DOE Bioenergy Technologies Office (BETO)
2019 Project Peer Review

White Dog Labs

Upgrading of Stillage Syrup into Single Cell Protein for Aquaculture Feed

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Advanced Development and Optimization: Integration and Scaleup

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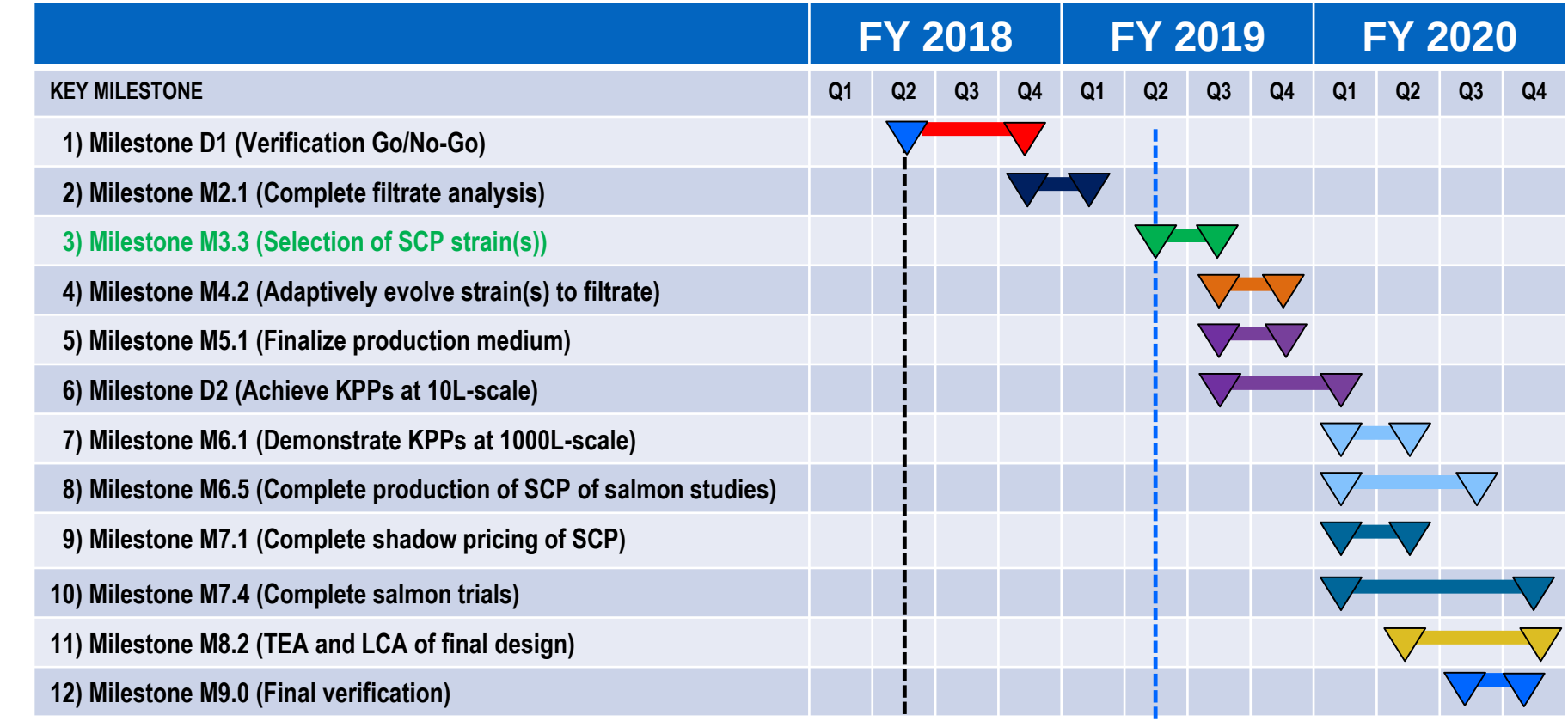
Goal Statement

- The goal of this project is to produce and validate a **Single-Cell Protein (SCP) product** from a cellulosic ethanol plant waste stream, specifically the stillage filtrate material.
- Our target is to produce enough SCP product (~100 kg) from actual filtrate material for salmon feeding and digestibility trials.
- Successful demonstration will provide cellulosic ethanol plants with a new valuable co-product from a current waste stream and improve the overall economics of the process.

Key Milestones



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	$17,000	$0	$0
BP2	$1,231,000	$308,000	$0	$262,000	$1,277,000	$0
- Strain tasks	$436,000	$109,000	$0	$184,000	$361,000	$0
- Fermentation tasks	$795,000	$199,000	$0	$79,000	$915,000	$0
BP3	$989,000	$763,000	$0	$0	$1,752,000	$0
- SCP production	$824,000	$703,000	$0	$0	$1,527,000	$0
- Salmon trials	$140,000	$35,000	$0	$0	$175,000	$0

Quad Chart Overview

Timeline

- January 1, 2018
- September 30, 2020
- 10% complete

Budget

	Total Costs Pre FY 17	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date
DOE Funded	$0	$0	$101k	$2,132k
Project Cost Share (Comp.)*	$0	$0	$25k	$1,049k

Barriers

- ADO-D – Technology Uncertainty of Integration and Scaling
- Ct-J – Identification and Evaluation of Potential Bioproducts
- At-D – Identifying New Market Opportunities for Bioenergy and Bioproducts

Partners

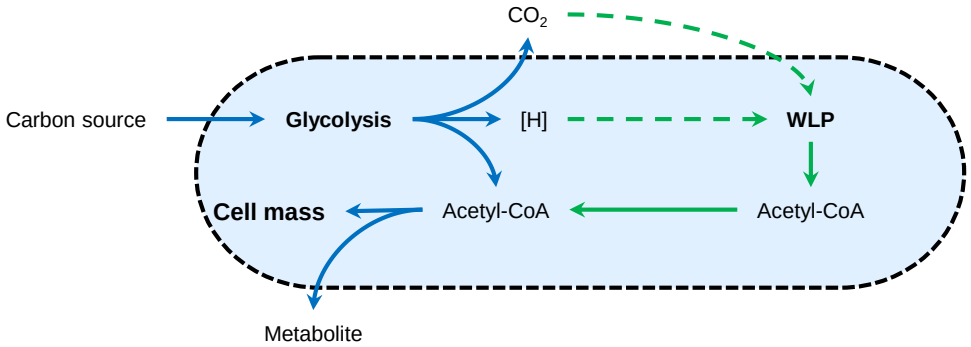
- White Dog Labs (WDL) – 94%
- Center for Aquaculture Technologies Canada (CATC) – 5%
- AdvanceBio – 1%
- POET/DSM Project Liberty ethanol plant (filtrate providers)

1 – Project Overview

FOA: DE-FOA-0001689 (Integrated Biorefinery Optimization)

Topic Area 2: High value products from waste and/or other under-valued streams in an IBR

WDL is currently commercializing a starch-based **Single-Cell Protein (SCP) product** as an aquaculture feed ingredient using its MixoFerm™ platform.



MixoFerm™ can improve cell mass yields for anaerobic SCP

1 – Project Overview

The feedstock for the project is the filtered whole stillage generated by the POET/DSM Project Liberty plant in Emmetsburg, IA.

For Proposal

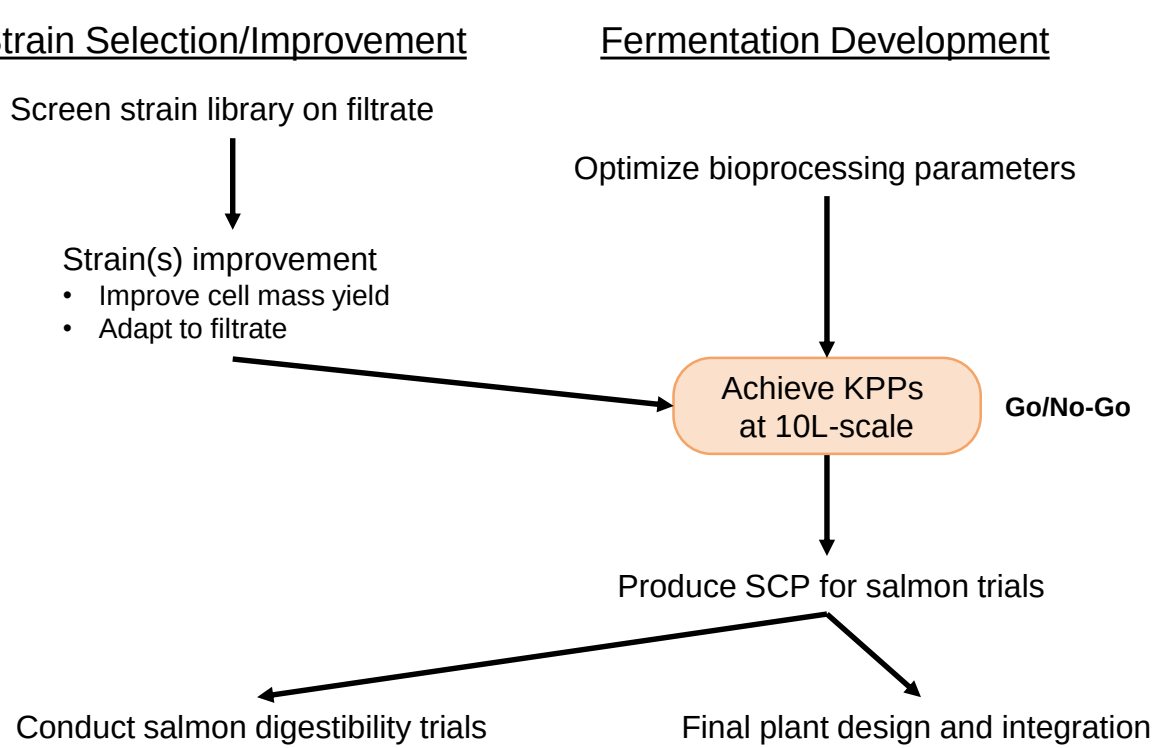
- Told filtrate contained residual C6 & C5 sugars and glycerol (unable to test before submission)
- Proposed a co-culture of a glycerol-consuming non-acetogen with an acetogen capable of MixoFerm™

Tested filtrate

- No residual C6 sugars
- Minimal C5 sugars with goal of total consumption
- No glycerol
- Primarily lactic acid and acetic acid

Need to identify different strain(s) for SCP

2 – Approach (Technical)

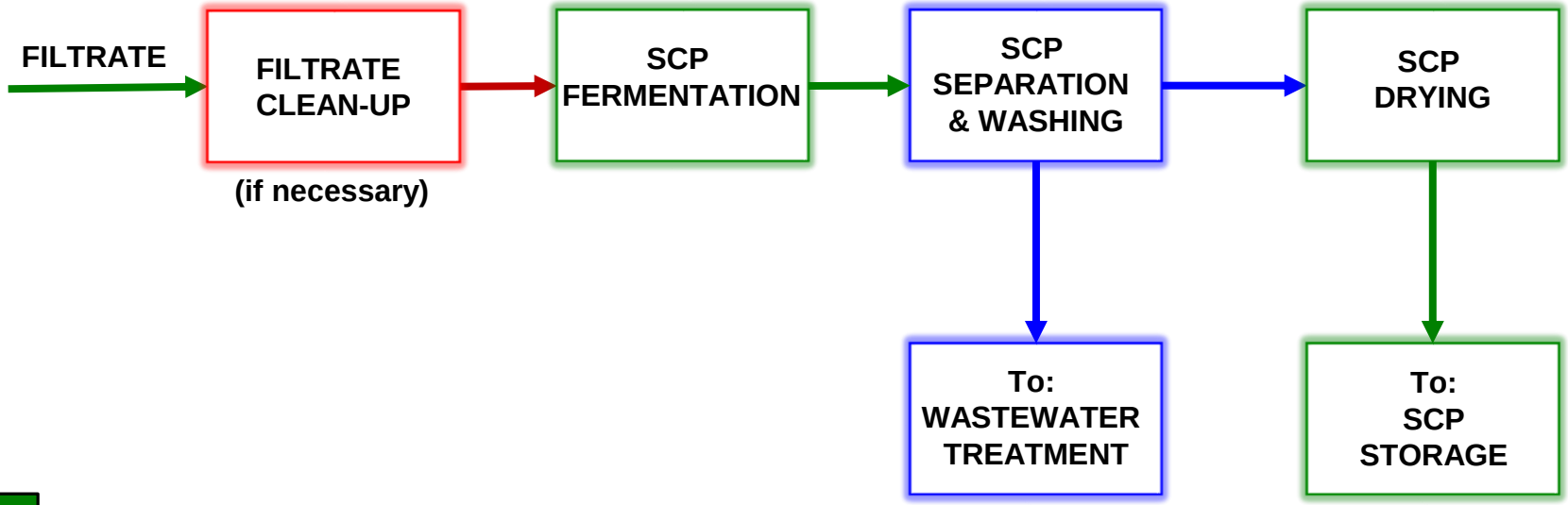


Major Challenges

1. Achieving growth on filtrate material. May contain inhibitory compounds or concentrations → Adapt strain(s) and/or additional processing of filtrate may be required

2. Palatability issues with SCP during feeding trials. Fish can be sensitive to changes in feed ingredients → Further purification of SCP or may need to include palatability enhancers

2 – Approach (Technical)



No Issues

Minor Issues

Moderate Issues

2 – Approach (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:
  - CATC – conduct salmon fish trials (have worked extensivity with on current SCP feed trials)
  - AdvanceBio – final plant modeling and integration (have worked with WDL previously on commercial plant design)

3 – Technical Accomplishments

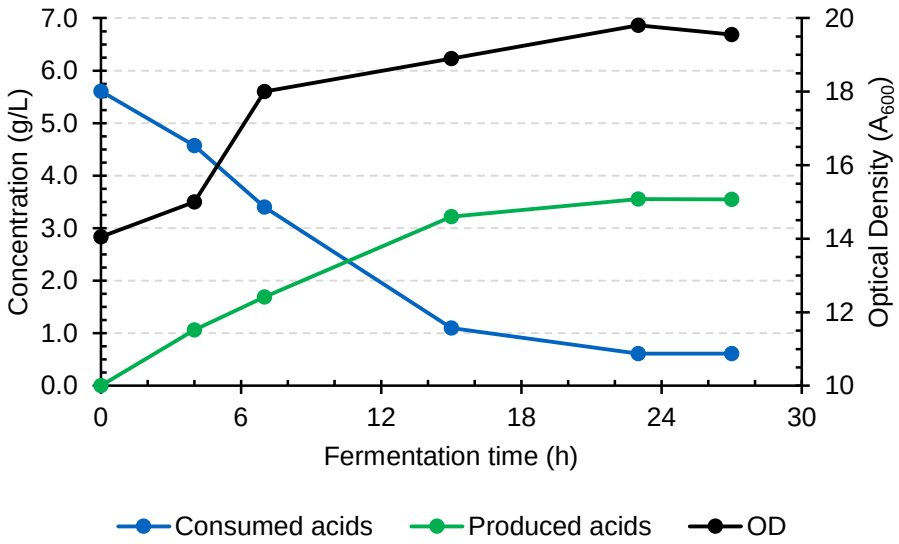
Achieved **GO** outcome from successful verification event

- Completed composition analysis of cellulosic filtrate material
- Based on compositional analysis, original proposed strains (glycerol-consuming and an acetogen) are not the ideal strains.
- Conducted a small screening of 11 potential strains for their ability to utilize the filtrate material as is (no additional components)
- Identified two potential strains:
  - *Megasphaera elsdenii* – isolated from a cow rumen)
  - *Clostridium* strain (termed Strain8) – novel isolate of WDL found during a chemostat evolution
- Strains could consume organic acids and grow on filtrate as is

3 – Technical Accomplishments

Test fermentation

Co-culture of Strain8 and *M. elsdenii*



Cell mass yield: 23.1% (g cells/g acid consumed)

Carbon balance: 85.2% (mol/mol)
(there is an unknown peak on HPLC chromatogram)

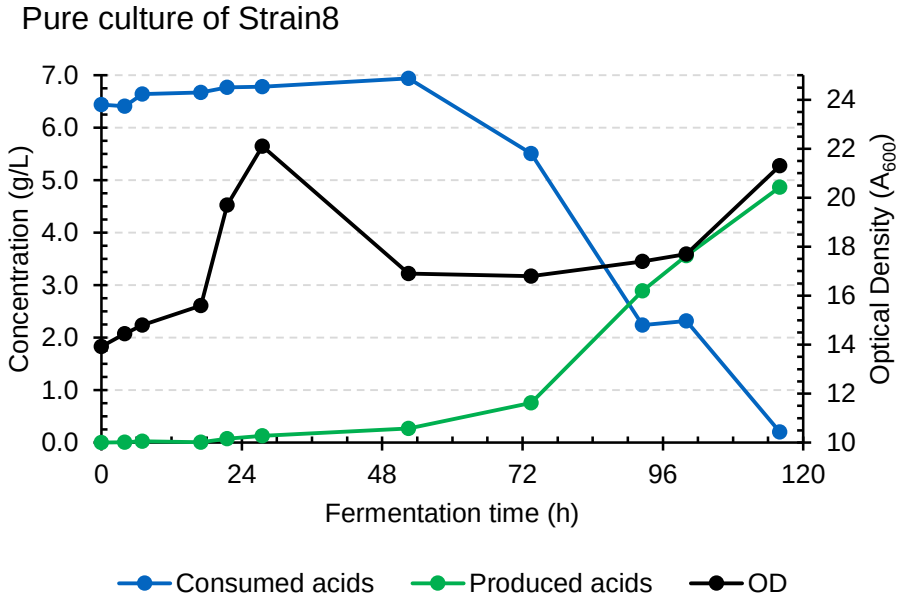
Proximate data

Component	As fed	Dried
Moisture	77.10%	-
Crude protein	2.88%	12.74%
Crude fat	0.70%	2.90%
Ash	5.27%	23.05%

Good growth but high ash makes for poor SCP

3 – Technical Accomplishments

Test fermentation



Cell mass yield: 27.2% (g cells/g acid consumed)

Carbon balance: 97.5% (mol/mol)

Proximate data		
Component	**As fed**	**Dried**
Moisture	76.30%	-
Crude protein	4.36%	18.25%
Crude fat	0.80%	3.60%
Ash	6.67%	28.14%

Good growth but high ash makes for poor SCP

4 – Relevance

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

- Secondary goal is to valorize all waste streams into a valuable co-product
- Currently, the filtrate is sent to an anaerobic digester for biogas production
- SCP could provide a significantly *more valuable co-product* to improve overall economics of the process and could *reduce wastewater clean-up costs*
- 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 validated with a salmon feeding trial

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

5 – Future Work

Strain Selection/Improvement

- Screen a larger library (>20 strains) for improved acid consumption
- Once strain is selected, determine if any pathways need to be deleted

Fermentation Development

- Scale-up fermentation to 10L and then 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 digestibility trials
- Estimate potential market value of SCP based on composition and digestibility results

5 – Future Work

Major Challenges to be addressed

- Improving fermentation (cell mass titers and yields)
  - Since only organic acids are available as feedstock, may need to add a more reduced feedstock to help improve titers and yields
  - Need to evaluate viability of different waste feedstocks, like biodiesel waste glycerol
- Decreasing ash content
  - Filtrate has high ash content and is purified with SCP to lower crude protein content
  - Need to reduce ash either before fermentation or better separate SCP from ash
- Impact on wastewater treatment costs
  - Fermentation broth will be sent to wastewater treatment
  - Need to determine if capable with current system at Project Liberty and if we impact treatment costs (either an increase or a decrease)

Summary

- This project aims to upgrade a current waste stream into a valuable SCP product to improve overall process economics
- The goal is to provide the process with another high-value coproduct diversified away from the biofuel market
- Project is underway and has demonstrated the filtrate stream is fermentable as is
- Major challenges to be addressed over the next year:
  - Improving cell mass yield and titer
  - Improving SCP product by removing ash

Additional Slides

Responses to Previous Reviewers' Comments

This is the first time this project is being reviewed, and no Go/No-Go Review meetings have taken place yet.

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 POET/DSM.

Project Scope Change Table

Scope Changes	Date	Logic / Reasoning	Approval / Rejection Date

No changes have been made to the Project Scope.

Risk Registry Table

Risk	Subtask	Impact on Project	Likelihood	Impact	Mitigation action	Current Status
High variability in the syrup	2.1	Significant changes in the composition of the syrup, including concentrations of substrates and/or inhibitors, will cause inconsistent fermentations	Unlikely	Significant	If the two tested batches show significant differences, additional batches will be tested to determine an average syrup composition to complete the project on. We will also discuss with POET-DSM their experience with variations in the syrup composition to see if these can be minimized.	Active (we evaluating additional filtrates)
High inhibitor concentration in cellulosic syrup	2.2	Delays selection of strain(s) and fermentation optimization	Unlikely	Critical	There are a number of treatments (both chemical treatment and process steps) that can be applied to the syrup to remove and/or neutralize inhibitors. Depending on the inhibitor and its concentration, we will select a treatment method.	Active (we will evaluate additional filtrates, though so far inhibitor presence has not been an issue)
Crude protein content of all strains <70%	3.3	Decreases the value of the final SCP product	Very unlikely	Significant	Additional strains will be screened on the syrup to identify ones higher protein content.	Active (need to improve ash separation from SCP)
Unable to achieve a doubling time of ≤2.5hrs	4.2	Extends fermentation time which could increase operating costs	Moderately likely	Marginal	The medium could be reformulated with additional nutrients to improve growth rates. This will have to be balanced between operating costs and media costs.	Active (will be addressed in strain screening)
Unable to achieve 25wt% cell mass yield	5.2	Increases operational costs as more feedstock will be needed to meet production goals	Moderately likely	Significant	Based on the metabolite profile, additional pathways would need to be deleted and/or overexpressed to direct more carbon towards cell mass creation.	Active (will be addressed in strain screening)
Unable to achieve 20 g/L cell mass titer	5.2	Increases capital costs as more fermentation capacity may be needed	Unlikely	Marginal	The medium can be reformulated to provide additional nutrients and/or removal of inhibitors from the broth.	Active (will be addressed in fermentation optimization)
Process behaves differently at 1000L than at 10L	6.1	Delays fish trials and final demonstration of the project	Very unlikely	Significant	Additional 1000L fermentations will have to be run and compared to the 10L fermentations to determine the impact of scaling. WDL has scaled the corn ethanol syrup process with no difficulties.	Active (will be addressed upon scale-up)
Unable to achieve 80% digestibility of SCP	7.3	Decreases the value of the final SCP product	Unlikely	Significant	Lysis procedures will be reevaluated to determine if increased lysis will improve digestibility.	Active (will be addressed in feeding trials)