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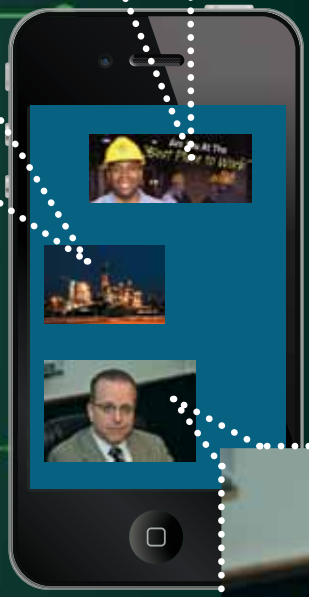

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Next Generation Enzymes for the Paper and Pulp Industry

Enzymes have long been part of the paper making process, but next generation enzymes use less energy, increase efficiency and operate at wider pH ranges

MARK EMALFARB

Next generation enzymes use genetic engineering to optimize the action of enzymes. The first step is the search for a gene that will produce the desired enzyme. High speed robots scan for genes using an assay that indicates the presence of the desired gene. The process is known as High Throughput Robotic Screening or HTRS (Figure 1).

Once a desired gene is located, it is extracted and inserted into a host organism to reproduce millions of copies of the cell containing the gene. Host organisms are single cell organisms that with genetic manipulation can be turned into little enzyme/protein factories. There are a variety of host organisms including Yeast, Chinese Hamster Ovaries, Viral Vectors, and Fungi such as C1—a proprietary fungal expression system from Dyadic.

Hosts are where research companies differ in their approach. While some companies extract the desired gene and insert it into a test host organism and produce laboratory quantities for further examination, others work directly with the commercialization host organism to ensure that the desired gene can be expressed on a commercial scale. This is an important difference because often the products produced by test hosts require further manipulation to make them suitable for the commercial host.

While working with the host organisms, genes that inhibit the production of the desired protein or enzyme are removed to optimize the production of the desired product.

Once a host strain is produced in sufficient quantities, it is inserted into large industrial fermentation vessels to produce the desired enzyme.

NEXT GENERATION ENZYMES AND PULP: ENDOGLUCANASE

Wood pulp fibers in a raw state contain both crystalline fibrils and less ordered cellulose structures on the surface. Endoglucanases help strip these fibrils from the surface of the wood and attack the less ordered structures that are to some extent degraded. Mild to moderate beating increases fibrillation on the fiber surface increasing fiber-to-fiber interactions and yielding a stronger pulp (Figure 2).

An improved next-generation endoglucanase interacts with cellulose in such a way that it facilitates degradation to the fiber wall structure allowing the pulp to be mechanically refined using lower energy input. The endoglucanase is also capable of hydrolyzing the small fibrils, or fines, that are released from the fiber wall without consuming the more ordered cellulose structures composing the bulk of the fiber. This effect can improve drainage when pulp reaches the paper machine, which may have a positive effect on productivity due to potential increases in paper machine speed.

The next-generation endoglucanase is also highly flexible—operating within wider temperature and pH ranges making it more adaptable in existing pulp mill processes. Dosage also is highly flexible yielding higher tensile strength than other cellulases (see Fig. 3). Typical cellulase enzymes often must be confined to a narrow dosage range or reaction time as overdose effects might occur if applied improperly due to excess degradation of the fiber wall prior to refining.

Following initial screening and industrial fermentation scale up, next generation enzymes go through extensive laboratory and mill trials to determine optimal dosage and effect under process-specific conditions.

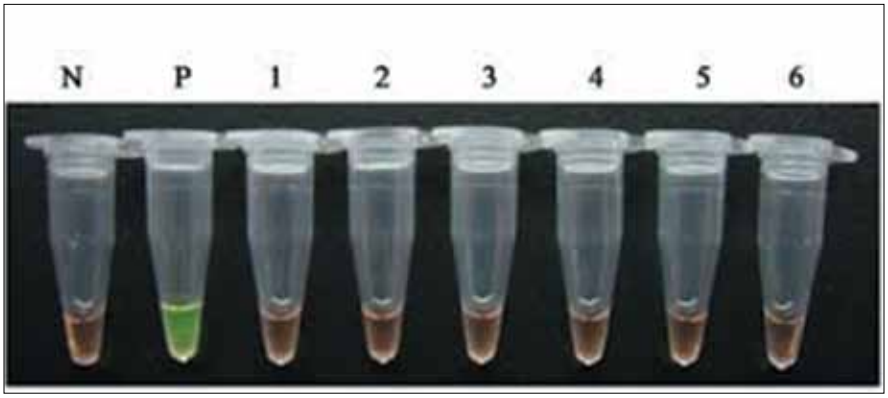

Figure 1. Vial P shows a positive assay.

MILL TRIAL RESULTS

Trial 1: Multi-liner board, Southeast Asia, 500 TPD capacity. The endoglucanase product was applied to the bottom layer of a multi-liner board at dosages ranging from 90-160 g/mT. As a result of successful treatment, machine speed was increased by 9 percent which in turn increased production by 44 TPD.

Trial 2: Six-ply coated and uncoated paper, 400 TPD capacity. The multi-ply paper was composed of a bleached eucalyptus top layer, an ONP bottom layer, and recycled

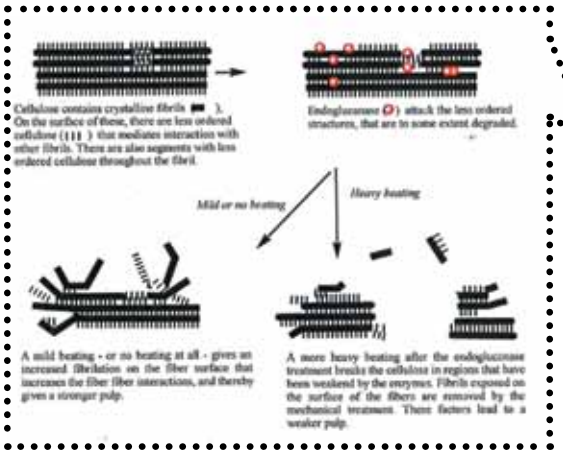

Figure 2. Mild to moderate beating increases fibrillation on the fiber surface increasing fiber-to-fiber interactions and yielding a stronger pulp.

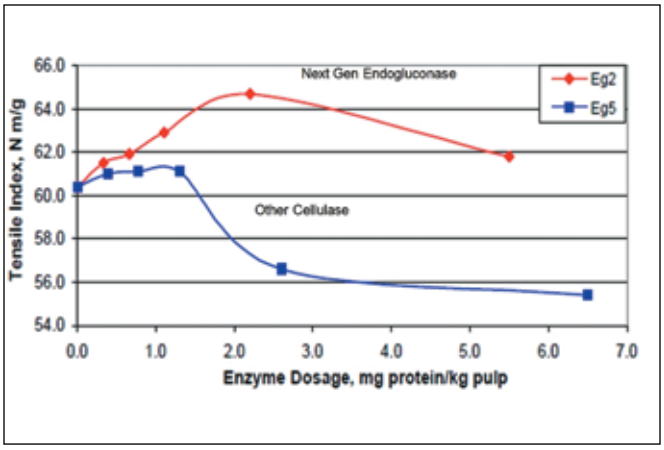

Figure 3. Enzyme dosage vs. Tensile Strength (BSWK).

mixed paper in the middle. Endoglucanase was applied to the top layer at a dosage of 200 g/mT. The results of this trial showed a 25 percent reduction in refining energy with no change in tensile strength.

Trial 3: Toilet tissue, Middle East, 100 TPD capacity. At a dosage of 200 g/mT, the endoglucanase product was added under the following conditions: pH 7.2-7.8, temperature 40 degrees Celsius, and reaction time 60-90 minutes. Results included a 20 percent decrease in refining energy while maintaining strength. The long term goal of this trial is to reduce dry strength and use lower strength recycled fiber.

Trial 4: Tissue (14 gsm)/Towel (33 gsm), Middle East, 40 TPD capacity (20 TPD each). A 78 percent bleached hardwood and 22 percent bleached softwood furnish blend was treated with 300 g/mT endoglucanase. The process featured a neutral pH, reaction time of 5 hours, and a temperature of 37 degrees Celsius. The tissue trial resulted in a 20 percent reduction in energy usage and reduced hood temperature while maintaining tensile strength. The towel portion of the trial showed a 17 percent energy reduction and a 14 percent increase in machine speed. Tensile strength was again maintained and a heaver weight towel speed limitation was debottlenecked.

ANALYSIS

When reviewing results from the next generation endoglucanase mill trials, one thing is readily apparent—using a next-generation endoglucanase has the potential to reduce pulp refiner energy on the order of 20 percent savings. Since energy is a significant portion of pulp and paper manufacturing costs, the reduction in refining energy can help bring down the total overall cost of the final paper product and increase profit margins.

Another advantage of the next generation endoglucanase is the increase in machine speed. This not only increases the mill's capacity, but may also help lower the final product cost.

Depending on the goals of an end user, additional benefits that can be realized by a product of this type might include improved bulk and softness, increased refiner capacity, and reduced picking.

IMPLICATIONS

Next generation enzymes, such as the endoglucanase outlined above, hold the potential to further reduce costs, use less energy, and reduce the overall environmental impact of the paper making process.

There are hundreds of thousands of genes that have already been identified and catalogued. However, only the smallest of percentage of these genes have progressed beyond identification into commercial development. While the novel endoglucanase has made its way to market and is beginning to play an important role in bio-refining, there are a number of other enzymes that can play a role in pulp and paper processing. These include hemicellulases, lipases, amylases, and esterases among others. Next generation enzymes are in development as pulp and paper process aids and represent a market that exceeds $1 billion annually.

Mark A. Emalfarb is the founder, President and CEO of Dyadic, a supplier of specialty enzymes used in various industrial applications. He is an inventor of over 25 U.S. and foreign biotechnology patents and patent applications resulting from discoveries related to the Company's patented and proprietary C1 fungus. Contact him at: memalfarb@dyadic.com.

WHAT TO LOOK FOR WHEN SELECTING AN R&D COMPANY

Should you find yourself in the position of looking for an enzymatic solution to a unique problem you are having or are interested in developing a next generation enzyme for a competitive advantage, there are a number of important questions you need ask a prospective research and development partner.

1. Do you have a gene you wish to develop? If not, does the prospective partner have a gene library to search?
2. Do you have an assay? If not, does the prospective partner have the capability of developing one?
3. Does the prospective partner work directly with the desired host organism early in the development process?
4. Is the prospective partner willing to invest his own money to help break through bottlenecks?