It is a complex task to optimize biotechnological production processes.
Bayer HealthCare and Bayer Technology Services closely cooperate in this work.

PROCESS OPTIMIZATION

Knowing What Hamster Cells Crave

At Bayer HealthCare in Berkeley cell cultures produce a very special protein that allows people with hemophilia A to live an active life. Colleagues at Bayer Healthcare and Bayer Technology Services are collaborating to find ways to make these cells even more productive.

"Our particular strength is testing first on a small scale. This is important as the high costs for the nutrient solution alone are reason enough not to test every change on a large reactor scale."

Dr. Thomas Daszkowski, Bayer Technology Services

They haven't got a contract of employment, and they don't ask for wages. And yet they work 24 hours a day, seven days a week for months on end. The workaholics in question are cells – whose parent cells, which are a stable, reliable and well-known source in the biotechnology industry, were originally extracted from the kidney of a baby hamster. These cells now thrive in head-high stainless steel containers in Berkeley.

Bayer HealthCare has several such fermenters at its Berkeley site in California. Each one holds 200 liters of a reddish brown solution brimming with billions of these hamster cells. And each cell functions like a tiny bio-factory.

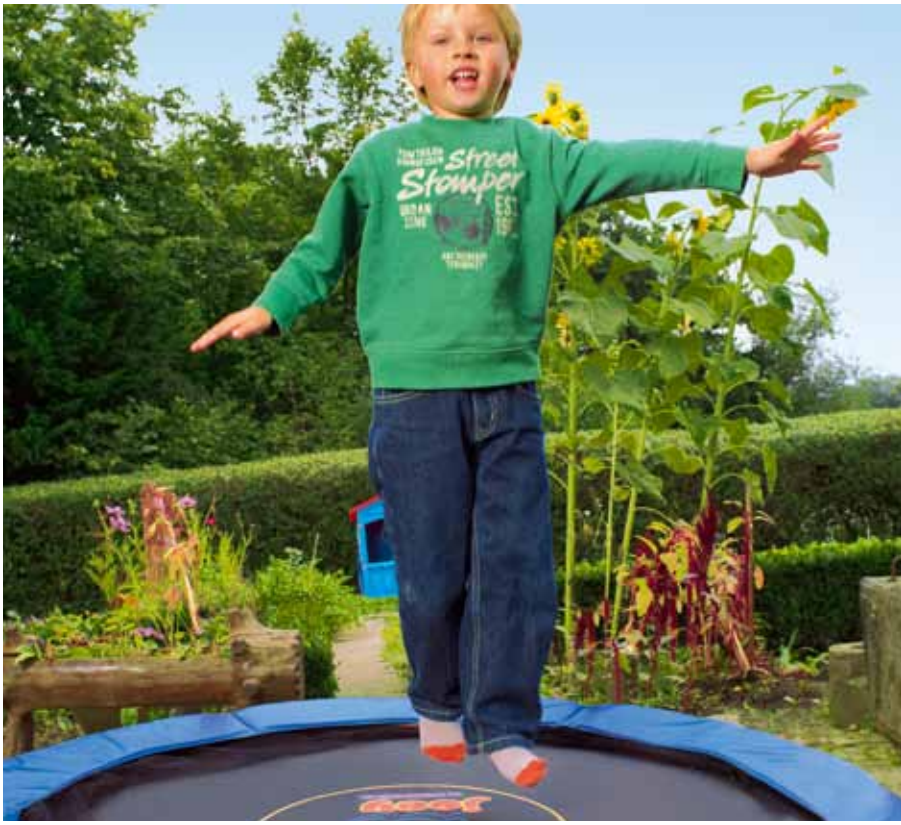
The special feature of these cells is a modification in their genotype. In the 1980s the genetic code of a particular protein was inserted into their precursor cells. On average, this protein, i.e. the blood coagulation factor VIII, is deficient in one of every 10,000 males, thus leading to hemophilia A. Thanks to this additional gene, these hamster cells now tirelessly produce the factor VIII protein, which is how Bayer HealthCare obtains the active ingredient for its hemophilia A treatment Kogenate FS. The company is one of the world's leading suppliers of hemophilia A products – and one of only three manufacturers of a genetically engineered product.

Although the cells carry out their duties without payment and unceasingly, they do have certain demands regarding their working conditions. They thrive at 37° Celsius, the pH value should be slightly below 7.0 and oxygen must also be present. In addition, they have some favorite food. So, a solution of nutrients including amino acids, sugar, vitamins, insulin and much more is constantly fed into the fermenters. The greater the well-being of the cells, the more eager they will be to multiply – and the more factor VIII they will produce.

But how much more are we talking about? The annual production in Berkeley amounts to just a few hundred grams of pure factor VIII, and for this the hamster cells consume many thousands of liters of nutrient solution every day – at an immense expense.

Bayer HealthCare has continuously improved productivity for many years – and not only because of cost efficiency. The company has also had to meet an ever-increasing demand, as Kogenate FS gained approval in more and more countries. To put their ideas to the systematic test and for the resulting optimization of the production processes, they found a partner in Bayer Technology Services.

Joint projects have been successful for more than six years now. During talks, Jörg Heidrich, Head of Product Supply Biotech at Bayer HealthCare, likes to show a chart that best illustrates the results. The curve in the diagram extends from the upper left-hand corner down to the right. It demonstrates how production costs have decreased significantly over the past years. Also, the annual write-offs due to defective batches have been reduced tremendously, Heidrich says. This de-



"The successful collaboration between Bayer Technology Services and Bayer HealthCare Product Supply resulted in efficiency gains and supported growth and innovation for Bayer HealthCare."

Jörg Heidrich, Bayer HealthCare

velopment has clearly improved Bayer HealthCare's competitiveness.

To achieve these cost reductions, the teams from Bayer HealthCare and Bayer Technology Services each pulled their weight very effectively. "We started by developing a very detailed Cost of Goods Map of the whole Kogenate manufacturing process and identified key areas for process optimization," Dr. Soumitra Ghosh explains. "Some typical examples of the things we found were optimizing material costs in the fermentation section and increased automation of the purification process." The chemical engineer is among the now 30 staff members employed by Bayer Technology Services in Berkeley. When Ghosh transferred to Berkeley in 2006, the team was smaller. But it grew when, as a consequence of the intensive cooperation and the good relationship among the colleagues, some of the Bayer HealthCare engineers joined the Bayer Technology Services team in 2009.

Help for an Active Life

Just a few decades ago there were still no treatments for people with hemophilia A. Blood transfusions helped in some cases, but this was not considered a standard therapy. Early in the 1970s saw the introduction of treatments containing the protein that is largely deficient in people with hemophilia A. Thanks to such products, children with hemophilia A, like four-year-old Julian (left), can lead an active life without fear of articular hemorrhaging.

Bayer HealthCare has been among the leading manufacturers of such products for more than 35 years. In 1993 the company launched Kogenate, a genetically engineered hemophilia A product.

One of the transferees is Chris Williams, who used to be Process Analytical Technology Manager with Bayer HealthCare. Working with Ghosh, he installed state-of-the-art inline measurement devices in the manufacturing process. Decreasing operator errors and reducing product wastage through sampling resulted in operational savings.

Ultimately, all this led to a significant reduction in production costs. But that is not all. The team has the ambitious goal to raise productivity even further in the coming years. "We are always looking for ways to increase each cell's production by improving the nutrient solution or, in other words, by providing a more balanced diet for the cells," Dr. Paul Wu from Global Biological Development at Bayer HealthCare explains.

This is where one particular strength of Bayer Technology Services comes in handy: first testing process parameters on a small scale. "The high costs for the nutrient solution alone are reason enough not to test every change on a large reactor scale," says Dr. Thomas Daszkowski, Head of the Process Technology Healthcare team at Bayer Technology Services in North America, to which Ghosh and Williams belong as well. The trick is to design the miniaturized tests so that the results can be transferred to large-scale dimensions.

As there is so much to test, the team in Berkeley has requested support from Germany. They now regularly send frozen packages to Leverkusen. One of the recipients of these packages is Dr. Volker Möhrle, who works in the Enzyme & Fermentation Technology Group at Bayer Technology Services in Leverkusen. His laboratories are outfitted with equipment that replicates the production process in Berkeley – except these units are scaled down by a factor of 200. Möhrle and his colleagues feed the cell cultures and the nutrient solutions from the packages into these devices. After concluding the tests, they in turn send parcels with their product yields back to Berkeley for evaluation.

Another project involves the recovery of the factor VIII protein. "Just one false condition, and it is damaged," Thomas Daszkowski observes about the sensitivity of the protein molecule. For this reason, it has not been possible so far to harvest all of the protein produced by the hamster cells safely for further processing into the finished product. A double-digit percentage is lost in the process.

A team consisting of colleagues from Global Biological Development and Product Supply at Bayer HealthCare and Bayer Technology Services is currently working to improve the yield. Initially, this work entailed painstakingly evaluating the data from the past three years. The data mining has

Transatlantic cooperation: research teams led by Dr. Thomas Daszkowski (left) in Berkeley and Dr. Volker Möhrle in Leverkusen are collaborating to optimize the production process for factor VIII. Lab experiments like the one shown here in Leverkusen play an important role. For instance, they test different compositions of the nutrient solution for fermentation.

already delivered some ideas to increase yields, which the team is implementing as we speak, says Daszkowski.

Other examples of improvements are related to the device through which air is permanently fed into the nutrient solution to supply the hamster cells with oxygen. "We developed a new mechanism that, firstly, clearly reduces the amount of work required for preassembling and secondly, allows an influx of 30 percent more oxygen," says Juri Seletzky from Bayer Technology Services. As a consequence, the cells are better supplied and raise their metabolism, thus increasing product yields.

Although each improvement is very different, one aspect remains the same: "We always begin in the laboratory," says Yuval Shimoni, who is with Manufacturing Science at Bayer HealthCare. The first level is at a scale of 50 milliliters; the next is a scale of one liter. Ultimately, the tests are performed in a 15-liter reactor. Only then is the most promising process upgraded to an industrial scale, i.e. the 200-liter fermenter. If all of the results are confirmed here, too, the company will then apply to global regulatory authorities for approval to use the modified process in regular production.

Clearly, all this is a complex and time-consuming process. The Bayer Technology Services team has therefore designed a new 15-liter reactor that replicates the conditions in the 200-liter production reactor. The final goal is to do this so perfectly that 15-liter runs might instead be accepted by the authorities. "We can then project the results and dispense with the test in the 200-liter reactor," Ghosh reports. That will save not only money, but also testing time. If all goes well, the 15-liter reactor will be available this year.

Admittedly, realizing this design has not been so simple. "Reactor geometry, mixing and also the sheer stress caused by the stirrer must correspond exactly to the conditions in the subsequent production reactor," says Ghosh. "The ultimate goal is to make sure that the cells experience the same 'micro-environment' in the smaller reactor as they would in a production-scale reactor."

Since Bayer HealthCare launched its first hemophilia product more than 35 years ago, it has remained committed to improving treatment. Bayer HealthCare is currently working on the next generation of treatment that will allow the factor VIII protein to remain effective in the body for a longer period so that it does not have to be administered so often. The clinical trials are already underway. When Bayer applies for approval, the company must also demonstrate its competency for production. Colleagues from Bayer HealthCare and Bayer Technology Services are working together to create the prerequisites for this task.

So, an extensive cooperation has now evolved from the initial projects. "This collaboration supports growth and innovation for Bayer HealthCare," says Jörg Heidrich. It is thus no surprise that Bayer Technology Services has a relatively big team at the Berkeley site. Although these employees may be separated from their customer both in terms of organization and spatially, Thomas Daszkowski attaches great importance to their teamwork: "One can truly say that Bayer HealthCare and Bayer Technology Services in Berkeley work together as a single large team." And this will no doubt continue for the time being, as the two companies signed a new three-year contract in 2010 for their projects in Berkeley.