Optimization of E. coli Fermented Synthetic Spider Silk for Commercial Viability

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ABSTRACT

Spider silk has impressive physical properties in the areas of strength, weight, toughness and elasticity, with commercial use currently limited as large-scale production has not been demonstrated. Transgenic work to synthetically replicate the spider silk proteins via *E. coli* has successfully been performed. Spider silk based products show promising characteristics, but the economic viability of synthetic spider silk production has not been evaluated. This work presents an analysis of the economic viability and environmental impact associated with the production of synthetic spider silk through *E. coli* fermentation. System models, validated with experimental data, were leveraged with economic and life cycle modeling to evaluate the sustainability of synthetic spider silk production. Results show protein yield, modeling scale, and processing technologies dramatically impact final silk protein cost as well as the global warming potential.

Keywords: spider silk, *E. coli*, economic analysis, life cycle, synthetic

1 INTRODUCTION

Until recently, synthetic spider silk has not been produced in sufficient quantities to facilitate integration into commercial markets. Production levels have been limited and only supported testing of material properties and development of small examples of spider silk based products. Advances in transgenic work have allowed for the synthetic creation of spider silk proteins in host organisms. Successful candidates for protein expression have included transgenic *E. coli*, silk worms, alfalfa, and goats. Continued research has lead to increased yield allowing for better analysis of the physical properties and more opportunities to investigate prospective products. A strain of *E. coli* capable of producing significant amounts (2.8 grams or purified synthetic spider silk protein per liter of cultured media (g/l)) of spider silk protien has successfully been created. This strain has also been demonstrated to maintain protein expression levels in cell culture volumes of up to 350 L. Several paths towards high volume output and varying levels of economic sustainability have been identified.

As a renewable resource, spider silk could have significant impact in the commercial sector. However, some of the processing methods used in production are energy and material intensive, potentially limiting the economic viability. This work focuses on the evaluation of the commercial feasibility and environmental impact of spider silk production through fermentation. Characterization of the material inputs and outputs coupled with sustainability modeling enables the quanitification of the cost as well as the global warming potential (GWP) associated with the production of synthetic spider silk. Data feedabck from techno-economic analysis (TEA) and life cycle analysis (LCA) of the current methods is used to identify target areas for further research and development, drivng towards an economical and environmentally favorable production pathway.

2 METHODS

The production process was modeled on each of the sub-process levels and integrated back together into a system model. Sub-process models were validated with experimental data. Experimental work was performed in large (500 L) fermentors to support validation of the modeling work performed at industiral scale. The system model represents the foundation for economic and environmental assessment. Mass flow rates are used to size processing equipment and determine the associated capital costs. All of these results are used to make an estimate of the actual cost of production and the minimum commercial sale price as produced in an industrial setting.

Several different production scenarios are considered. All of the production scenarios estimate the price of silk as produced in industrial quantities, which range from one to twelve million kg per year, depending on the protein expression level. The TEA includes standard financial considerations such as capital expenses, staffing, maintenance, internal rate of return, an installment loan, taxes and depreciation. Sensitivity analysis has been done to identify processes that most significantly affect product cost.

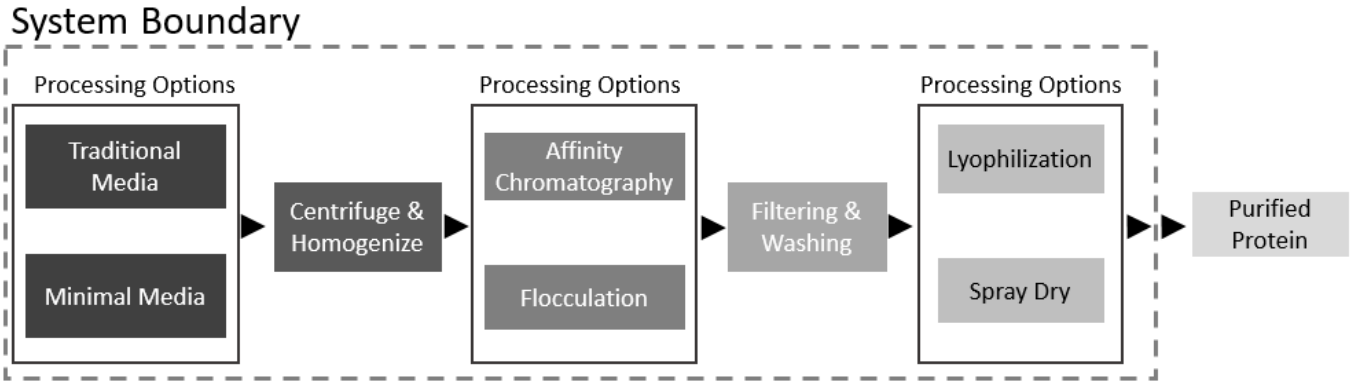

Figure 1: The major sub-processing steps for the production of synthetic spider silk via *E. coli*. Boxes represent various processing options at a specific stage

2.1 *E. coli* Process Modeling

Production is composed of several stages: fermentation, harvesting, purification, and product formation. Fermentation involves the feeding and development of the transgenic *E. coli* for exponential growth and expression. Harvesting includes removing the *E. coli* from the culture media and lysing the cells. Purification of the protein is done to separate the spider silk proteins from the bio-waste and the remaining media. This can be accomplished by either affinity chromatography or flocculation. The purified protein is then washed with alcohol and water before being dried. Purified dry synthetic spider silk protein powder can then be mixed with water for the application of many different products. These major processing steps with the various processing options are shown in Figure 1.

2.1-1 Fermentation

Fermentation of *E. coli* has two major phases, exponential growth and induction. During exponential growth the *E. coli* are fed all the nutrients needed for cellular division until an optimal cell density is reached. Induction triggers the expression of the spider silk protein in the single-celled organism. The level of protein expression is very important in determining economic viability, and will be discussed further in the results section. The baseline expression level is assumed to be 2.8 g/l.

Several variations are considered for the fermentation broth recipe, with a rich media and a basic media, as well as two different carbon sources. Traditional media provides all of the basic nutrients to encourage optimal growth, while minimal media excludes some of the amino acids and only provides the most basic nutrients to sustain life. Glycerol is also considered as an alternative carbon energy source to glucose. Glyceol represents a by product of bio-fuel refineries and would be a low cost carbon source with increasing biofuel production.

Induction of the media causes the *E. coli* cells to begin to express the spider silk protein and is done in the laboratory using Isopropyl β-D-1-thiogalactopyranoside (IPTG). An alternative method to chemical induction is heat induction. Heat induction is done by temporarily raising the temperature of the media to 45 °C [1] which similar to chemical induction causes the *E. coli* to begin to express the protein. Heat induction may or may not be possible with this strand of *E. coli*, but the economic and environmental affects have been analyzed.

2.1-2 Harvesting & Purification

Harvesting and purification are necessary to separate the protein from the *E. coli* cell. After fermentation, the *E. coli* culture is passed through a centrifuge to harvest the *E. coli* cells in the form of pellet from the media. The pellet is mixed with various salt solutions which help the protein to dissolve after the cells are lysed. In industrial settings lysis is done with a homogenizer which uses the sudden pressure change to bursts the cells, releasing the protein. A second pass through the centrifuge removes much of the solid bio-waste, while the spider silk protein stays in solution. Affinity chromatography and flocculation are two methods that can be used to remove additional waste and purify the protein solution, both are modeled in this work.

2.1-3 Product Formation

Spider silk as purified protein powder is useful for many applications. Mixing the protein powder in water with heat and pressure solubilizes the protein into an aqueous form. Fibers can be extruded from the solution. Other products can be made from the solution like hydrogels, lyogels, glue, coatings and microcapsules. Other hydrophilic materials can also be added to the solution. Spider silk is hypoallergenic in nature, making it suitable for many medical purposes such as replacement ligaments joints, antifungal coatings, and slow time drug-release medical implants. The econmic viability of the process will be dependent on the end use. This work focuses on the cost of producing the protien in a raw form that can then be integrated into advanced processing.

2.2 Techno-economic Analysis

Operational and capital expenses were combined to evaluate cost over the life of a production facility. Net

present worth was used in conjuction with loan repayment, and other operational costs to identify the minimum sale price incorperating an internal rate of return of 10%. Where possible, commercial pricing and estimates were used for equipment, material and labor costs. Aspen plus economic analyzer provides equipment estimate costs based on mass flow rates. In some cases, equipment costs were calculated based on literature reviews assessing industrial equipment costs. Staffing estimation is based off of industrial averages for employee density per square meter for hightech/R&D industrial settings. [2]

Tax rates, internal rate of return, depreciation and other standard financial expectations were considered in the economic evaluation. The facility was assumed to take 3 years to build and, following it, a 10 year pay back period on the capital loan. Interest on the loan was assumed to be 10%. Working capital and investment capital are 5% and 40% of the initial capital costs, respectively. Depreciation over an eight year period was also calculated in. Income tax was assumed to be 35% of the annual profit.

2.3 Life Cycle Analysis

Material and energy inputs are quantified through the engineering system model and combined with life cycle inventory (LCI) data to quantify the GWP of the production system. LCI data values were obtained primarily from GREET and other literature sources. [3] In some cases, LCI data values were not always available, so alternative sources were used that have similar production methods and are therefore likely to have similar emissions. Component emissions were included for all products that contributed at least 1% of the total mass consumed, when excluding water, as water makes up over 80% of the consumed mass.

3 RESULTS & DISCUSSION

3.1 Pilot Plant TEA

The results from a scale up model, which uses all of the current laboratory material input and output values as provided by Lewis et al., indicate that the material costs dominate other monetary inputs in the production process, as shown in Figure 2. In determination of the sale price of purified spider silk protein, material consumption makes up over 80% of the associated costs. The majority of these expenses come directly from purification by chromatography followed by fermentation.

The material consumption rates for fermentation and the affinity chromatography processes are dependent solely on the amount of *E. coli* pellet that is being processed. This is due to the "batch" nature of these techniques. The same amount of materials are consumed every batch, even though the yield varies due to varying levels of protein expression in the *E. coli*. The chromatography column requires several buffer solutions to prepare it, charge it, strip it, and to precipitate out the protein. Some of the components used are very expensive even in industrial quantities. The nickel solution used to charge the column contributes the largest portion of material cost associated with affinity chromatography.

IPTG is also a very expensive material, with commercial wholesale pricing listing as high as $850/kg. The contribution to material costs from IPTG is over 8% of the total cost even though it makes up less than 0.01% of the mass consumed. In fermentation, the material costs for Hy-express and yeast extract are significant contributors.

3.1.1 Process Optimization

Process optimization for this project focuses mainly on using alternative processing methods that either consume less material or energy or use less expensive materials. Material costs can be reduced by replacing some of the current laboratory processing methods such as the use of minimal media over traditional media, using heat induction over IPTG, heat treatment of waste over chemical sterilization, and flocculation over affinity chromatography.

Not all of these methods have been fully demonstrated with spider silk recombinant proteins produced by the transgenically modified *E. coli*, but they do represent procedures common to industrial applications. The two most impactful methods of process optimization are the use of minimal media and flocculation.

The substitution of minimal media over traditional media has been validated. The preliminary results have shown little to no decrease in protein expression, while dramatically reducing the material costs. These results correspond to protein expression levels of 2.8 g/l and do no represent increased yield.

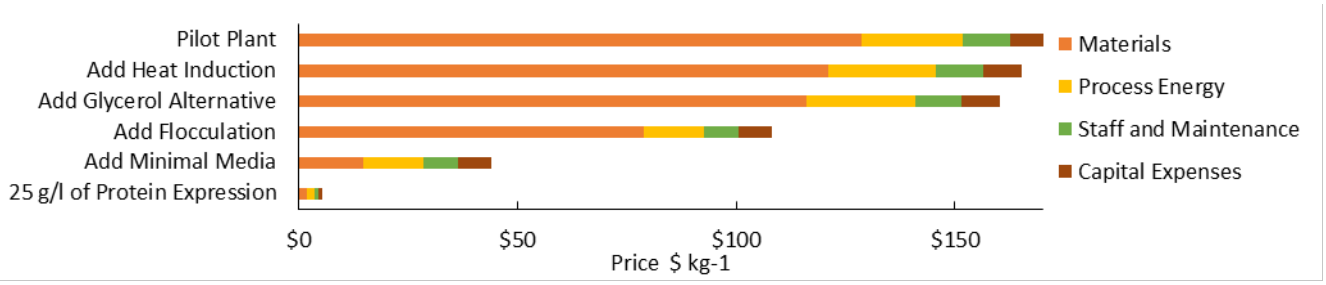

Figure 2: Estimated sale price for spider silk protein by *E. coli* with the substitution of various alternative processing methods. Each descending bar gives the estimate of the pilot plant with an additional optimized processing method

Glycerol has been demonstrated as a viable substitute for glucose during fermentation. In the laboratory, the yield has not been observed to drop. Glycerol has also been known to help recombinant protein growth and prevent the creation of acetate.[4] Acetate is a natural byproduct of the *E. coli* and its presence inhibits further growth. The use of glycerol could help prevent this buildup as high cellular densities are necessary to move towards greater economic viability. In the case that a cheap source of glycerol is available the substitution of glycerol for glucose would impact the econmic viability through multiple advantages.

Flocculation has been verified for batch sizes of only a few liters with very high protein purity, but continued efforts are being made to maintain purity levels in larger batch sizes. High purity may not be an issue in some cases, depending on the product use. In a material consumption comparison flocculation uses several of the same components used in affinity chromatography, but it eliminates some of the more expensive components. Of the components that are used in both processes, less material is used for flocculation than affinity chromatography.

Quantification of material properties with varying levels of protein purity have not been fully documented. With little to no loss in strength, lower purity could result in additional product, with less cost. For cases in which the protein is used as a structural member of a composite or adhesive, quantification of the properties at low purity may be enough. For pharmaceutical or medical purposes higher purities may be required that could eliminate flocculation as a method for industrial application. If sufficient protein purity can be reached with flocculation to maintain high strength and elasticity, the economic benefits from flocculation may be enough to overcome loss in value due to a decrease in material properties of the product.

3.1.2 Optimization of Protein Expression

Based on the results from a sensitivity analysis, the level of protein expression in the *E. coli* has the single greatest impact on reducing the cost to benefit ratio, and decreasing the projected minimum, or “best case scenario” sale price. Figure 3 shows the predicted sale price based on the cost of operations for varying levels of protein yield. At protein expression levels of 25 g/l the minimum sale price estimate is $\$5.53 kg^{-1}$.

The estimate of the sale price drops in an exponential decreasing manner with increased protein expression, as shown in Figure 3. This is because increasing protein expression doesn’t require additional material consumption, equipment, or labor for most all of the processing methods. The only processes affected by increasing protein yield are the washing with alcohol and the drying of the protein. However, since these represent only a small portion of the the total material and energy, its contribution only becomes significant at high yields.

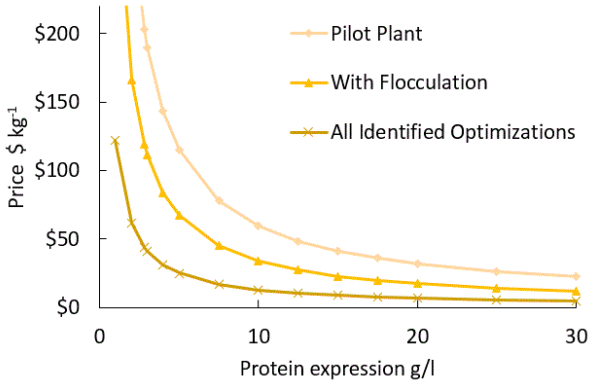

Figure 3: Product cost as a function of protein expression during fermentation with various levels of processing optimization.

3.2 Pilot Plant LCA

The emissions associated with the production of synthetic spider silk are very similar to the price as expected. GWP decreases as protein expression increases and with process optimization. Preliminary results show emissions are as high as 261 kg CO_2-eq kg^{-1} for the scaled up experimental laboratory processes, but as low as 22.7 kg CO_2-eq kg^{-1} for a more optimized plant operating with high levels of protein expression. The majority of emissions are associated with the processing energy, as large batches are processed with relatively small yield. Continued modeling efforts will focus on identifying additional process optimization methods to reduce the energy consumption and drive towards and environmentally friendly product.

REFERENCES

[1] Mason, C. A., Dünner, J., Indra, P., and Colangelo, T., 1999, "Heat-induced expression and chemically induced expression of the Escherichia coli stress protein HtpG are affected by the growth environment," Applied and environmental microbiology, 65(8), pp. 3433-3440.
[2] Council, W., 2015, "Wiltshire Workplace and Employment land Strategy Appendix 6: Employment and Development Density Assumptions,"Wiltshire, UK.
[3] ANL, A. N. L., 2015, "GREET Version 1_2015," Argonne National Laboratory, Lemont, IL, USA.
[4] Eiteman, M. A., and Altman, E., 2006, "Overcoming acetate in Escherichia coli recombinant protein fermentations," Trends in biotechnology, 24(11), pp. 530-536.