzyme products made from a biological production process replaces a relatively large amount of harsh chemicals made using a relatively large amount of energy derived mainly from coal.

Contributions to other environmental impacts (acidification, nutrient enrichment and smog formation) are also reduced. In fact, the environmental impacts resulting from enzyme production are at least 20 times lower than the impacts that are avoided by the savings in chemical and electricity consumption.

Sulphide savings in the unhairing/liming process are considerable compared with other chemicals. Since a large quantity of coal is used to produce sulphides and a large quantity of carbon dioxide is emitted during production, the savings in sulphides turn out to be the most important environmental effect of enzyme use (see Table 1).

A comparison of conventional and enzyme-assisted bovine soaking and unhairing/liming processes indicates that the application of enzymes in the tanning industry results in considerable reductions in the contribution to global warming. The environmental advantages of enzyme application are primarily due to savings in sulphides in the unhairing/liming process because a small quantity of enzyme replaces a considerable amount of sulphides, which have a relatively high environmental load.

Novozymes is continuously developing enzymatic solutions. In future, the company envisages that new, more efficient enzymes will be able to virtually replace conventional chemicals in the soaking and unhairing processes.

Global impact

The global supply of bovine hides for leather production was about 8.8 million tons in 2005. Today, less than 10% of bovine hides are soaked and unhairing in enzyme-assisted processes. Assuming that the environmental improvements observed in this study by switching from conventional to enzyme-assisted soaking and unhairing/liming are applicable worldwide, the global potential saving is in the order of 8 million GJ of energy and 0.7 million tons of carbon dioxide per year. The saved carbon dioxide emissions are equivalent to the annual load from 75,000 average world citizens or 170,000 cars. ●

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Making creams and lipsticks greener

The environmental impact of Degussa's enzymatic process for producing cosmetic esters is much lower than that of the conventional chemical process, as measured by a Life Cycle Assessment. The products also have a higher purity.



Oliver Thum sees the LCA results as a new way to position Degussa's cosmetic esters made by biocatalysis.

It is one thing to say that a process is environmentally sustainable; it is another to prove it. That is exactly what the international specialty chemical company Degussa has done. Within one of the company's 12 business units called Care & Surface Specialties, the business line Goldschmidt Personal Care has conducted the first



Karen Oxenbøll of Novozymes helped Degussa to conduct the detailed investigation and number-crunching necessary to make a Life Cycle Assessment.

ever LCA study on a cosmetic ingredient. LCA stands for Life Cycle Assessment - an approach that differs from the traditional way of viewing a company's environmental work. An LCA looks at the big picture - the whole of a product's life cycle from cradle to grave, not just the part behind the company's factory gates.

The LCA was conducted in 2005 to compare the biocatalytic and conventional chemical synthesis of cosmetic ester oils known as emollient esters. They are sold by Degussa under the brand name Tegosoft®

and used by cosmetic manufacturers as the oil phase in emulsions such as creams, lotions and lipsticks. Their main function is to create a pleasant feeling of the cosmetic on the skin.

The production of myristyl myristate (Tegosoft MM) was chosen for the LCA as a model process for these types of cosmetic ester. Hundreds of tons are produced every year by Degussa, and this is one of the most popular emollient esters. Apart from myristyl myristate, three other esters - cetyl ricinoleate, decyl cocoate and isocetyl palmitate - are produced by biocatalysis at Degussa's factory in Duisburg, Germany. All of them are produced with the unique lipase Novozym® 435, which Degussa has been using in commercial production for more than ten years. The remaining cosmetic esters in the Tegosoft range are produced by chemical routes. Degussa therefore has experience of both the biocatalytic and chemical synthesis processes.

LCA expertise available

However, Degussa Care & Surface Specialties did not have experience of conducting an LCA, so they enlisted the help of Novozymes' expert Karen Oxenbøll.

She is Director of Environment, Health and Safety at Novozymes and the person responsible for a small group of LCA specialists. Since 2004 they have been comparing the overall environmental impact of enzymatic methods with the traditional way of making products in a number of different industries.

"LCAs are a strong tool because customers can use them in their marketing and environmental reporting," says Karen Oxenbøll. "Degussa is publishing the results and making use of them in promotional material. This is just the way it should be."

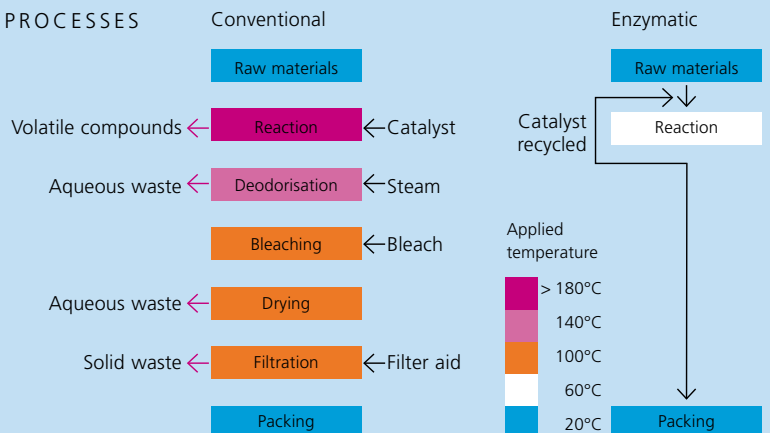
Preparing the LCA required close co-operation and a lot of investigative work. Degussa made all the necessary data available to Novozymes such as the types and quantities of chemicals used in their conventional and enzymatic processes.

Biocatalysis is best

When comparing the environmental impact of the two processes, biocatalysis showed clear advantages (see table). Energy consumption was reduced by more than 60%, and emissions of different pollutants by between 60% and 90%. Emissions of greenhouse gases were reduced from 1,518 to 582 kg CO₂-equivalents when mak-

CONVENTIONAL AND ENZYMATIC ESTERIFICATION PROCESSES

These are the steps in the production of cosmetic esters such as myristyl myristate. The conventional process requires high temperatures and a chemical catalyst. The product becomes discoloured and therefore requires bleaching plus some other purification steps. Degussa's enzymatic process is much simpler and takes place in a fixed-bed reactor under mild conditions. The end-product is ready to pack without purification.





degussa.

Emollient esters give creams a pleasant feel on the skin.



The smooth feel of lipstick is partly due to emollient esters.

TABLE 1. A SUMMARY OF THE RESULTS OF THE LCA

		Chemical	Enzymatic	Savings
Energy	GJ	22.5	8.63	62%
Global warming	kg CO ₂ -equivalents	1,518	582	62%
Acidification	kg SO ₂ -equivalents	10.58	1.31	88%
Nutrient enrichment	kg PO ₄ -equivalents	0.86	0.24	74%
Smog formation	kg ethylene-equivalents	0.49	0.12	76%

The results for five key environmental parameters based on the production of 5 tons of myristyl myristate, a popular emollient ester.

ing 5 tons of esters - a reduction of 62%. Significant reductions were also measured in sulphur dioxide (acidification potential), phosphates (nutrient enrichment potential) and volatile organic compounds (smog formation potential). The major reasons for the reductions are savings in energy for heating and substitution of the tin catalyst.

Fewer steps

Oliver Thum is head of Biotechnology Research at Degussa Care & Surface Specialties based in Essen, Germany. He took the initiative to conduct an LCA after hearing a seminar on the subject given by Karen Oxenbøll. "Esters for care applications are commonly produced under classical chemical conditions," he points out.

"The raw materials are mixed with a conventional catalyst such as strong acids or metal salts. With the catalyst tin oxalate, the reactions take place at 240°C for several hours, and the oil can turn malodorous and brown. In order to be used in a cosmetic, the end-product has to be odourless and colourless. That's why a lot of downstream processing is needed: first malodorous by-products have to be removed by steam-stripping, followed by bleaching, and finally filtration to remove the catalyst. Afterwards the product has to be dried to remove residual water. In contrast, in the biocatalytic process all these steps can be avoided. As long as the raw material is of a high quality, no refinement is needed after the reaction in order to obtain a cosmetic-grade product."

The most important raw materials used by Degussa for making cosmetic esters are derived from coconut oil, palm oil and palm kernel oil.

Quality improvements

Oliver Thum of Degussa has analysed four

myristyl myristate products made by competitors by conventional chemical synthesis and compared them to Degussa's own product made enzymatically. He found that Degussa's product has a superior quality. "The biocatalytic process gives a 2-17% higher content of the active ingredient myristyl myristate, less unwanted by-products, a better colour and a better odour," he comments. "Our customers know that we have the highest-quality myristyl myristate on the market."

The most dramatic example of improved yield is the production of cetyl ricinoleate (Tegesoft CR), a cosmetic wax that melts at skin temperature giving a silky but non-oily feel to the skin. During production by conventional synthesis, significant side reactions occur, including the formation of dimers and polymers of ricinoleic acid. This is due to the unspecificity of the chemical catalyst and the extremely high temperatures.

The amount of undesirable dimers of ricinoleic acid decreased by 75% in the biocatalytic process, while the content of the desired product, cetyl ricinoleate, increased from 61% in the conventional process to 93%. The main reason for this is the regioselectivity of the enzyme Novozym 435 giving a high conversion rate.

Works for weeks

The immobilised enzyme Novozym 435 works at 60°C and is fixed in a packed bed, so it is easily removed from the final product without an additional filtration step. After a few weeks of continuous operation, the enzyme activity becomes exhausted and the old enzyme bed needs replacing.

"Novozym 435 is a tremendous enzyme; it works under almost every condition you can imagine and we do not need any special treatments to make the reaction work.

It is a very simple process," Oliver Thum explains.

New selling point

The LCA results are being used as part of Degussa's strategy to promote their emollient esters obtained by biocatalysis. After presenting the LCA results to key customers, they showed the findings to a wider audience at a poster presentation at the 24th IFSCC (International Federation of Societies of Cosmetic Chemists) Congress held in October 2006 in Osaka, Japan.

"By preparing the LCA, we have gained additional marketing arguments for these products that we can use to differentiate them from competitive products. As far as we know, we are the only company using biocatalysis to make cosmetic esters," says Oliver Thum. "Environmental arguments are slowly becoming more important, and these products could be of interest to companies making natural cosmetics or those emphasising the sustainability of their work and products."

Novozymes hopes that the LCA study at Degussa will lead to a greater interest in biocatalysis in the cosmetics industry. This is a prime example of a sustainable technology because energy consumption is reduced and the use of chemicals can be minimised. ●

In the scientific literature there are thousands of examples of the lipase Novozym® 435 being used for biocatalysis. It is a real workhorse of an enzyme suited to biocatalysis. Samples are available from Novozymes.

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Goodbye to gums with Mannaway®

The mannanase Mannaway is what detergent manufacturers have been waiting for. It is an innovative detergent enzyme that does what other enzymes cannot do: it removes stubborn stains containing gums.



“Every high-performance detergent should contain five enzymes,” says Tommy Lykke Husum, Customer Solutions Application Manager at Novozymes, who has conducted tests on Mannaway®, seen here with Kirsten Væver Jokumsen, Senior Launch Manager for Mannaway.

Some of the most common stains on clothes are also among the most difficult to remove. These stains often contain mannan, the general term for the group of carbohydrates called galactomannans but often simply referred to as gums.

Mannan is widely used as a thickener in everyday foods and personal care products such as shampoo and body lotions. It is very hard to keep clothing free from occasional stains caused by these products, and once the stains are there, they are difficult to remove. In fact, even if the stains appear to have been washed out, the mannan is still present and may even have been distributed to otherwise clean fabrics. These invisible mannan residues on the garment act as a kind of glue and attract particles of dirt that cause white fabrics to go grey.

These problems can be solved with Mannaway, the original mannanase from Novozymes with a well-documented effect when tested in detergents around the world. For example, Mannaway has been tested in commercial European detergents at the well known Hohenstein Institute in Germany using their standard range of mannan-based stains. On a number of these Hohenstein stains, Mannaway has a significant effect on stain removal even in detergents already containing a number of enzymes, irrespective of whether they are colour detergents or bleach-containing detergents.

“A basic requirement”

“Detergent manufacturers have been waiting with great interest for a mannanase because it is regarded by many as a basic enzyme requirement for their detergents. Mannaway is not only for the high-end brands which already contain a number of enzymes. It can work equally well in a detergent that simply contains a protease or amylase,” says Kirsten Væver Jokumsen, Senior Launch Manager for Mannaway at

Novozymes. Mannaway was launched in January 2007 as an enzyme granulate. A liquid product will be available a year later.

By integrating Mannaway into a detergent formulation, a customer can make some strong brand claims such as removal of common, tough stains, better whiteness by preventing soil redeposition on white fabrics, or in-depth cleaning by removing mannan that acts as a glue for soil. Mannaway can thus improve the positioning and competitiveness of a brand.

What is mannan?

Two of the most commonly used mannans are guar gum and locust bean gum. It is surprising just how many everyday foods and personal care products contain these additives, which are classified in Europe by the E-numbers E412 and E410.

Because enzymes are highly specific, other carbohydrases such as amylases and cellulases do not recognise the structure of mannan and cannot therefore remove mannan from fabrics. In contrast, mannanases are galactomannan-specific carbohydrases, so they are able to degrade the mannan.

Fig. 1. Chocolate ice cream stain removal

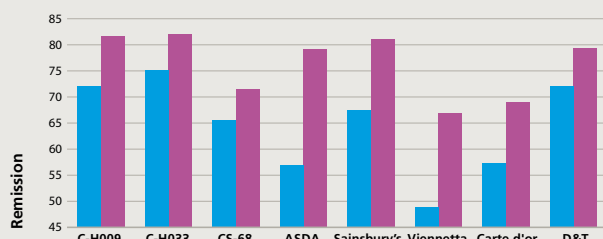
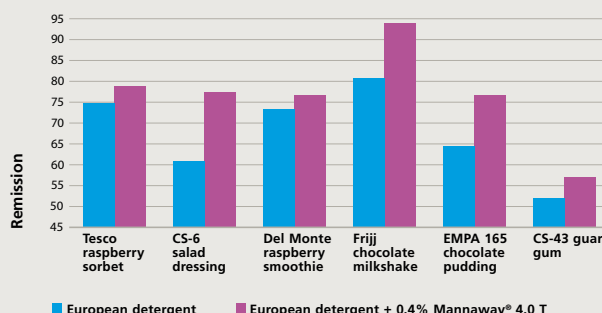


Fig. 2. Mannan stain removal

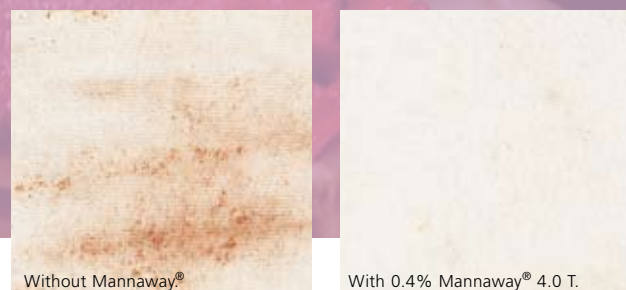


Figures 1 & 2. Mannaway® provides a significantly improved stain removal effect (high remission) in a commercial European colour powder detergent on a range of standard stains. (European washing machine, detergent dose 5 g/l, 40°C.)

■ European detergent ■ European detergent + 0.4% Mannaway® 4.0 T

Ice cream often contains guar gum and produces a troublesome stain. Not any more with Mannaway.®

FIG. 3. ANTI-REDEPOSITION EFFECT OF MANNAWAY® ON TENNIS COURT CLAY



Mannaway® prevents deposition of tennis court clay on 100% cotton fabric soaked in a mixture of commercial US detergent, 0.1% guar gum and 0.5 g tennis court clay. (300 ml detergent solution, 10 minutes, room temperature.)

Mannaway in particular has proven its ability to break down mannans. It is therefore very effective at removing stains containing guar gum or locust bean gum. In addition, it increases in-depth cleaning by removing the mannan that adheres to fabrics.

Improved stain removal

Mannaway efficiently removes stains containing mannan such as chocolate ice cream (see Figure 1), chocolate pudding, fruit sorbet, milkshake and salad dressing (see Figure 2).

The figures show the performance of Mannaway when tested in a commercially available European colour powder detergent. When dosed with 0.4% Mannaway 4.0 T, a far superior stain removal effect is clearly visible. Remission is a measure of stain removal. The higher the remission value, the cleaner the test swatch. As can be seen in Figures 1 and 2, the increases (delta) in the remission values before and after adding Mannaway are very high.

“We are not used to recording such high delta remissions in the range of 20-25. This means that there has been a significant change in the cleaning performance, and you can see this clearly with the naked eye,” says Tommy Lykke Husum, Customer Solutions Application Manager for the detergent industry at Novozymes, who has conducted tests with Mannaway.

Similar effects were observed with US detergents. Tests performed using commercial US detergents show that Mannaway can increase stain removal dramatically. In two different commercial powder detergents, a dose of as low as 0.2% Mannaway 4.0 T was able to give a far superior effect

on chocolate ice cream. When adding 0.4% Mannaway 4.0 T, an even higher level of stain removal was obtained.

Synergy with Stainzyme®

Amylases are very effective at removing stains containing starch, but by design they are ineffective at degrading mannan. Even the highly effective amylase Stainzyme from Novozymes is not able to remove chocolate ice cream containing guar gum. However, by combining Stainzyme with Mannaway, the stain-removing power of a detergent can be boosted on food stains containing both starch and mannan. When combining the two enzymes, synergy effects can be obtained. Mannaway releases the mannan, giving Stainzyme access to the starch and providing superior stain removal.

Deeper cleanness

Mannan binds strongly to the cellulose fibres in cotton fabrics due to the high affinity between the mannan and cellulose polymers. This can be demonstrated by comparing the removal effect on chocolate ice cream soiled on a cotton fabric and on a polyester fabric. Tests show that Mannaway is necessary for efficient stain removal on cotton fabric, whereas a Mannaway-free detergent can remove the ice cream stain from polyester because the mannan does not bind to it.

In addition to having a high attraction to cotton fabrics, mannan also has a gluing effect on particulate soils released during the wash cycle or subsequent wear. Mannaway removes the mannan, thus preventing soils from redepositing. Regardless of whether a colour or bleach-containing detergent is used, Mannaway makes a visible difference.

The whitening effect of Mannaway on cotton can be demonstrated with a number of different outdoor soils such as red tennis court clay.

To illustrate how Mannaway prevents soil redeposition on fabrics, swatches made of 100% cotton were treated with a commercial US detergent in the presence of guar gum and tennis court clay. After just ten minutes, it is very clear that with no Mannaway in the detergent solution, the cotton becomes heavily soiled with tennis court clay. When Mannaway is present, the guar gum is degraded, preventing the clay from gluing to the fabric. The fabric therefore remains white (see Figure 3).

“Mannaway definitely has a whitening effect. Without Mannaway white shirts tend to become more and more grey after every wash,” comments Tommy Lykke Husum. “Mannaway is truly special. You can’t match its performance with other enzymes and you can really see the difference. Every high-performance detergent should contain five enzymes.”

Proteases, amylases, cellulases and lipases are regarded as four fundamental types of enzyme with clear benefits for detergents. Now there is a fifth. ●

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The next generation of fuel ethanol



Good progress is being made towards converting biomass to ethanol in an economical way. But even though the cost of suitable processes and enzymes has been cut, there is still some way to go.

Some obstacles still need to be overcome before a fully commercial process is developed, and so far biomass has only been converted into ethanol in a few pilot plants. However, this could be the humble beginnings of a huge new branch of industry that could grow to become even bigger than the existing first-generation fuel ethanol industry. 'First-generation' is used to refer to the conversion of sugar crops and cereals that are rich in starch, for example corn (maize). 'Second-generation' refers to the conversion of lignocellulose, more commonly known as biomass - a very abundant substrate found all over the world.

The high cost of enzymes for converting lignocellulose into sugars was once considered one of the main obstacles to commercialisation, but costs have been reduced. In 2001, the US Department of Energy awarded a three-year research contract to Novozymes worth USD 14.8 million to reduce the cost of enzymes by tenfold. By the end of the government-sponsored research in 2004, the cost had been successfully reduced to the target level.

"Enzyme cost is no longer the dominating economic barrier to producing ethanol from biomass," comments Joel Cherry, Director, Biotechnology/Bioenergy, who led the biomass research project at Novozymes' research centre in Davis, California. The research work is continuing there under his leadership, and he predicts that even more efficient enzymes will be developed and process optimisation will take place at partners with whom Novozymes has allied.

Major opportunity

"The conversion of biomass to ethanol is not yet a commercial industrial process,

but it is likely to be a very interesting opportunity for Novozymes in the future," predicts Emmanuel Petiot, Global Business Development Manager for biomass applications at Novozymes. "This is an industry in the making. We believe it will take another four to five years to develop and commercialise the enzymes for this new field."

Even if commercial sales of enzymes have not started, the conversion of biomass-to-ethanol is already receiving full attention at Novozymes, where efforts are being increased significantly within R&D, Marketing and other areas. In fact, the search for high-performance cellulases and hemicellulases represents a global mobilisation of Novozymes' research resources and expertise in this field. Novozymes also engages actively in key conferences on the subject and is seeking more government funding to speed up developments across the globe. Biomass research is being conducted at Novozymes' laboratories worldwide, while biomass marketing is headquartered at Novozymes North America led by Emmanuel Petiot.

Pilot partnerships

Novozymes has a long history of mutually beneficial partnerships. The partnerships

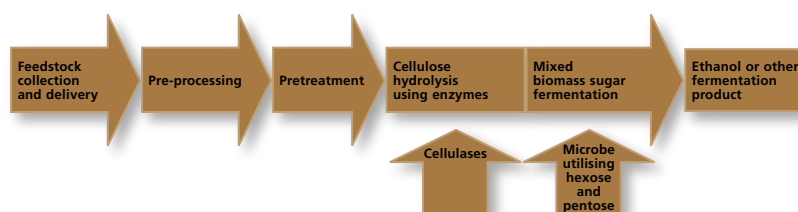
that Novozymes is entering into are visible signs of the research efforts. Implementing and fine-tuning Novozymes' enzyme processes through collaborations with industrial partners that are bringing the technology to the pilot-scale testing phase is the current focus of biomass R&D.

For example, in June 2006 Novozymes signed a three-year research agreement with China Resources Alcohol Corporation (CRAC) on the development of biomass for biofuel in China. CRAC is a business area within China National Cereals Oils & Foodstuffs Corporation (COFCO) and has interesting technologies within the production of biofuel. CRAC has set up a pilot factory for the production of ethanol from corn stover in Zhaodong in Heilongjiang province.

In the long term, China is expected to become an important market for biofuel. The consumption of fuel has increased significantly in recent years in line with the increasing number of cars, and this has brought a focus on sustainable energy, especially biofuel.

It is commonly agreed that the production of first-generation ethanol based on food crops will not grow very much in China in the coming years because China

CONVERSION PROCESS STEPS



The steps in the conversion of biomass need to be carefully integrated.



“Enzyme cost is no longer the dominating economic barrier to producing ethanol from biomass.”

Joel Cherry, Director, Biotechnology/Bioenergy, Novozymes

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Corn stover is a prime candidate for conversion into ethanol, but Emmanuel Petiot of Novozymes predicts that it will take another four to five years to develop and commercialise the enzymes for biomass conversion.

largely imports corn and other cereals as foodstuffs. In contrast, China is expected to focus on the development of non-food crops such as cassava and sweet sorghum, and on second-generation biofuel from biomass.

In the USA, Novozymes has recently signed a partnership with Broin with the aim of taking the next steps needed to bring cost-effective ethanol derived from corn stover to market. The collaboration is an extension of the close partnership between the two companies; it is an opportunity for Novozymes to put its unique biotechnology platform to work and for Broin to aim for fast commercialisation.

These are just two examples of what is happening in the drive to bring biomass-based ethanol into the commercial arena.

Integrated approach

The production of ethanol from biomass can be broken down into the steps shown in the diagram. For a successful commercial process, all these steps need to be looked at in a holistic way because there is a complex interplay between them.

Lignocellulosic fibres are composed of three major fractions: cellulose, hemicellulose and lignin. Enzymes are able to attack the polymeric sugar chain in the cellulose and hemicellulose fractions, releasing monomeric sugars for fermentation. Pretreatment is needed to open up the fibres and make the lignocellulosic substrate accessible to enzyme action. Obviously the enzymes need to match the substrate. If a dilute acid pretreatment is used, most of the hemicellulose is degraded and a hemicellulase will not be needed. However, if an alkaline or neutral pretreatment is used, the hemicellulose still needs to be hydrolysed and hemicellulases will be needed. For researchers or companies that want to test enzymes in the laboratory, a Novozymes Biomass Kit is available with small samples of enzymes.

Another challenge is being faced at the fermentation stage because the 5-carbon (C5) sugars produced by the degradation of hemicellulose are not fermented by the yeast currently in use. Research is proceeding to develop organisms that can effectively utilise these sugars in order to increase

A major R&D effort is in progress at Novozymes to find enzymes to economically degrade lignocellulosic feedstocks such as straw into sugars.

ethanol yields and make the whole process more economical.

Collection of feedstock is a logistical challenge. Corn stover, which has been the subject of most research in the USA and China, is often not collected and is left in the fields to decompose. Systems of collection need to be established. That is why trials with biomass are often based on what are known as 'captive streams' - existing residues or by-products from first-generation ethanol plants. Corn fibre left over after the processing of corn starch and bagasse left over from sugar cane processing are examples of lignocellulosic raw materials that are readily available for conversion. Wood chips and wood trimmings could also be used.

Renewable fuel

Biomass provides a renewable feedstock for producing biofuel as an alternative to petrol (gasoline). With rising oil prices and strong government incentives, producing ethanol from biomass is becoming more viable step by step. With a growing awareness of global warming, biofuels could have a bright future. With political, economic and environmental factors favouring biofuels, there is now a strong will in certain countries to develop the conversion of biomass to ethanol.

Novozymes is engaged in an unprecedented research effort to find the cellulases and hemicellulases that the second-generation fuel ethanol industry will need in order to succeed. ●

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Aquaculture farmers around Asia buy into the power of PondPlus®



PondPlus has gained popularity in Asia - and it is being used extensively and successfully in India, Thailand, Vietnam, China and the Philippines.

In 2007 the world will produce 12% more shrimp than it did in the previous year. This increase in production, changing cultural practices, price pressures and the increasing cost of production have made shrimp farming more challenging than it used to be.

It has now become vitally important for shrimp farmers to get maximum value from their farms by improving survival, increasing yield and producing larger-sized healthy animals that fetch a better price.

"The shrimp farmer is getting squeezed, and even more so when his crop is hit with disease," says Sameera Gujarathi, Business

Manager for Aquaculture, Asia Pacific, at Novozymes. "Good pond management is very important for shrimp farmers, and that's why Novozymes' PondPlus and PondProtect® are gaining market recognition in Asia."

Better pond with fewer chemicals

Proper pond management can help control disease, and a healthier shrimp means a better yield. Microbial biology offers an alternative to chemicals and antibiotics; and with PondPlus and PondProtect, farmers can get proper pond management with natural resources.

PondProtect is a nitrifier consisting of an ammonia and a nitrite-oxidising strain, and it is highly effective at controlling ammonia and nitrite, which can build up and make a pond unsuitable for healthy shrimp growth.

PondPlus is a *Bacillus* consortium of seven specifically selected bacterial strains; it has a broad range of waste-degrading enzymatic activity and helps clarify water. PondPlus helps control critical nutrient levels in the pond, including nitrate. This creates a better balance with essential phytoplankton, helps avoid destructive 'blooms and crashes', and ensures a consistently healthy 'green water'



A bountiful harvest in Thailand with PondPlus®



Dr Pornlerd Chanratchakool (left) and Dr Dave Drahos discussing shrimp health problems at a farm in Thailand.



“Good pond management is very important for shrimp farmers, and that’s why Novozymes’ PondPlus® and PondProtect® are gaining market recognition in Asia.

Sameera Gujarathi, Business Manager for Aquaculture at Novozymes

colour. PondPlus can also remove pond-bottom sludge caused by the accumulation of organism waste matter.

“PondPlus cleaned out my severely damaged pond bottom,” says Atal Rout, who does semi-intensive farming in Orissa, India.

PondPlus helps farmers by reducing the build-up of black soil, giving good colour to the pond and ensuring a stable bloom of phytoplankton.

Certain bacteria in PondPlus form ‘bio-flocs’ around uneaten feed and organic wastes, and help convert this to usable food for the shrimp. This helps in reducing the feed conversion ratio.

Due to the benefits that PondPlus offers, the shrimp are on average about 10% larger in size, and the animal looks better, which means that farmers using PondPlus can potentially get a better price for their product.

“This is a high-level product,” says Chen Zhou Yu, a farmer in China. “Other products were unstable and over-stated their advantage.”

PondPlus is preferred over competitor products in Asia because of its stability and ease of use. Similar microbial products available in Asia are unable to safeguard the pond against phytoplankton crashes, which lead to the need for early harvest and poor pond bottom conditions - problems that do not occur with PondPlus.

“I will continue to use PondPlus because it maintains the bloom through the whole crop and maintains the condition of the pond bottom after the crop,” says Kantibhai, a medium-sized farmer from Gujarat, India.

What are they saying about PondPlus?

In a previous article in the March 2006 edition of *BioTimes*, Novozymes declared

its dedication to develop new products that can help aquaculture and test existing products. In this regard, Novozymes collected feedback on PondPlus from 125 shrimp farms with a total of 546 ponds in Thailand, India, China and Vietnam.

“The results were spectacular,” says Dr Pornlerd Chanratchakool, Technical Manager for Aquaculture, Asia Pacific, at Novozymes. “81% of the ponds reported good results, and 14% of the ponds reported fair results with farmers saying that they will continue to use PondPlus.”

Ponds treated with PondPlus showed an 18% increase in yield and a 22% improvement in survival rate. The feed conversion ratio (FCR) dropped by 9%, which meant that the farmers needed less feed to grow the same size shrimp.

Farmers are especially pleased with the ease of use that PondPlus offers. Another concern for shrimp farmers that PondPlus alleviates is vibriosis, which is caused by *Vibrio* spp - bacteria that can result in the death of almost 90% of a crop, with the most significant impact on post-larvae and juvenile shrimp.

“There was a drastic reduction in *Vibrio* count,” says Nilesh Patel, a corporate farmer in Gujarat, India. “We could see a visible difference in the nursery within 60 days of using PondPlus.”

About 5% of the ponds that Novozymes received feedback about did not benefit from PondPlus. Most of these ponds were in small-scale farms where PondPlus had been used in the early part of the first crop. Overdosing of the product caused phytoplankton crash, which led to increasing dead matter at the pond bottom, creating anaerobic conditions in the pond and resulting in severe stress and even mortality.

However, improved product application, timing and dosage information provided to the farmers have resolved this matter. The second crop in the ponds where PondPlus failed initially - even those that were infected with viral white spot disease - did better because of PondPlus.

What’s next?

Though awareness of microbial products does exist in Asia, penetration of the technology is limited due to lack of proof of performance - something that isn’t an issue with the highly successful PondPlus. However, dubious claims of products termed as ‘microbial’ have made some farmers wary of all microbial products.

Novozymes brings scientific backing and a world-class reputation to an industry in a region that is desperately seeking this new level of expertise. Novozymes is a biotech world leader in microorganisms and is committed to biological solutions that can enhance the aquaculture business.

“Currently, we have two microbial treatment products in shrimp and aquaculture ponds,” says Dr Dave Drahos, R&D Manager for Aquaculture Business at Novozymes.

“More exciting products are under development, one aimed at hydrogen sulphide control and others providing a second generation of *Bacillus* strains to tackle a broader range of aquaculture needs.” ●

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Winemaker of the year uses Novozymes' enzymes

One of Australia's most respected and innovative winemakers, Peter Lehmann Wines, uses Novozymes' enzymes to create wines that delight wine lovers around the globe.



The weighbridge at Peter Lehmann Wines.

Peter Lehmann Wines was named International Winemaker of the Year and Australian Producer of the Year at the 2006 International Wine & Spirit Competition in London for the second time in the last four years.

With wine operations in the heart of the internationally famous Barossa Valley in South Australia, Peter Lehmann Wines has always used enzymes to make both red and white wine. Almost all Australian winemakers use enzymes to enhance their white winemaking processes, and many use enzymes for their reds as well.

"I've always used enzymes to make wine," says Andrew Wigan, Chief Winemaker at Peter Lehmann Wines. "We started using Novozymes' enzymes because our trials with them went very well, and we've been using them successfully for 14 years now."

Wine is made differently in Australia than in Europe because of the hot climate, which requires processing times to be short to avoid infection. Sometimes, winemakers have just a half hour to move wine from crushing to the clarification tanks.

"In Australia, the vintage start varies from region to region due to the different climatic conditions. In each region the vintage duration tends to start at a relatively firm date and also ends at a relatively firm date, so the process has to keep moving. This is not like juice processing where you can keep the apples and pears in cold storage; with grapes you process them as they come," says Peter Dunn, Business Manager for Wine at Novozymes.



"We're very happy with Novozymes' enzymes, and we'll continue to use them," says Andrew Wigan, Chief Winemaker at Peter Lehmann Wines.

Novozymes is sensitive to the Australian wine market and its needs, and the fact that Novozymes has a strong market position underscores this. The one major factor for Novozymes' success has been excellent customer service.

"We guarantee delivery," says Peter Dunn. "And when you're on a limited time schedule, you want your enzymes to be there when you start making your wine. With Novozymes, the enzymes are always delivered on time and the enzymes delivered always do their job."

White wine production

Clarification of the must is generally difficult due to the presence of pectins extracted during winemaking. Pectins are natural compounds found in grapes and act like a sponge. High viscosity makes the release of juice difficult and the cloud particles are kept in suspension. Novozymes' Ultrazym® CP-L and Novoferm® C-L resolve these issues.

"We use Ultrazym CP-L with white wines because they help with clarification, and a nice clear juice accentuates the characteristics of the fruit," says Andrew Wigan.

"Ultrazym CP-L speeds up the clarification process, which is important to us. We have limited settling space and if we leave the wine on day one, we want it to be settled by day two."

Ultrazym CP-L helps winemakers by reducing viscosity, releasing free-run juice easily, allowing must clarification and settling, and speeding up the process for obtaining clear musts before fermentation.

"The enzyme is added during the crush so that we get quick contact time and a quick reaction. Ultrazym CP-L helps release free-run juice and enhances settling by working in the clarification tanks," says Peter Dunn.

Red wine production

In addition to ease of production and enhanced settling, enzymes are used in red wine processing to assist in the release

of the available colour from the grapes, and improve lees compaction. Though not everyone in the Australian wine industry is convinced about the advantages of using enzymes in reds, Peter Lehmann maintains that enzymes have always been part of their red winemaking process.

"In our reds, we use Ultrazym EX-L to get better clarification and extraction - we've always done this," says Andrew Wigan.

Ultrazym EX-L is added at the beginning of the winemaking process and offers all the same advantages as Ultrazym CP-L; as well as improved extraction of phenolics and aroma compounds, enabling excellent compaction of lees, improved throughput and improved filtration.

"Red wine is filtered several times and during each filtration step, the wine loses some colour, tannins and flavour," says Peter Dunn. "With Ultrazym EX-L, winemakers can look at reducing at least one filtration step - this helps to maintain the quality of the final product."

High-quality wine production

Good wine can only be made from good grapes and Peter Lehmann Wines in Barossa Valley does not compromise on quality at any step of their winemaking process. Barossa Valley, today, is the beating heart of Australia's wine industry and it's up to winemakers like Peter Lehmann Wines to maintain the area's reputation.

"At Peter Lehmann Wines, near enough is never good enough, but we temper the high demands of winemaking with fun, mateship, and a glass or two at the end of the day," says Andrew Wigan.

Premium winemakers like Peter Lehmann Wines partner with Novozymes because of their high-quality service and products. ●

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