

Department of Agriculture, Trade and Consumer Protection
Division of Agricultural Development
Agriculture Development and Diversification Program
Grant Project Final Report

Contract Number: 21003

Grant Project Title: Development of Novel Enzymes to Facilitate Commercial Bioethanol Production from Soy Meal Carbohydrate

Amount of Funding Awarded: \$45,000

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Please use the following questions as a guide for writing your grant project final report. In your final report, please answer each question as it relates to your grant project. Information submitted in this survey will be public record, unless it contains trade secret information that you request (in writing) to be kept confidential. The Department intends to post final report on the DATCP webpage.

- 1) What was the original intent of the grant?
- What did you want to accomplish with the grant?
 - How was it expected to benefit the Wisconsin bio-based industry i.e agriculture and forestry?
 - What makes this project work important or significant?

The intent of the grant was to provide funding to further the development of novel enzymes designed to convert the carbohydrate portion of soy meal to ethanol. The funding from the grant was intended to forward project development work and help move the project to the proof of concept stage.

The project could potentially benefit Wisconsin by helping to commercialize this technology which, for the first time, would facilitate the production of ethanol from soy substrate. Currently, a biodiesel facility and a proposed soy crush facility are planned for a campus site in Evansville, Wisconsin. A soy ethanol/protein facility would be a natural addition to this campus with many synergies among the three potential participants. Such a facility with a biodiesel plant, a soy ethanol/protein plant and a crushing plant would be the only facility of its kind in the world. In addition to producing both ethanol and biodiesel at a low price, the facility would provide concentrated soy protein that could be sold into various markets that are important to Wisconsin such as dairy feed and forest products adhesives.

What steps did you take to reach your goal?

Thermophilic organisms that degrade soy carbohydrates were identified and isolated from samples obtained at hot springs. DNA libraries were constructed from these organisms in a plasmid capable of producing the enzymes at useful concentrations. The correct clones were identified and fermentation conditions were optimized to generate maximum enzyme yield. The cloned enzymes were able to release fermentable sugars from both the soluble and insoluble carbohydrate in soy.

- What worked?

A number of enzymes were identified that rapidly degrade soy carbohydrate under the desired conditions without pretreatment of the sample.

- What challenges did you face?

Genes for a number of the enzymes obtained were unstable in the cloning vector used for the screening work.

- What would you do differently?

We would utilize a low copy vector that produced less enzyme, but that which would be more stable.

2) What were you able to accomplish?

We demonstrated conversion of soy carbohydrates to fermentable sugars under mild conditions.

- What are the results from this project?

Two enzyme product candidates, SoyBuster 1A and 1B are currently being evaluated for conversion of soy carbohydrates in ethanol fermentations.

- Include any analysis of data collected or materials developed through the project.

3) What conclusions can you make based on project work or on the analysis of collected data?

The data collected to date shows efficacy for SoyBuster 1A and 1B. Either of these two enzymes should allow conversion of 30% to 40% of the carbohydrates to fermentable sugars.

4) What do you plan to do in the future as a result of this project?

The Company is continuing with the effort to develop additional enzymes to increase the conversion of the soy carbohydrates from the 30% to 40% range to the 80% to 90% range.

5) What information or additional resources are needed to commercially develop this enterprise?

The Company is presently developing the additional enzymes needed to reach the conversion goal of 80% to 90%. When the goal is reached, lab scale ethanol production trials will be performed at C5-6 Technologies to verify the performance of the enzyme product. After verification, a pilot facility will be required for the following work:

1. Obtain rate and mass balance data.
2. Define design criteria for full-scale production facility.
3. Prepare samples of soy protein for customer evaluation.

6) How should the agricultural or forestry industries use the results from your grant project?

The agricultural industry will benefit from increased demand for soy, as well as having a local source of high-protein feed for dairy and other applications. Soy protein is currently being developed as a replacement for petroleum-based adhesives in the forest products industry for products such as OSB, particle board, etc. Potential partners to sell the protein produced by this process are also in Wisconsin.