Pharmaceutical
MANUFACTURING

Control, Coordination Help Scale Up New Enzymes

Optimizing Fermentation in Its Pilot Plant, and Working Closely With Its Contract Partner, Diversa Has Increased Yield Five-fold, and Reduced Costs by 75%

By Agnes Shanley, Editor-in-Chief

After years of focusing on research and development, Diversa Corp. (San Diego, Calif.) is scaling up production of its first commercial enzymes, using recombinant techniques to express them in different strains of yeast. Bacterial expression systems will also be used in the future, according to the company. Fermic S.A. de C.V., a contract manufacturer in Iztapalapa, near Mexico City, is dedicating a portion of its manufacturing capacity to Diversa, which, in turn, is co-investing in capital equipment at the site.

A new downstream processing facility has been built at Fermic's site, dedicated to Diversa's new enzymes. At this point, one enzyme is already in commercial production, and another two have been scaled up and are ready to move into production pending FDA approval. Within the next five years, both partners expect to be making over $100 million worth of enzymes per year at the site.

Theirs is no standard toll arrangement, says Patrick Simms, Diversa's senior vice president for commercial process development and operations. In fact, the groundwork for the scale-up project was laid about two and a half years ago, says Susan Oliver, Diversa's assistant director of manufacturing and engineering, who currently divides her time between San Diego and Iztapalapa. All told, about 25 people in California and Mexico, including operators, are now dedicated to the enzyme manufacturing project, she says, including four Fermic staffers, who work full-time on the Diversa project and report directly to her.

Diversa had worked with a number of companies in the U.S. that offer contract fermentation services, but their focus tends to be on large volume, low-cost projects, Simms says. Typically, after three to five years, such companies want to do something else with their facilities. "There's a low ebb of available capacity out there," he says. The new agreement with Fermic, a certified cGMP facility that specializes in fermentation, will lock in production capacity for the long term. Initially, hundreds of thousands of liters in fermentation capacity and an equivalent amount of separations capacity, will be involved, but the total will be increased as needed, by adding modular equipment.

Currently, Phytase, an enzyme that Diversa codeveloped with the Danish drugmaker Danisco, is being produced in full-scale 90,000-L quantities at Fermic's facilities. The enzyme helps degrade phytic acid in animal feeds so that animals can absorb phosphate more readily. Diversa has applied for FDA approval of Quantum, another phytase enzyme, and plans to ramp up production upon approval; other enzymes are also ready to move into full-scale production, once they receive a green light from FDA and other global regulatory agencies.

Finicky Organisms

Scaling up these bioprocesses has presented a number of challenges because the host organisms tend to grow, thrive and reproduce in very specific environments. "Processes coming from the lab are finicky," says Samun Dahod, director of process development at Diversa. "In the lab, you might be using nutrients that cost $5 per kilo, or you might use deionized water, neither of which is cost-effective or practical in manufacturing."

Any changes, from switching raw material suppliers to using water with a different mineral composition, can reduce protein expression and limit production, Simms says. "Downstream, in recovery, the scale of the plant equipment is so different from that in the laboratory or pilot plant," he says. "The processing time for each step is quite different—for example, many days in the plant versus one to two days of recovery in the pilot plant. Chemical engineers working on scale-up have to work through issues such as microbial contamination and stability."

To improve results, it is critical to make the fermentation process as robust as possible. The proper "scale down" approach is essential, Dahod says, and all processes at Diversa's fermentation pilot plant (Photo) duplicated Fermic plant conditions closely, using equipment and materials as similar as possible to those that would be used at the manufacturing site. Being able to use a second, larger pilot plant onsite at Fermic's facility helped with the scale-up process, says Fermic engineer Nazario Neira.

Computer control has also been essential to developing, and improving, a robust fermentation process. However, optimizing control systems for bioprocesses can be tricky. "You have to consider the living organism's growth cycle and metabolic characteristics," says Diversa process development scientist Song Liu. The control system must be responsive enough to sense changes in a timely manner and take corrective actions. In some cases, he says, sampling times as short as 3 seconds are required.

The computer control system must also be able to distinguish between changes due to external mechanical factors such as aeration, agitation or pressure, and biological changes occurring due to the metabolism of the organisms involved. "Typically, we have to use very simple variables, such as dissolved oxygen, oxygen uptake rate, CO_2 evolution rate, and pH changes, to control something that's very complex," says Dahod. "It's essentially coordination and keeping up with the organism," he adds. "You have to figure out, as the process moves along, how to keep mechanical parameters in synch with what's happening, biologically, within the cell."

Diversa and Fermic use a two-tiered system for control. The first tier establishes setpoints for process variables such as temperature or back pressure on the fermenter, and then uses basic feedback loops to control these variables. For example, a back-pressure sensor sends readings to the computer, and, if any of them exceed or fall short of setpoint values, the computer sends a signal to a control valve on the fermenter's exhaust line, increasing or decreasing the pressure.

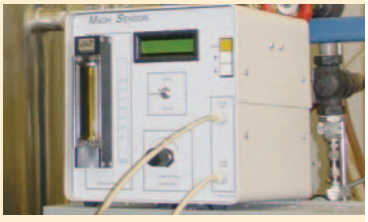
Algorithms Aid Control

Above this basic control level, a supervisory control system responds to changes

Online Methanol Sensor Helps Optimize Fermentation

Methanol is essential for fermentation reactions involving the yeast strain *Pichia pastoris*. Not only do the organisms feed on it, but methanol triggers a promoter critical to their growth. However, too much of it can also kill the cells.

In the past, controlling methanol concentration meant using liquid or gas chromatography, typically off line, to monitor methanol concentrations. Alternatively, concentrations could be measured indirectly, by interrupting the feed and measuring the time required for methanol in the batch to be exhausted, indicated by a spike in the level of dissolved oxygen. This technique was labor intensive, though, only worked for some types of *Pichia* cells, and could reduce protein expression.



Raven Biotech (Vancouver) developed its methanol sensor about five years ago, says company principal Doug Kilburn, professor emeritus at the University of British Columbia. The sensor uses well-established semiconductor technology used in breathalyzers and chemical "sniffers," Kilburn says. It can also measure ethanol and benzene and other volatile vapors.

The company has also developed a monitoring and control system (Photo) for the sensor that can be used in stand-alone mode, or interfaced into a computer control system. Its output can be fed into BioXpert software, the company says, using the Applikon ADDA converter and other equipment, allowing real-time data acquisition.

in dissolved oxygen, oxygen uptake or other variables, processes them in computer programs and then sends out new setpoints for key process variables—for example, changing the feed rate of a feed pump or the speed of a mixer.

Diversa senior scientist Kangfu Gu developed the two algorithms for the supervisory control system. The first program was developed to control glucose or methanol feed rates to the fermenter very closely, using respiratory quotient (RQ) as the signal. The RQ is the ratio between the rate of carbon dioxide evolution from the fermenter, and the rate of oxygen uptake by the fermenter. To calculate RQ, fermenter offgas is analyzed every 5 to 10 minutes for oxygen and carbon dioxide using a mass spectrometer. The change in RQ can be correlated to feed rate.

The second algorithm controls the concentration of methanol in the yeast culture. An on-line methanol sensor developed by Raven Biotech (Vancouver) was critical to implementing this control, Dahod says. "Methanol level is very important to some yeast fermentations, since it controls the growth of the organism and drives the promoter for the recombinant protein," says Raven principal Doug Kilburn, professor emeritus at the University of British Columbia, which spun off the company and the technology five years ago. (Box)

Diversa has placed the sensor so that it measures methanol concentration within the fermenter online and feeds data into the computer system. The algorithm then regulates the amount of methanol fed to the culture.

Diversa's supervisory control system has already achieved results, Gu says. Originally, Phyzyme yields were very low, but the feed-control strategy has increased fermentation yield by more than five fold, he says, and lowered costs by 75% so far.

Computer control has made scale-up more convenient, says Gu, and the basic control platform, coupled with the algorithms developed inhouse, has allowed for more precise control. By monitoring mechanical and metabolism-related variables, and responding to them online, the control system has allowed users to more readily distinguish between the two during fermentation, says Dahod.

While the enzymes were still in the development stage, Diversa had evaluated a number of control options for the fermentation process, including PC-based systems and a variety of proprietary programs offered by various reactor manufacturers. The company ultimately selected Delta V, the process automation platform from Emerson Process Management (Austin, Texas) for its robustness, power and speed, and installed its first system in 2000, Gu says.

Fermic had installed Delta V the year before, and the fact that both partners' control systems are "in synch" has eased scale-up considerably, and allowed it to proceed faster, Simms says. "We've seen very good, rapid tech transfer and scale-up with the first three products so far."

The next hurdle will be bringing new enzyme recovery capacity on line, to recover the new enzymes from the fermentation broth. This portion of the project has posed the logistical challenges inherent in any start-up, Oliver says, and good project management has been necessary to coordinate design, purchasing and equipment installation. A basic building shell was completed early last year, and capacity, currently enough to handle 200,000 L of fermentation volume, will be expanded as needed, she says.

Communication Challenges

Two-way communication has been essential to this project, which is both international and bilingual in scope. Protocols for fermentation and recovery were developed in San Diego, Oliver says, then transferred to Fermic, and translated into Spanish, becoming part of Fermic's quality systems. Teams on each site communicate with each other daily, and Diversa engineers frequently travel to Fermic for troubleshooting.

Batch reports have been critical in improving the process. Fermic's Neira generates comprehensive reports on each run that are published and shared with Diversa. Results so far have been encouraging: for the three new enzymes, activity and expression levels in production runs were brought up to pilot plant levels very soon after production start-up.