DOE Bioenergy Technologies Office (BETO)
2021 Project Peer Review

White Dog Labs

Upgrading Cellulosic Ethanol Fermentation Streams into Butyrate Enhanced DDGS for Broiler Chickens

March 26, 2021
Advanced Development and Optimization
Process Integration and Scale-Up

Carrissa Wiedel
White Dog Labs

Goal Statement

- The goal of this project is to produce and validate a **Single-Cell Protein (SCP) product** from a cellulosic ethanol plant waste stream, which may contain fermentation broth, whole stillage, thin stillage, or a combination thereof.
- Our target is to produce enough butyrate enhanced SCP product from cellulosic ethanol streams, that can be mixed with DDGS, for broiler feed trials.
- Successful demonstration will provide cellulosic ethanol plants with a new valuable co-product from their current cellulosic ethanol stream and improve the overall economics of the process.

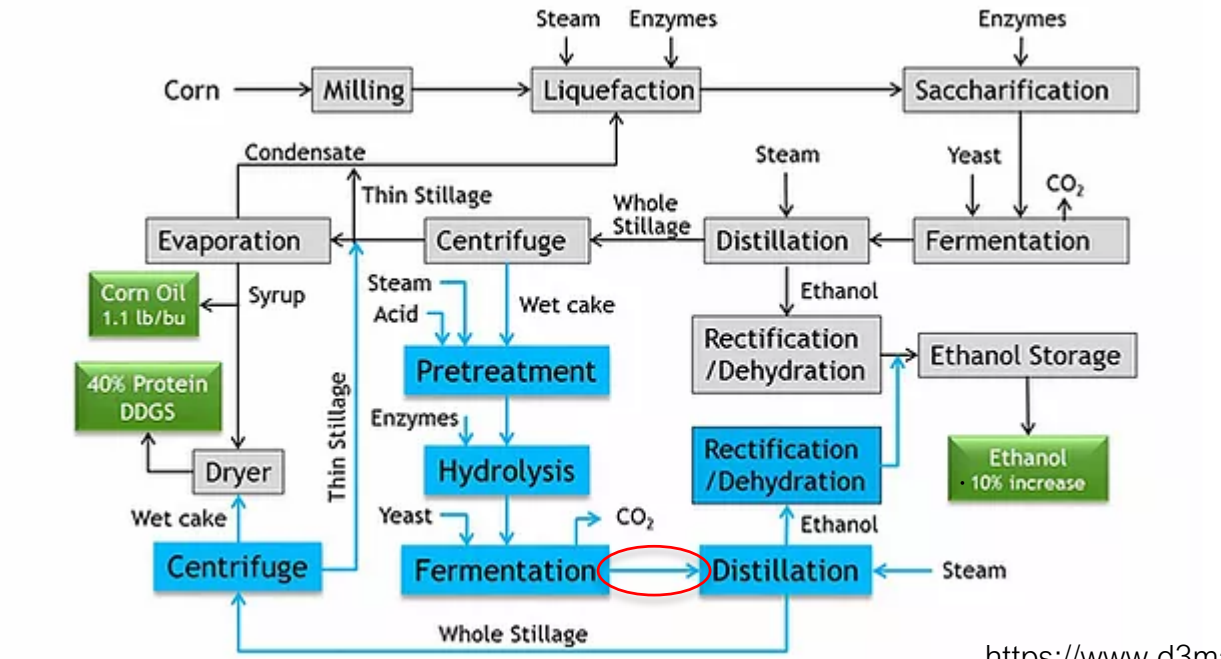
Key Milestones

KEY MILESTONE	FY 2018				FY 2019				FY 2020				FY 2021			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1) Milestone D1 (Verification Go/No-Go)																
2) Milestone M2.1 (Complete filtrate analysis)																
3) Milestone M3.3 (Selection of SCP strain(s))																
4) Milestone M4.2 (Adaptively evolve strain(s) to filtrate)																
5) Milestone M5.1 (Finalize production medium)																
6) Milestone D2 (Achieve KPPs at 10L-scale)																
7) Milestone M6.1 (Demonstrate KPPs at 1000L-scale)																
8) Milestone M6.5 (Complete production of SCP for broiler studies)																
9) Milestone M7.1 (Complete shadow pricing of SCP)																
10) Milestone M7.4 (Complete broiler trials)																
11) Milestone M8.2 (TEA and LCA of final design)																
12) Milestone M9.0 (Final verification)																

01/01/2020

Project Overview

The feedstock for the project is a cellulosic streams generated by Ace Ethanol operating the D3MAX technology in Stanley, WI .

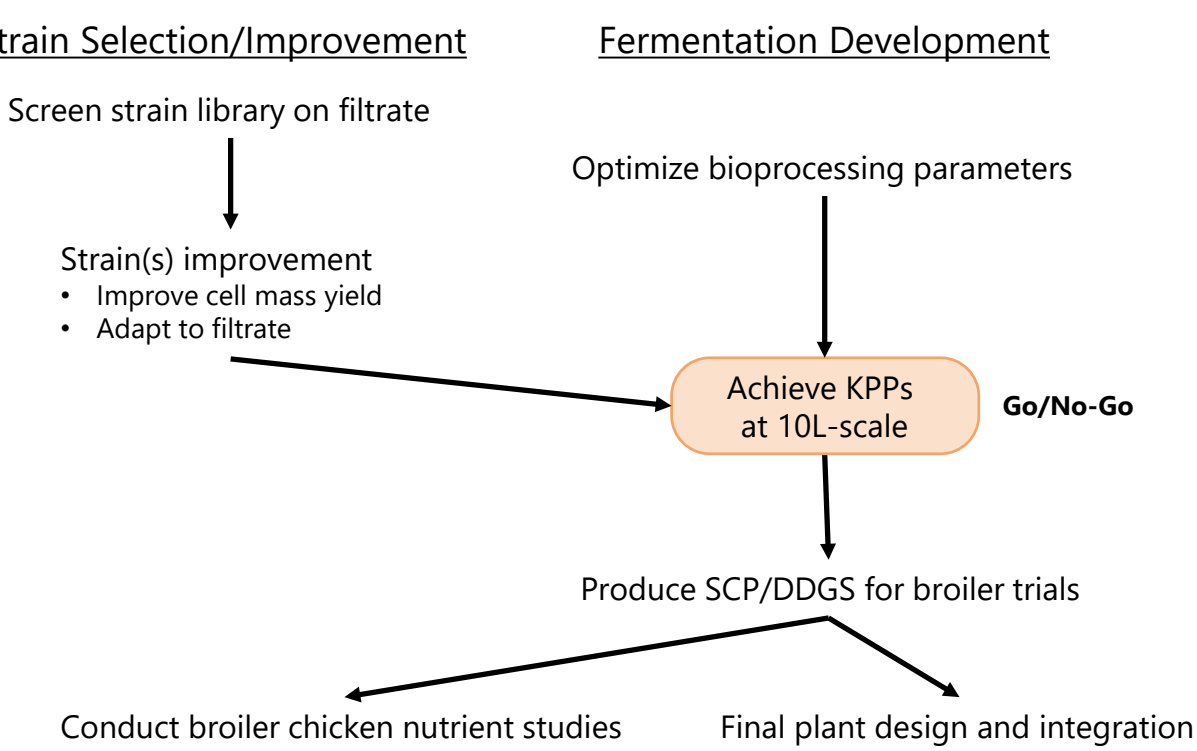


https://www.d3maxllc.com/technology

1 – Management

- WDL performs the vast majority of the tasks
- Two WDL locations:
  - Microbiology lab – strain selection and improvement
  - Fermentation facility – fermentation optimization and SCP production and processing
- Weekly meetings between PI and project leads
- Monthly project update meetings of all team members to update on progress and discuss critical issues
- Two key partners:
  - AHPharma – conduct broiler trials (WDL has worked extensivity with them on previous feed trials)
  - AdvanceBio – final plant modeling and integration (they have worked with WDL previously on commercial plant design)

2 – Approach



Major Challenges

1. Achieving high cell titers on filtrate material. Organic Acids are the main source of feedstock we are limited on the cell titers that can be achieved. Removing the filtration step before fermentation and removing the wash step after fermentation will give us higher cell titers and a desired metabolite
2. Overall fermentation time. Adapting Strain8 to the Ace Ethanol feedstock. By doing several transfers in the seed train with the Ace Ethanol material it allowed us to cut down on the overall fermentation time in the bioreactors.

3 – Impact

A goal for BETO is to help develop co-product production to **enable cost competitive biofuels.**

- Currently, the filtrate is dehydrated to produce a low fiber, high protein DDGS
- WDL proposes a quick fermentation on the beer that can boost the protein content of the DDGS already being produced by the plant
- Butyrate enhanced SCP could provide a significantly *more valuable co-product* to improve overall economics of the process
- Project targets cellulosic filtrate material but could have applicability to the wider biofuel and bioproduct industry
- By the end of the project, the SCP product will be blended with other DDGS to deliver an efficacious dose of butyrate to broiler chickens

Improve overall process economics of cellulosic ethanol to see greater industry adaption

3 – Impact

The higher value DDGS that will be produced in our fermentations will be fed to broiler chickens which based upon previous studies should demonstrate multiple health benefits:

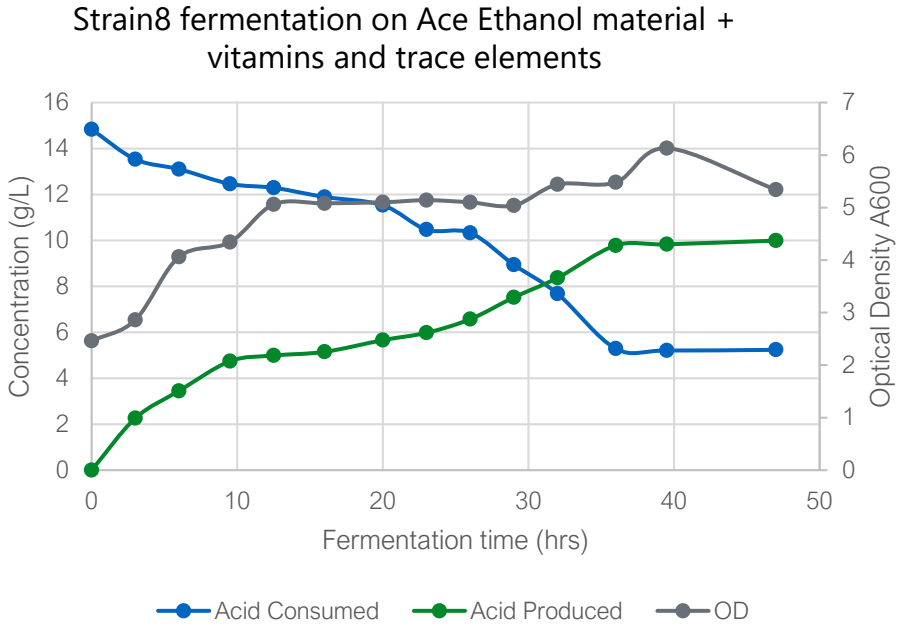
- Inclusion of 1500mg/kg of butyric acid has previously demonstrated an increase in blood globulin which indicates a higher concentration of antibodies in circulation (Barnas et al., 2020).
- Butyrate is considered to be important for normal development of epithelial cells (Pryde et al., 2002)
- A study by (Fernández-Rubio et al., 2008) showed that butyric acid enrichment has led to a significantly lower percentage of *Salmonella* in fecal shedding of broilers.

4 – Progress and Outcomes

- Original provider of the feedstock for this grant stopped production for the foreseeable future after we had already completed a 2L and 10L fermentation
- Completed composition analysis of new cellulosic filtrate material from Ace Ethanol
- Conducted a screening of potential strains for their ability to utilize the filtrate material as is and discovered Strain8 – a novel isolate of WDL found during a chemostat
- Strain8 could consume organic acids and grow on filtrate as is
- Conducted 2L and 10L fermentations on the Ace Ethanol material - cells were harvested, washed, dried, and analyzed
- Instead of trying to separate out a small fraction of protein to go into shrimp feeds WDL is moving toward using all the protein and butyrate produced to go into a specialty DDGS with focus on broiler applications

4 – Progress and Outcomes

2L Fermentation



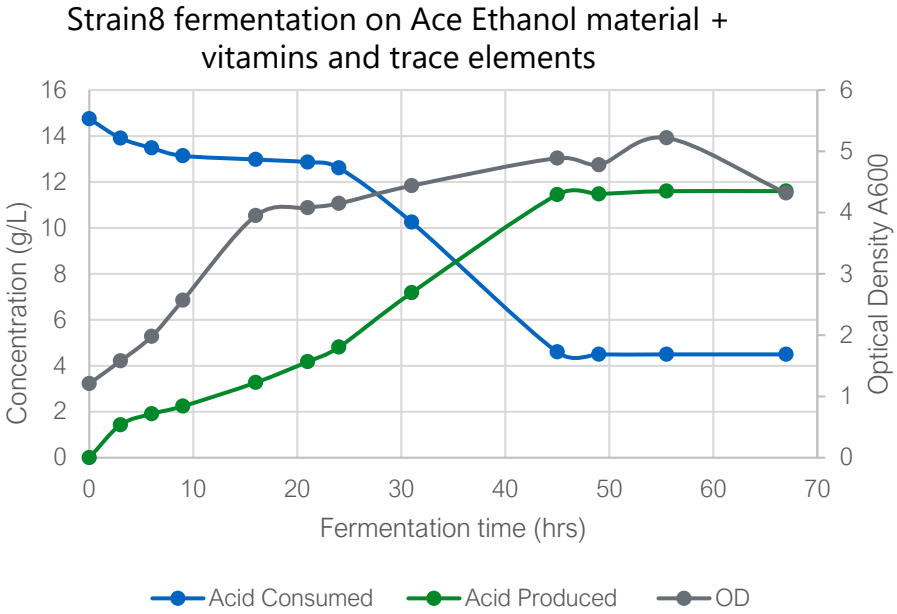
- The fermentation reached exhaustion just under 48 hours after inoculation
- Carbon balance: 78% (mol/mol) (excluding cells and CO2)

Proximate data		
Component	**As fed**	**Dried**
Moisture	89.1%	-
Crude protein	9.4%	86.3%
Ash	1.4%	12.2%

High protein content but low cell titers make for poor SCP feed by itself

4 – Progress and Outcomes

10L Fermentation



- The fermentation reached exhaustion just under 70 hours after inoculation
- Carbon balance: 95% (mol/mol) (excluding cells and CO2)

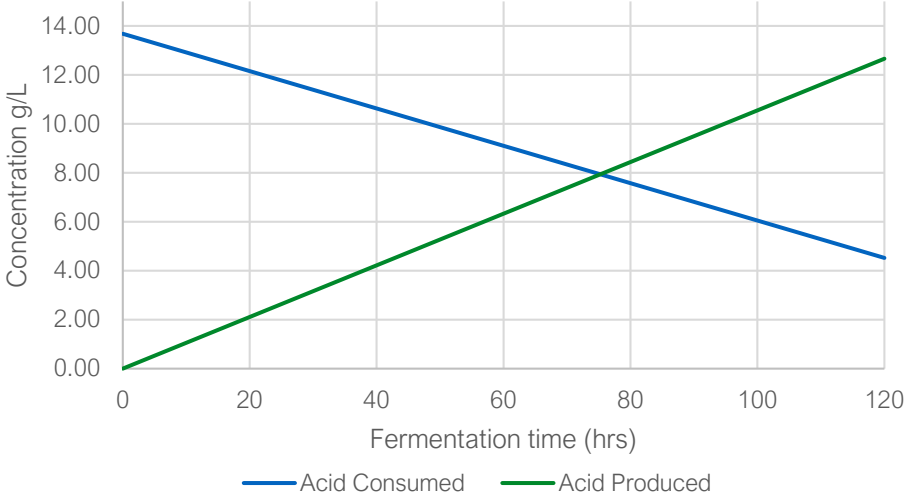
Proximate data		
Component	**As fed**	**Dried**
Moisture	88.3%	-
Crude protein	10.1%	86.6%
Ash	1.42%	12.15%

High protein content but low cell titers make for poor SCP feed by itself

4 – Progress and Outcomes

Bottle-Scale Fermentation

Strain8 fermentation on unfiltered Ace Ethanol material with no vitamins and trace elements



- This bottle-scale fermentation was concentrated but not washed afterwards
- 5.7 g/L of Cell Dry Weight + DDGS were produced
- We are requesting more material from Ace Ethanol to repeat this on the 10L scale before we scale up to production for the feed trials

High protein cell mass will boost the protein content in the DDGS and the butyrate salts will make the final product a high-value feed

Future Work

Fermentation Development

- Repeat 10L fermentation without filtering or washing the material and then scale-up to 1000L for SCP production
- Integrate fermentation, SCP separation, and drying

SCP Testing

- Determine final composition of SCP (crude protein, crude fat, amino acid make-up, etc.)
- Conduct nutritional feeding trials
- Estimate potential market value of SCP based on composition and feed trial results

Summary

- This project aims to upgrade a current cellulosic fermentation stream into a valuable SCP product to improve overall process economics
- The goal is to provide the process with a higher-value product diversified away from the biofuel market
- Project is underway and has demonstrated the filtrate stream is fermentable as is
- Immediate action items for the next quarter is to repeat the 10L fermentation and quickly scale up to begin production of the butyrate enhanced SCP for broiler feed trials

Quad Chart Overview

Timeline

- January 1, 2018
- December 30, 2021

Budget

	FY20 Costs (10/01/2019-09/30/2020)	Total Award
DOE Funded	$396,085.49	$2,233,290
Project Cost Share (Comp.)*	$99,021.34	$1,073,999

Project Goal

To further develop utilization of cellulosic-based fermentation streams into a high value SCP for broiler feed applications

End of Project Milestones

Integrate the proposed SCP process into Ace Ethanol's current model to increase the value of their current DDGS that are used in animal feed

Partners

- White Dog Labs (WDL) – 94%
- AHPharma – 5%
- AdvanceBio – 1%
- Ace Ethanol (filtrate providers)

Additional Slides

Responses to Previous Reviewers' Comments

- The proposal did not identify potential problems with dual culturing of bacteria.

We are no longer are using a dual culture. When the feedstock changed, we rescreened our library to find a strain capable of consuming organic acids and grows on the material as is. We have been able to move forward with using only Strain8 which grows well on the Ace Ethanol material.

- Reducing ash level is a significant challenge to make this a valuable co-product or a blendable product.

The Ace Ethanol feedstock that we are now using has a lower ash content than the material we were previously obtaining from POET. 10.15% now vs 21.2% that we were receiving previously

Publications, Patents, Presentations, Awards, and Commercialization

No publications, patents, presentations, or awards have resulted from this work yet.

Commercialization efforts are in the very early stages with Ace Ethanol/D3MAX.

Project Budget Table

	Original Project Cost (Estimated)			Project Spending and Balance		Final Project Costs
Budget Periods	**DOE Funding**	**Project Team Cost Shared Funding**	**Contingency**	**Spending to Date**	**Remaining Balance**	**What funding is needed to complete the project**
BP1 – Verification	$13,000	$3,000	$0	$16000	$0	$0
BP2	$1,215,000	$308,000	$0	$1523000	$0	$0
- Strain tasks	$436,000	$109,000	$0	$545,000	$0	$0
- Fermentation tasks	$795,000	$199,000	$0	$994,000	$0	$0
BP3	$989,000	$763,000	$0	$148,900	$1,378,100	$0
- SCP production	$824,000	$703,000	$0	$148,900	$1,378,100	$0
- Broiler trials	$140,000	$35,000	$0	$0	$175,000	$0
- Plant design	$25,000	$25,000	$0	$0	$50,000	$0