Fusion Enzymes for Biofuels

C2 Biotechnologies, LLC
Germantown, New York

Research & Development Focus: Fusion Enzymes

C2B harnesses the power of biology to fuse different proteins, specifically, enzymes with desirable characteristics into one product. We call this process our fusion enzyme technology platform.

All organisms require the use of proteins to function and survive. Generally, each protein is encoded by one gene, which is a central dogma of biology: one gene, one protein, one activity. Evolution occurs naturally by the introduction of mutations in genes that result in proteins with biological activities that confer better chances for organism survival when challenged by the environment. Evolutionary processes have resulted in the development of many organisms with enzymes adapted to various environments.

C2B uses protein engineering and molecular biology to screen and select enzymes that have biophysical properties compatible with operation in manufacturing environments and combines them to produce products that have multiple functions, are easier to use and improve yields.

Combining multiple enzymatic activities into one protein reduces manufacturing requirements and provides our customers with products that are cost effective, simpler to use, reduce variability and increase yield in manufacturing processes.

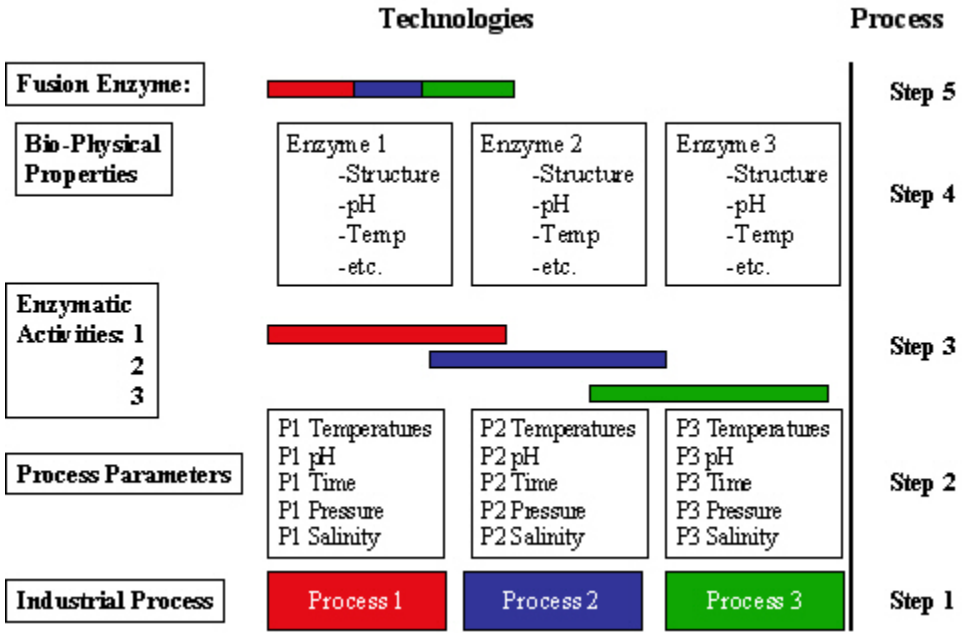


Figure1: Graphical representation of fusion enzyme technology platform.

Our technology process uses a series of steps to identify, screen, engineer and construct a single enzyme with multiple functions for a specific industrial process.

Specific Fusion Enzymes:

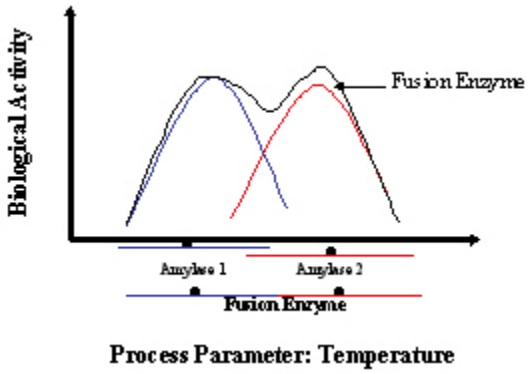
I. C2B's first project: Amylase Fusion Enzyme

Description:

This technology encompasses an expression vector encoding a bi-functional amylase fusion enzyme. The amylase fusion enzyme is unique because two temperature related activities, starch digestion at low and high temperatures, have been combined. The resultant fusion enzyme works over a wider range of temperatures and therefore is more robust to industrial processes. The robust character of the product makes it easier to use.

Applications:

- Conversion of starch into sugar
- Mashing
- Bio-fuel ethanol production
- Fermentation processes
- Waste management
- Corn sweetener production



II. Other Areas of Fusion Enzyme Research:

Other active areas of research include the development of cellulosic fusion enzymes.

Cellulosic fuel producers require at least three enzymatic activities; in a synergistic sequence of events, endo-ß-glucanase acts randomly on the cellulose chain, while exo-ß-glucanase acts on exposed chain ends by splitting off cellobiose or glucose. Cellobiose is subsequently hydrolysed by b-glucosidase to glucose.

The glucosidase activity is important because the accumulation of cellobiose inhibits the cellulose degradation.

Combining these three essential activities into one fusion enzyme has the potential to reduce enzyme costs by 30% or more while increasing mashing efficiencies (Figure 1). Both of these properties work synergistically to lower the cost to produce ethanol fuel from renewable resources. For enzyme manufacturers, bio-fuel has been projected to be a $5 billion enzyme market.

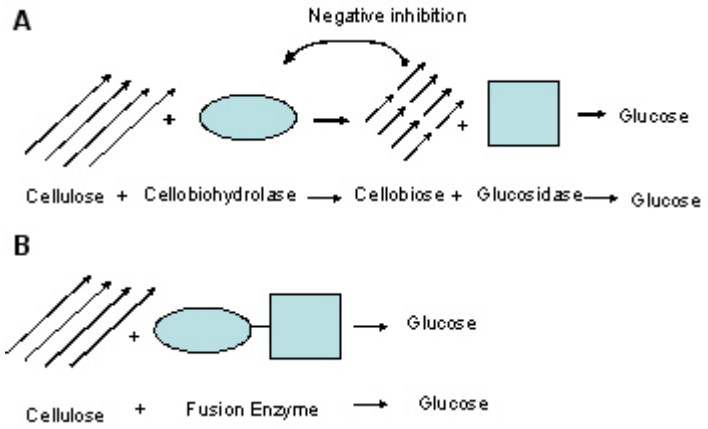


Figure2: Graphical representation of proposed fusion enzyme activity.

Cartoon of two enzymatic activities used for hydrolyzing cellulose.

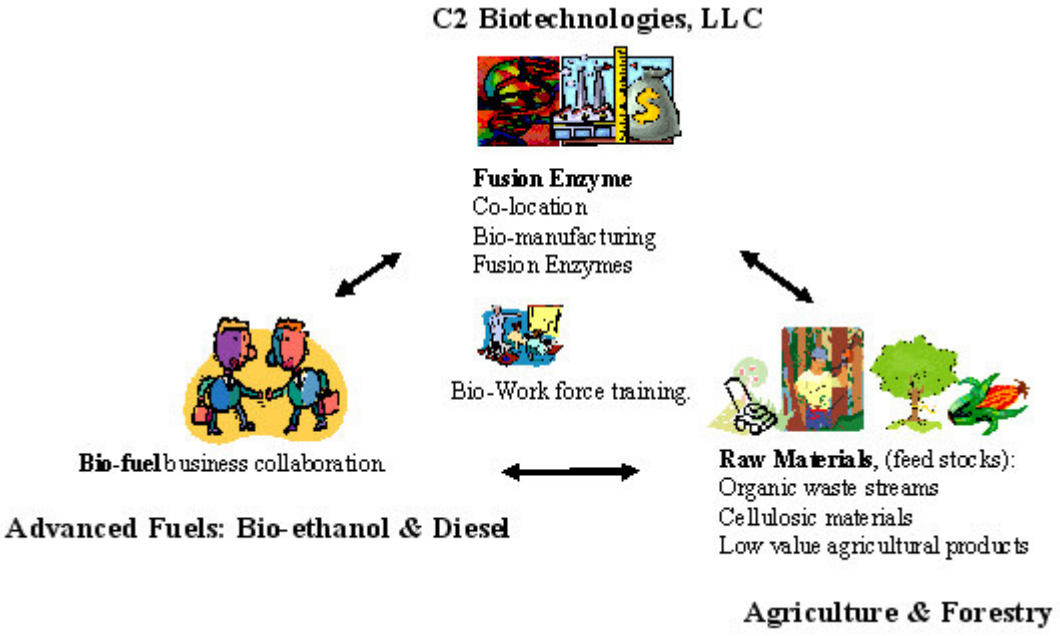
Panel A: Cartoon of native enzyme activities used in converting cellulose to glucose.

Panel B: Cartoon of how cellulosic fusion enzyme is anticipated to function. Negative inhibition of cellobiohydrolase is shown with reverse arrow. Fusion enzyme is represented by having both cellobiohydrolase and glucosidase activities physically linked with a line.

Other benefits of this project are the development of new tools for cellulosic fuel producers.

C2's vision of the future decentralized biorefineries

We envision production of biofuels in a grid of "small" volume plants where our fusion enzyme bio-manufacturing is co-located with biofuel plants that target or specialize in local feed stocks.



C2 Biotechnologies, LLC

C^2 Biotechnologies (C2B) is a Limited Liability Company (LLC) formed in Ulster County, New York, in 2006 that uses biotechnology to improve the quality of life through a distributed bio-manufacturing organizational structure producing consumables for the energy market.

Our fusion technology platform allows us to produce enzyme products with multiple biological activities. Producing one product with multiple activities reduces manufacturing requirements and results in value adding products at reduced costs for our clients.

Our idea of a distributed bio-manufacturing structure is unique. We will offer seamlessly integrated on site enzyme production facilities guaranteeing un-compromising customer service satisfaction and product reliability.

By co-locating our enzyme manufacturing next to client operations reduces product packaging and translocation costs.

The modular design of our production facilities will also allow for unique franchising opportunities.

C2B has developed a three tier business strategy that involves selling customer service and support, unique enzyme technology and franchises worldwide for the production of biofuel. These operations are environmentally friendly, economically viable and politically timely.

For more information about C2 Biotechnologies, contact Larry Cosenza, Ph.D. at lcosenza@c2biotechnologies.com.