SYNTHETIC SPIDER SILK SUSTAINABILITY VERIFICATION BY

TECHNO-ECONOMIC AND LIFE CYCLE ANALYSIS

by

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Major ampullate spider silk represents a promising biomaterial with diverse commercial potential ranging from textiles to medical devices due to the excellent physical and thermal properties from the protein structure. Recent advancements in synthetic biology have facilitated the development of recombinant spider silk proteins from Escherichia coli (E. coli), alfalfa, and goats. This study specifically investigates the economic feasibility and environmental impact of synthetic spider silk manufacturing. Pilot scale data was used to validate an engineering process model that includes all of the required sub-processing steps for synthetic fiber manufacture: production, harvesting, purification, drying, and spinning. Modeling was constructed modularly to support assessment of alternative protein production methods (alfalfa and goats) as well as alternative down-stream processing technologies. The techno-economic analysis indicates a minimum sale price from pioneer and optimized E. coli plants at $761 kg\(^{-1}\) and $23 kg\(^{-1}\) with greenhouse gas emissions of 572 kg CO2-eq. kg\(^{-1}\) and 55 kg CO2-eq. kg\(^{-1}\), respectively. Spider silk sale price estimates from goat pioneer and optimized results are
$730 \text{ kg}^{-1}$ and $54 \text{ kg}^{-1}$, respectively, with pioneer and optimized alfalfa plants are $207 \text{ kg}^{-1}$ and $9.22 \text{ kg}^{-1}$ respectively. Elevated costs and emissions from the pioneer plant can be directly tied to the high material consumption and low protein yield. Decreased production costs associated with the optimized plants include improved protein yield, process optimization, and an Nth plant assumption. Discussion focuses on the commercial potential of spider silk, the production performance requirements for commercialization, and impact of alternative technologies on the sustainability of the system.

(68 pages)
PUBLIC ABSTRACT

Synthetic Spider Silk Sustainability Verification by
Techno-economic and Life Cycle Assessment

Alan M. Edlund

Spider silk has many material properties that make it a desirable product, but large scale production has not been demonstrated. Natural spiders are difficult to farm, and the end products are limited to fiber derivatives. Feasibility of large-scale production is being explored through several other transgenic alternatives such as E. coli, alfalfa and goats, all of which have the capability of producing raw spider silk protein. A raw protein has the possibility to be converted into a variety of products with fibers being just one. The production techniques are proven fields of application, but have not been demonstrated with synthetic spider silk production. No analysis regarding the economic feasibility of synthetic spider silk production through these alternative methods has been performed. Techno-economic modeling and life cycle assessment, which have been successfully used to evaluate emerging technologies on the metrics of cost and environmental impact where developed and used to understand the sustainability of synthetic spider silk production. Modeling results indicate a high sale price for spider silk from pioneer plants, but reveals a pathway towards economic feasibility through system optimization. At the current sale price estimates, spider silk based products are likely to be integrated into specific markets such as the medical and aerospace industries, with greater market penetration resulting from additional optimization. Environmental
impact of pioneer plant operations is significant, but great reductions can be achieved through optimization. Modeling work has supported identifying target areas for research and development to drive towards a sustainable production platform.
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I am also indebted to my fellow colleagues in the office at which I have worked for the last two years. Their willingness to review my work and give their opinion has been extremely valuable. Many of them have also set a great example of a dedicated work ethic.

I am again appreciative of Dr. Jason Quinn, Dr. Randolph Lewis, and Dr. Thomas Fronk for reading my thesis and being a part of the council for my defense.

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Alan M. Edlund
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<tr>
<td>BEC</td>
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<tr>
<td>TOC</td>
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CHAPTER I
INTRODUCTION

Spider silk has remarkable properties in the areas of strength, weight, sonic vibrations, toughness, elasticity, and thermal and electrical conductivity [1-4]. Additionally, spider silk is hypoallergenic making it more biocompatible and a promising material for a variety of products ranging from the medical to textile industries [5, 6]. However, industrial production of spider silk fibers has been limited. Spiders are cannibalistic and territorial which makes farming a difficult operation [7]. These challenges have driven research in the area of transgenic manipulation where the basic spider silk proteins can be synthetically created and harvested. Advancements in synthetic biology have facilitated the production of recombinant spider silk proteins (RSSPs) in alfalfa, goats, mice, \textit{Escherichia coli} (\textit{E. coli}), silkworms, yeast, tobacco, and potatoes [8-12]. Of these methods, field experts previously suggested that transgenic silkworms represented the only economically viable pathway for the production of spider silk [13, 14] but do not support this with economic modeling. Spider silk from transgenic silkworms is also limited to fiber production. Other synthetic protein production methods allow for the harvesting of raw protein, which is a versatile product. The silk’s extraordinary properties are directly related to the structure of the large proteins (250-350 kilo Dalton- \textit{kDa}) [1, 15]. Laboratory protein constructs from \textit{E. coli}, goats, and alfalfa have been found to maintain a relatively large structure (>75 kDa) which is required for high strength [10]. For these reasons production through \textit{E. coli},
goats, and alfalfa have significant advantages. In addition, the raw protein obtained can be used for multiple commercial applications ranging from silks to gels or adhesives.

1.1 Spider silk Protein

As a raw protein, spider silk is suitable to numerous industries and would compete with many other high strength materials such as Kevlar, carbon fiber, stainless steel, aluminum, polypropylene and others. The raw major ampullate spider silk (MaSp) protein can be mixed with water and formed into countless products including films, gels, coatings, fibers, adhesives and more [16]. Laboratory work has found that small amounts of MaSp give nylon threads increased stiffness and strength at concentrations of less than 5% [17]. The ability to be mixed with other materials makes it appropriate for several medical purposes such as time-release drug implants and anti-fungal coatings, both of which have been preliminarily verified [16, 18]. Laboratory fabrication of silk and its derivatives are labor and material intensive, leading to high costs. This has supported the conclusion that most of the current laboratory production methods are not commercially viable. In the medical industry, where only small material quantities are used, price is less consequential than in other industries and spider silk based products may still find great acceptance. Aerospace projects, which are highly weight dependent, often rely on expensive materials to meet their high-strength lightweight needs. In spite of the range of projects that could benefit from spider silk, even at high costs, the sustainability through techno-economic analysis (TEA) and life cycle assessment (LCA) of large-scale spider silk fabrication has not been analyzed and reported.
1.2 Large-scale Feasibility

The purpose of this work is to identify the industrial feasibility and environmental impact of large-scale synthetic spider silk production through multiple production pathways. Laboratory data was leveraged in combination with an engineering process modeling to characterize large-scale manufacturing. The engineering process model was constructed modularly to support the assessment of alternative processing technologies. The model served as the foundation for evaluation and focuses on identifying the sustainability of the processes through the metrics of economic viability and environmental impact. Multiple scenarios are evaluated and include: pioneer, substitute technology scenarios, and optimized cases. The baseline pioneer plant is built on *E. coli* production, and considers the effect of a low but demonstrated productivity as well as the use of the more proven yet more costly processing methods. Alternative processing techniques are considered independently on the metrics of economic impact. An optimized case, which considers the benefits of all the alternative technologies, high protein yield, and technology maturation is also analyzed. Goat and alfalfa silk production are also modeled using the same downstream processing methods used with *E. coli* production. Modeling results identifies key areas for research and development to support driving towards a commercially viable production platform. Discussion focuses on the potential of the various modeled pathways, sensitivity analysis to model inputs, exploration of the potential of substitute technologies, and the importance of protein expression.
1.3 Thesis Objectives

Research objectives have been created to focus modeling efforts and to determine standards for completion of the work. Outlined objectives detail goals for both economic and environmental evaluation.

1.3-1 Objective 1: System Modeling

Integrate sub-process models that accurately capture the production of synthetic spider silk constructed in a modular fashion that facilitates the evaluation of alternative processing technologies.

1.3-2 Objective 2: Techno-economic Analysis

Perform techno-economic assessment of the production of synthetic spider silk to determine the economic viability of various production pathways.

1.3-3 Objective 3: Life Cycle Assessment

Perform a life cycle assessment based on the material and energy inputs for each of the design case and determine the environmental impact of the system.
CHAPTER II

LITERATURE REVIEW

2.1 Silk in Nature

Spider silk has intrigued mankind for several centuries. A speech given by Monsieur Bom in 1710 recites the usefulness of the “silk of spiders” [19]. Small strands of silk are capable of holding insects much larger than the spider itself and with relative ease. The properties vary between species as well as between the diverse types of silk that a spider can produce. Some spiders can produce up to seven types of silk [20]. Each of the silks have unique qualities with regards to stiffness, elasticity, smoothness, toughness, and stickiness and as such are used for different purposes such as the structure of the web, capturing prey, absorbing the kinetic energy, and protecting the egg sack [5].

2.2 Golden Orb Weaving Spider *Nephila Clavipes*

The strongest of silks comes from the major ampullate gland and is identified as dragline silk. This silk is used as the foundational part of the web for structural strength. The Golden Orb Weaving spider, also known as *Nephila clavipes* (*N. clavipes*), is large in comparison to most spiders which makes it an easy candidate to study. It also a suitable candidate as it has strong dragline silk from which it benefits by occasionally catching large prey such as birds [21].
Figure 1: A Golden orb weaving spider crawls across the hands of a laboratory researcher.

Photo taken by Alan Edlund
The strength of the *N. clavipes* dragline silk varies with each strand but averages around 1.3 GPa which is 4X the strength of AISI 1010 cold rolled steel [22]. Dragline silk can also extend 40% before failure. The high elongation and stiffness of silk result in very high toughness, $1.6 \times 10^5$ J kg$^{-1}$, which is greater than that of Kevlar 49 [2].

Natural fibers are hard to obtain in large quantities. The spiders have to be taken manually, fastened down, and have their silk spooled while it is pulled from their spinnerets. This is not harmful to the spider and can be repeated periodically but the amount of thread obtained is minimal [23]. Spiders also have to live in individual containers as they are both territorial and cannibalistic [7]. The high capital costs associated with individual cages along with the high labor costs for harvesting make large scale farming impractical. As a result, much research has been done to understand the structure of silk, and how to replicate it.

2.3 Transgenic Production of Silk

The protein structure gives spider silk its tremendous properties [1, 15, 24]. The proteins that make up spider silk are known as fibroins, or more specifically spidroins, of which there are many types [25]. Dragline silk from the *N. clavipes* is mostly made up of two spidroin types: major ampullate spidroin 1 MaSp1 and major ampullate spidroin 2, MaSp2 [26]. For the rest of this work, both MaSp1 and MaSp2 are referred to as MaSp. Some of the first transgenic hosts used to express the MaSp were goats and *E. coli* [27, 28]. Goats produce whey protein in their milk. Through transgenic manipulation the genes which indicate protein expression can be altered to express spidroins instead of whey
protein. *E. coli* are simple single celled organisms which can also be manipulated. Under a genetically determined stress factor, transgenic *E. coli* will begin to express the RSSPs. Similar work has been done with a host of other organisms including alfalfa, mice, silkworms, yeast, tobacco, and potatoes [8-12].

One challenge with RSSPs is the small size that is produced in comparison to the native spidroins. Natural spider silks range in size from 250 – 350 kDa [29]. Some work with *E. coli* has led to native sized proteins as large as 284.9 kDa [3]. However, RSSPs often only have a range of 30-100 kDa [14]. The result is reduced strength. In spite of this challenge, the properties of artificial silk are still quite impressive. Fibers from RSSPs have been tested and found to have an average tensile strength of 192 MPa with an elongation of 28.1% before failure [16].

2.4 Feasibility

TEA and LCA are common techniques for analyzing large-scale feasibility of laboratory scale technologies. These techniques give clarity to the actual cost and environmental impact of laboratory research without the significant financial investment that comes from pilot and scale-up facilities. This method of analysis has been heavily used for identifying the cost of biofuels, biogas, and other bioproducts from algae but have never been performed with spider silk [30-35]. Laboratory production of synthetic silk is labor and material intensive suggesting high production costs. However, TEA and LCA give information regarding optimization over laboratory procedures through the use of commercial techniques.
Scaling up of laboratory procedures for estimation of commercial production is a topic that has been extensively studied. Capital cost estimation of manufacturing equipment is available through U.S. national laboratory capital cost estimating documentation, ASPEN plus, and other online equipment cost estimating resources [36-38].

Life cycle inventory (LCI) data provides the basis for LCA. LCI data has been compiled by several private and public entities. Emissions for many materials have been provided by the NREL LCI and ANL GREET databases and are free to use [39, 40]. LCI data can be combined with results from the Intergovernmental Panel on Climate Control (IPCC) to obtain CO₂ equivalent emissions [41]. LCA focuses on emissions during the production and life of a product. Since spider silk produces no known emissions during its life time, only the emissions from production are considered. Emissions from plant infrastructure are excluded.
CHAPTER III

METHODS

Laboratory data provided the foundation for the engineering process flow and system model. The system model was modularly constructed to facilitate the analysis of alternative processing methods. Multiple production pathways are assessed including varying levels of technology maturation and protein generation, Figure 2. The baseline production pathway is based on a pioneer *E. coli* plant representative and validated with laboratory-scale data. Industrial scale production systems from *E. coli*, goats and alfalfa were also analyzed. The system model serves as the foundation for TEA and LCA calculations which provide the basis for determining sustainability.

3.1 Baseline: E. coli Process Modeling

*E. coli* production is divided into five sub-processes: fermentation, harvesting, purification, drying, and product formation. Mass balances were tracked on a sub-process level and integrated to form the process model. Within most sub-process’, several alternative processing methods were considered and include alternative protein production methods through goat and alfalfa as well as alternative downstream processing technologies, Figure 2. Many of the process flows and equipment used were modeled in ASPEN Plus V8.6. Transportation for consumed goods was outside the system boundary and was not included in the analysis. All of the *E. coli* scenarios represent manufacturing
from the same facility, with varying output capacities ranging from 350 to 8,750 metric tons per year, depending on the level of optimization. Goat and alfalfa pioneer facilities were also sized to output 350 metric tons per year.

Figure 2: Process flow diagram synthetic spider silk manufacturing from E. coli. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1-1 Fermentation

Fermentation of *E. coli* for RSSPs production has three phases: batch phase, exponential growth and induction; all of which take place in a heated, stirred, and aerated bioreactor. During the batch phase the *E. coli* are provided with an initial amount of nutrients. Cell density starts to climb entering the exponential growth phase. During exponential growth additional nutrients are added to the bioreactor until an optimal cell
density is reached. Typical laboratory growth periods for batch and exponential growth periods were 12-14 hours with the media reaching an optical density (OD\textsubscript{600}) of 100 as measured at 600 nm. Six paddle Rushton impellers were used to mix the 500 liter tank, starting at 200 rpm linearly increasing the speed up to 1000 rpm at full culture density. A dissolved oxygen set point of 45\% was maintained by varying mixing rate, gas flow, and percent oxygen. Inoculum volume for the batch phase was 2\% of the final culture volume.

Laboratory induction methods included using Isopropyl β-D-1-thiogalactopyranoside (IPTG) at concentrations of 1-2 mM. After induction, an additional 4 hour period was provided to allow the \textit{E. coli} to express the RSSPs. A laboratory strain of \textit{E. coli} capable of producing as much as 2.8 grams of RSSPs per liter of cultured media (g l\textsuperscript{-1})) has been demonstrated (Unpublished Data). However, typical laboratory expression levels can be much lower at 1.0 g l\textsuperscript{-1} with other researchers reporting levels of expression at 0.04 – 0.3 g l\textsuperscript{-1} [42]. This high yielding strain has been demonstrated to maintain protein expression levels in cell culture volumes of up to 400 liters (Unpublished Data).

Large-scale fermentation was modeled to occur in 36 identical 25,000 liter bioreactors. Each modeled bioreactor would be inoculated at 5\% volume per volume ratio (v:v) from a 1,250 liter bioreactor. The smaller reactors were assumed to be inoculated from a genetic maintenance laboratory having negligible material consumption. Due to the larger inoculation size, time to reach optical density was expected to be shorter. Each large-scale fermenter was assumed to complete a fermentation cycle every 16 hours. A spiral tube heat exchanger was modeled as the primary method for cooling and sterilization, with water at 15 °C providing the necessary cooling and recycled into the fermentation process. An
agitation unit using Rushton turbine blades was modeled along with air at 1 volume per volume-minute (VVM) from a compressor.

Alternatives to the standard laboratory procedures were considered for their economic and environmental impact. An alternative method to chemical induction is heat induction, which is done by temporarily raising the temperature of the media to 45 °C [43]. Several fermentation broths were tested including K12 media [44], R/2 minimal media, and a glycerol based media. Minimal media excludes some of the unnecessary nutrients provided in K12 and has been demonstrated to not dramatically impact productivity (*Unpublished Data*). Glycerol has also been tested with the *E. coli* strain with no notable decreased protein production [45]. For modeling purposes, the glycerol based media recipe is exactly the same as the K12 or minimal media, except for the substitution of glycerol over glucose. Global production of glycerol is also predicted to increase in future years due to the increase in biofuel refineries and represents a promising carbon source for large-scale integrated production [46]. For economic evaluation, biofuel refinery waste glycerol was set to cost half the price of standard glucose.

### 3.1-2 Harvesting

After fermentation, the media is centrifuged to harvest the *E. coli* cells in the form of pellet. A GEA FSE 10-06-077 stacked disk centrifuge processed the media at 70 liters per hour operating at 10,000 rpm. This speed helped prevent premature lysing of cells. *E. coli* pellet was harvested at a concentration of approximately 45% volume per volume of the initial media, at 6.8% solids. The harvested pellet was mixed with a lysis buffer at a 2:1
v:w ratio. The economic benefit of skipping this first centrifuge stage and adding the buffer components directly to the media was considered.

Laboratory methods for lysis were limited to sonication based on equipment availability. In industrial settings high pressure homogenization (HPH) is more common [47, 48]. HPH involves releasing the pellet and lysis solution through a nozzle, causing a sudden pressure change which bursts the cells and releases the protein. For system modeling purposes, HPH energy consumption was calculated by raising the pressure to 1500 bar. After lysis, heating the flow to 80 °C was found to cause some of the bio-waste to precipitate out of solution. Significant heat buildup is obtained during HPH (as much as 25 °C increase) reducing some of the additional heating load in the model. A shell and tube heat exchanger was also modeled for additional heat recovery. Heating combined with an additional pass through the centrifuge removes most of the solid bio-waste.

3.1.3 Purification

Affinity chromatography was used to purify the protein solution. For this process, an eight-liter column was loaded with 1.5 liters of Ni-NTA agarose beads. The solution was then sent through the column at a flow rate of 0.350 liters per minute. The beads were charged with a Nickel (II) sulfate hexahydrate solution prior to filtering the protein. Part of the transgenic manipulation included a histidine tag on the RSSPs which bound to the nickel as the lysed cells and protein were pushed through the column. Protein was then concentrated in the column while bio-waste was pushed out. Additional salt solutions containing tris-HCl, NaCl, imidazole, and urea were used to strip the protein from the column as well as precipitate it out of solution. Precipitated protein could be strained with
a basic mechanical filter. Wet protein was washed with isopropanol and water before being dried.

The alternative purification method evaluated with the model was flocculation, or salt precipitation which has been demonstrated by researchers (Unpublished Data). The process requires 12% weight by volume of ammonium sulfate to be added to the lysed media. The ammonium sulfate causes the RSSPs to precipitate out of solution while solubilizing bio-waste. An additional centrifuging then removes the media and bio-waste. After this it can be filtered and washed with the same procedures used in sequence with chromatography.

3.1-4 Drying

In the laboratory, the washed protein is lyophilized to remove water and alcohol, leaving a dry protein powder. Wet protein was cooled to -40 °C for 24-48 hours, at 0.1 mBar in a 900 ml container filled with 500-600 ml of wet protein. After drying, the protein was stored at -80 °C up till it was further processed. An industrial freeze dryer, that spreads the protein in a thin layer rather than as a bulk material, was modeled for large-scale drying. Another potentially viable method for moisture removal is spray drying. Typically, proteins are in danger of being denatured by the high heat used for spray drying, but laboratory work has found that RSSPs have high heat resistance, suggesting that spray drying may be practical [6, 42]. In a continuous flow system, where the protein is made directly into final product, the protein may only need to be partially dried or may not need to be dried at all. This modeling work assumes the protein is dried to less than 5% water content.
3.1-5 Product Formation

Spider silk as purified protein powder is useful for many applications. Many of the spider silk derivatives are made from aqueous solution. To achieve this in the laboratory, protein powder was mixed at an 8:1 ratio in a glass vial with water at 120 °C using a conventional microwave. Many products have been made from the solution including fibers, hydrogels, lyogels, glue, coatings and microcapsules [16].

Fiber formation is achieved by extruding the solution through a small syringe into an alcohol bath. Different baths cause the fibers to have varying stiffness and elasticity. The fiber is then stretched twice, first in an 80/20 bath of either methanol or isopropanol and water, and then in a 20/80 bath of methanol or isopropanol and water. Modeling for this sub-process includes the pressurized microwave mixing system, extruder, stretcher, spooler, and alcohol recovery system.

3.2 Goat Process Modeling

Transgenically modified spider goats produce MaSp protein in their milk in addition to natural whey protein. Modeling work includes all aspects required for goat milk production. Farms sites containing 1,000 goats, operated by five employees, were modeled as the basic farming unit. This size facilitated farming and milking, while maintaining a small enough herd to minimize the dangers and effects from sickness [49]. Housing stalls, milking stations, and a fenced roaming area were included in the costs of each farm site. A total of 422 farm sites would be necessary to provide the milk to meet annual production capacity of 350 tons of spider silk.
Through research and development it was observed that not all of the protein present in the milk was expressed as RSSPs. Much of the protein in the milk was still expressed as whey protein. Laboratory concentrations of MaSp averaged around 1.5 g l$^{-1}$ \textit{(Unpublished Data)}, but natural goat milk can have as much as 40 g l$^{-1}$ of whey protein \cite{50}. Work over the last decade has struggled to bring the amount of RSSPs expression up in the milk. The baseline model is based on the demonstrated protein yield of MaSP but the economic impact of increased protein expression is explored.

After the milk is collected, it is strained to remove large impurities. The milk is then defatted and mixed with Arginine at 10.53 g l$^{-1}$ of milk while water is added at a 1:1 ratio. Laboratory methods of purification for goat milk differed from alfalfa and \textit{E. coli} by the use of tangential flow filtration (TFF) instead of chromatography or flocculation. TFF uses the disparity between the size of the protein and the other bio-waste to harvest the spider silk proteins. The proteins of interest are between 35 and 100 kDa. Drying and product formation of the harvested proteins is done in the same fashion of the baseline \textit{E. coli} process.

3.3 Alfalfa Process Modeling

Alfalfa naturally contains large amounts of crude protein in the leaves, making it a valuable feedstock and a promising host for MaSp production. Alfalfa farming is a commercially demonstrated technology in agriculture. As alfalfa farming is already highly understood, farming operations would be contracted out and alfalfa purchased at a set price. A competitive price of the 2015 season was $200 ton$^{-1}$ \cite{51}. Harvesting of the synthetic...
protein starts with the separation of the fresh leaves from the stems. Available farming techniques allow for the harvesting of the leaves separate from the stem while still in the field. Stems would also be harvested and could be sold as feedstock for cattle or as a base for bio-oil production [52]. A system boundary was established including only the protein harvesting stage.

The collected leaves are ground with binding buffer of NaCl, Tris-HCl, imidazole, and water which solubilizes the protein after it is extracted from the plant matter. The plant solution mix is then centrifuged and the pulp removed. The protein solution is then mixed with a chlorophyll removal solution. Chlorophyll removal solution is made of heptane, acetone, water, and chlorophyllase at v:v ratios of 3:3:3:1. Mixing at 37 °C for 30 minutes is sufficient time for separation. The chlorophyllase is necessary for purification or the protein retains a greenish tint, indicating a lack of purity in the protein. After a multistage separation using settling tanks, a protein solution is obtained similar to what is obtained post harvesting in the baseline E. coli process. The protein in solution can be purified by chromatography but flocculation was also considered. It is then dried and turned into product. Results show relatively low yields compared to the total protein content in the leaf, at 1.5% or 2 g MaSP kg⁻¹ alfalfa leaf (Unpublished Data). E. coli methods for purification, drying, and product formation were leveraged for the downstream processing.

3.4 Sensitivity Analysis

A sensitivity analysis was performed to identify model inputs that most significantly affect product sale price. Model inputs were varied by +/- 20% while the effect on resulting
sale price ($ kg$^{-1}$) was recorded. The results were then statistically analyzed to obtain a two-tailed 95% confidence based on t-ratio to determine inputs that statistically affect the model. Model inputs that are sensitive can then verified for accuracy and ensure greater confidence in the results. The findings were used to inform researchers of factors that most significantly affect product sale price and are able to increase economic viability.

3.5 Techno-economic Analysis

Multiple design case processing facilities and power production systems have been evaluated for economic feasibility following a standard set of financial assumptions [53, 54]. These design case rules facilitate economic analysis and were leveraged to complete this work. The economic assumptions are coupled with the process model to create the entire picture of industrial production. The engineering process model served as the foundation for the economic assessment. Operational costs were determined from the energy and mass balance of the engineering process model. The total flow rates in the production system were used to size the facility and determine the capital costs. Standard economic assumptions include an internal rate of return of 10%, capital investment of 40%, income tax of 35%, and a 3 year facility buildup time with a 10 year loan term upon completion. Depreciation of equipment under the modified accelerated cost recovery system (MACRS) schedule was also considered [55]. A complete list of economic inputs is listed in Table 3 in the appendix. The financial modeling was performed based on a discounted cost flow rate of return (DFCROR). Results from the modeling work were focused on determining a minimum product selling price over the 30 year life of the
production facility with results presented in 2016 USD. Cost estimates for consumables were based on commercial and equation based cost estimates and averaged [38, 56]. Operational costs include labor, materials, taxes, maintenance, and energy requirements for production. Capital costs account for the all of the infrastructure including equipment and facilities to support manufacturing.

3.5-1 Capital expenses

The engineering process model was leveraged to ascertain equipment sizing for the various pieces of infrastructure. The total installed cost, or bare erected cost (BEC), can be extrapolated from the equipment purchase price and includes all other required infrastructure [54]. Several resources were leveraged to estimate capital cost. Sources used for estimating purchase price included commercial quotes, ASPEN Plus capital cost estimates, and national laboratory reports [36-38]. Individual capital estimates for each unit operation were averaged in order to obtain a more accurate estimate, Table 4 in the appendix. Commercial quotes for equipment that were similar in size and power to ones required for the model were recorded, and a linear cost curve created, from which a purchase price for a specific size could be obtained. Equipment estimated by Loh et al. (2002) allowed for variation in size and included equipment such as pressure vessels, agitators, heat exchangers, storage tanks, pumps, centrifuges. Additional literature was also used to estimate the cost of a distillation column [57]. ASPEN plus was used to provide equipment cost estimates for bioreactors, pumps, heaters, mixing tanks, and the distillation column. Commercial estimates were not available for all unit operations, such as the continuous flow microwave heating unit modeled for product formation. In this situation a
bill of material was generated, a simple and basic design constructed and a module price estimated. A list of all the modeled equipment, number needed, and relative size is shown in

Table 1.

Installed cost was estimated as a percentage of the purchase price and included a breakdown for labor and purchasing of all auxiliary components such as buildings, infrastructure, piping, paint, insulation, electrical wiring, instrumentation and other miscellaneous items [36]. All non-current cost estimates were brought to 2016 USD using the chemical engineering plant cost index (CEPCI) [36, 58]. Since the CEPCI index is only intended to be used to obtain estimates for time periods no greater than 10 years apart, the averaging of CEPCI adjusted estimates with other current estimates allows for greater confidence in the resulting BEC. Minor equipment has not been included in this list. Depreciation of equipment under the MACRS schedule as defined by the IRS (2015) was assumed to occur over a 5 year period.

Process contingency costs varied with each equipment, depending on the level of novelty or uncertainty that each process possessed. For example, there are no commercially available HPH systems that are within an order of magnitude of the given flowrate. In this case, an HPH manufacturer was contacted who provided a very basic quote of a hypothetical system. The contingency cost for this process is 50% of the purchase and setting cost. In contrast, pumping water is a relatively simple process and easy to calculate. Contingency costs for more established procedures, such as pumping, were estimated at much lower percentages.
Table 1: List of equipment required, number needed, and the relative sizing

<table>
<thead>
<tr>
<th>Equipment &amp; Relative Sizing</th>
<th>Sizing</th>
<th>Units</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity Column -3,000 liter/hour</td>
<td>4,000</td>
<td>liters</td>
<td>2</td>
</tr>
<tr>
<td>Aqueous Extruder &amp; Stretcher</td>
<td>400</td>
<td>liters/hour</td>
<td>1</td>
</tr>
<tr>
<td>Bioreactor-small</td>
<td>1,250</td>
<td>liters</td>
<td>36</td>
</tr>
<tr>
<td>Bioreactor-large</td>
<td>25,000</td>
<td>liters</td>
<td>36</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>101</td>
<td>kW</td>
<td>54</td>
</tr>
<tr>
<td>Distillation Column</td>
<td>600</td>
<td>liters</td>
<td>1</td>
</tr>
<tr>
<td>Filtration Unit</td>
<td>120</td>
<td>liters/minute</td>
<td>1</td>
</tr>
<tr>
<td>Genetic Maintenance Equipment</td>
<td>200</td>
<td>square meters</td>
<td>1</td>
</tr>
<tr>
<td>Homogenizer</td>
<td>25,000</td>
<td>liters/hour</td>
<td>1</td>
</tr>
<tr>
<td>Liquid Freezer</td>
<td>1,500</td>
<td>kg/hour</td>
<td>1</td>
</tr>
<tr>
<td>Microwave Heater</td>
<td>400</td>
<td>liters/hour</td>
<td>1</td>
</tr>
<tr>
<td>Mixing Tank</td>
<td>various</td>
<td>liters/hour</td>
<td>17</td>
</tr>
<tr>
<td>Pump</td>
<td>various</td>
<td>liters/hour</td>
<td>97</td>
</tr>
<tr>
<td>Shell &amp; tube heat exchanger</td>
<td>250</td>
<td>square meters</td>
<td>2</td>
</tr>
<tr>
<td>Spooling Machine</td>
<td>50</td>
<td>kg/hour</td>
<td>1</td>
</tr>
<tr>
<td>Storage Tank</td>
<td>various</td>
<td>liters</td>
<td>28</td>
</tr>
<tr>
<td>Water boiler</td>
<td>6,000</td>
<td>kg/hour</td>
<td>3</td>
</tr>
<tr>
<td>Water purifier</td>
<td>50,000</td>
<td>liters/hour</td>
<td>2</td>
</tr>
</tbody>
</table>
3.5-2 Operational expenses

Operational expenses were broken up into four categories including material purchasing, energy consumption, maintenance, and labor. Wholesale pricing estimates were used to determine product pricing [38]. A use tax on all purchased goods was included but shipping costs were assumed to be included in the wholesale price. Both electrical energy from the grid and natural gas were used as process inputs. The 2015 national average for both electrical and gas energy were used at $0.0676 kW\textsuperscript{1}h\textsuperscript{-1} and $2.61 MMBtu\textsuperscript{-1} thermal, respectively [59, 60]. Labor costs have been simplified and were estimated using production floor space, average employment density information for high-tech/research and development industrial settings and wages from the Bureau of Labor Statistics [61, 62]. The maintenance category included estimates for equipment and building maintenance as well as waste management. Equipment maintenance was simplified to be a percentage of the asset replacement value while building maintenance cost is based on average annual cost per square foot [63]. Waste was estimated as being a fixed cost per cubic meter of waste [64].

3.5-3 \(N\text{th} \) plant

Spider silk represents a new technology where little is understood about the large-scale flow rates and exact sizing specifications. The lack of process definition has an effect on capital cost estimation causing designs to be generic, and hard to optimize. For modeling purposes, pioneer plants are considered to be first of a kind (FOAK) facilities. The technologies are hard to troubleshoot this early on, so the first plant is expected to have high capital costs from lack of proper optimization that could be realized as greater process
definition occurs. As additional plants are built, a technology becomes better understood and optimized, resulting in lower capital costs due to reduced engineering, and lower contingency fees [65]. Considering this consequence, the benefit of being a more mature plant was included as a part of the optimized scenarios. The mature plant scenarios were considered to be the 17th plant constructed of their type under the assumption of a 0.06 learning rate as defined by NETL (2013).

3.6 Life Cycle Assessment

The environmental impact of the baseline *E. coli* process was evaluated through LCA. This method focuses on identifying global warming potential (GWP) in terms of greenhouse gases (GHGs). The GWP metric for this study combines three emissions, namely CO₂, CH₄, and N₂O as a carbon dioxide equivalent (CO₂-eq) through the IPCC 100 year equivalency factors of 1, 24, and 298, respectively [41]. Quantification of the emissions can be done by determining the required energy and mass consumption from the engineering process model and then coupling it with life cycle inventory data (LCI). LCI data was provided by the National Renewable Energy Lab (NREL) and Argonne National Lab (ANL) including the U.S. LCI database and GREET 2015, respectively [39, 40]. Emissions for electrical energy and natural gas came from GREET 2015 which was based on the 2015 US energy grid mix and US natural gas production [39].

LCI data was obtained for all materials that contributed at least one percent of the total mass consumed (excluding water) during fabrication. Water is the primary material used in production, and its environmental impact is also considered. Emissions from the facility
infrastructure were excluded. While there is considerable waste, which has the potential to be sold as a co-product for fertilizer, only some of the waste (i.e. fermentation media and solubilized urea) has been considered for co-product allocation.
CHAPTER IV
RESULTS AND DISCUSSION

Economic and environmental results are provided for multiple scenarios including a baseline *E. coli* pioneer plant (Case 1), alterations of the Case 1 (a-g), and an optimized plant (Case 2). A sensitivity analysis of Case 1 inputs is performed including an evaluation of protein yield on final cost. Pioneer and optimized plant economic results for goat (Case 3(a,b)) and alfalfa (Case 4(a,b)) are included. Results for all three protein production methods are discussed and summarized in their potential to meet various current commercial demands. Environmental impact results for Case 1 and Case 2 with resolution at the sub-process level are presented.

4.1 Case 1: Pioneer Plant

The baseline pioneer plant is intended to represent current production cost estimates based on demonstrated technologies in the laboratory and thus represents a conservative estimate for production. The DCFROR results were obtained using the capital and operational cost estimates from the *E. coli* engineering process model at an annual production capacity of 350 tons. Capital costs results for the TOC which includes the BEC, project and process contingency, engineering procurement & construction cost, inventory, and financing costs [54], and other owner’s costs as presented in Figure 3. The BEC was estimated at $161 million and represents the equipment purchasing, setting, piping,
building, and other installation costs. Equipment purchasing and setting make up the largest portion of the BEC at almost $73 million or 24% of the total overnight capital cost (TOC). Equipment costs serve as a critical capital cost input as other related costs, overhead, building construction, are determined as a percentage of this cost estimate. As a result, purchasing costs from multiple industrial sources and ASPEN plus modeling where averaged to ensure accuracy of the BEC. TOC cost for the scaled processing equipment necessary for Case 1 came to $314 million. Part of the TOC, such as inventory capital and pre-production costs, were derived from the cost of operation but represent a small fraction of the total cost, 16%. The TOC serves as a primary input to the DCFROR analysis with the plant assumed to be constructed over a 3 year period.

Figure 3: Total overnight capital (TOC) cost and bare erected cost (BEC) breakdown in 2016 USD for the construction of an E. coli based synthetic spider silk production facility, Case 1.
Operational costs were broken up into four categories: materials, process energy, labor, and maintenance. These annual costs were combined with the initial capital investment to perform the DCFROR economic assessment over the life of the plant, Figure 4. Based on the annual production capacity, a TOC of $314 million, and an operational cost of $223 million, the resulting minimum product sale price is $761 kg$^{-1}$ of spider silk. This high estimate is driven by the large annual operational costs and the low level of protein expression. The material costs of production dominate the economics of the pioneer plant, with the majority of the operational costs derived from the fermentation and purification sub-processes. However, significant economic improvements can be made through optimization.

*Figure 4: Contribution to the sale price estimate of synthetic spider silk, Case 1, from E. coli categorized by the production sub-processes and by operational and capital expense items.*
For the fermentation sub-process, IPTG, which is used for induction of the media and represents 67% of the material expenses and 20% of the total operational costs. As the primary carbon source for the \textit{E. coli}, glucose makes up the next highest cost at 16% of the material cost or 5% of the total operational costs. Antibiotics also significantly contribute to operational costs, but are required to maintain the health of the \textit{E. coli} system. These high material costs are common to fermentation of recombinant proteins, yet improvements in protein production provide a viable pathway for decreasing the impact of these costs [48].

Purification costs are also high due to the large process volumes. The nickel (II) sulfate and NTA beads used in chromatography make up 42% and 23% of the sub-process material costs of purification, respectively. These materials are required for purification by chromatography but can potentially be eliminated with an alternative technology such as flocculation which is explored. Similarly, fermentation costs can be reduced with the substitution of a cheaper carbon source and an alternative induction method. The combined cost of these processes yield a sale price for spider silk that demonstrates the need for alternative technologies to improve the economic viability. The estimate of $761 \text{ kg}^{-1}$ is expected due to low yields and the use of scaled laboratory processing techniques. However, significant economic improvements can be made through optimization and targeted research.

4.2 Sensitivity Results

A sensitivity analysis was leveraged to identify statistically significant inputs that can be improved to support the identification of a commercial viable pathway. Based on a 95%
confidence interval, inputs with a t-ratio less than 2.12 are considered to be insignificant. Only five input parameters were identified as substantial. Protein yield has the single greatest impact on sale price, Figure 5. Case 1 represents a low, but demonstrated protein yields of 1.0 g l\(^{-1}\) (*Unpublished Data*). Laboratory protein expression has reached higher levels but has not been consistently demonstrated. Increasing the yield by 3X corresponds to a 67% reduction in sale price, bringing the cost to $254 kg\(^{-1}\). Protein yield’s dramatic influence on price is largely due to the batch nature of the first three sub-processes (fermentation, harvesting, and protein purification), which also make up 99% of the operational expenses. Increasing protein yield has a direct impact on increasing the amount of available product with minimal change in process volumes resulting in effectively no additional cost for downstream processing. Fermentation time is an important model input with a reduction representing an improvement in economic viability. IPTG pricing, capital costs, and NTA beads are also hefty financial burdens. Other individual material pricing estimates and model inputs had lower t-ratios and have relatively low impact on sale price. Results from the sensitivity analysis were used to identify the alternative scenarios explored in this modeling work.

4.3 Optimization

The commercialization of synthetic spider silk through *E. coli* fermentation will require the integration of alternative technologies to those of the pioneer plant as well as improved protein yield. Alternative technologies are explored that represent advancements in strategic areas. Modeling improvements come from three sources: 1) process optimization, 2) experienced design for subsequent plants, and 3) increased protein expression (10 g l\(^{-1}\)).
Individually, each of these methods are considered for their effect on pioneer plant economics. All of these advancements combined are considered for their mutual economic benefit in an optimistic scenario, Case 2.

Figure 5: Sensitivity analysis of Case 1 sale price with a 20% variation of model inputs.

Inputs that have a t-ratio greater than 2.12 are considered to be significant based on a 95% confidence interval.

4.3-1 Process Optimization

The pioneer plant, Case 1, represents a robust production platform for which all technologies have been demonstrated at pilot scale. Alternative processing technologies have been identified with modeling work used to understand the potential impact on the economic viability and therefore justify experimental evaluation, Figure 6.
Figure 6: Product cost for RSSPs by E. coli with the substitution of various alternative processing methods. (1a-use of minimal media, 1b-use of glycerol carbon source, 1c-use of heat induction, 1d-remove the first centrifugal process and add lysis buffer components directly to fermented media, 1e use of flocculation over chromatography, 1f-remove the drying stage, 1g-all process optimizations used concurrently)

The fermentation process has intensive material requirements based on the current media requirements. Three alternatives to address this issue include a minimal media recipe (Case 1a), the replacement of glucose with glycerol (Case 1b), and the use the heat induction in place of IPTG induction for triggering protein expression (Case 1c). The minimal media recipe has been tested in the laboratory and shown to have minimal impact protein expression levels (Unpublished Data). The laboratory E. coli strain as well as other E. coli strains have been shown to effectively utilize glycerol instead of glucose [66]. These two improvements respectively result in a 4% and 6% improvement in sale price compared
to the baseline, Case 1. Integration of the first two improvements represents minimal risk in terms of impact on the function of the facility. The use of glycerol could also help prevent acetate production in large-scale operations, which is a natural byproduct of *E. coli* and is undesirable [66]. IPTG induction is the process of triggering the *E. coli* to produce RSSPs and is a costly component of fermentation process. Since the price of IPTG is high, the implementation of heat induction (Case 1c) results in a significant benefit. A 28% sale price reduction is observed. The application of this method would have significant benefits towards achieving economic viability. While heat induction has not been demonstrated with the laboratory strain, it has been demonstrated on *E. coli* [43]. Some possible concerns with the implementation of heat induction are reduced protein expression or protein degradation but this has not been explored. The combined impact of integrating all the fermentation improvements (1a, 1b, and 1c) result in a 33% decrease in the sale price over Case 1.

Post fermentation, the *E. coli* are harvested via a centrifuge, and are then mixed with a salt solution. By skipping this first centrifugation step, and adding the salt components directly to the media (Case 1d), a 1.7% cost reduction is observed. Additional optimization during harvesting could come from reducing the pressure of the HPH process. Based on a literature review, successful lysis occurs between 1000- 1500 bar. Since 1500 bar was used in the model, using only 1000 bar would cut $1.75 kg^{-1}$ in production cost resulting in a 0.23% reduction in total cost (not shown in Figure 6). The integration of these two processes have minimal impact on the economics of the system.
Protein recovery is a critical step in the production process. Lysed cells are sent through a chromatography column for purification. Flocculation, also known as salt precipitation, represents a viable alternative and is commonly performed on large scale with water treatment [67]. By adding various salts to the protein solution the bio-waste is precipitated out while the protein remains solubilized. Additional salt components are then added to precipitate out the protein while keeping any residual waste suspended in the liquid. Flocculation is advantageous compared to chromatography, resulting in lower capital and operational costs. The economic effect of implementing flocculation (Case 1e) results in a 33% cost reduction below Case 1 results, Figure 6. After chromatography or flocculation, another pass through a centrifuge and a filter are required before obtaining a nearly purified RSSPs.

At this point the protein paste is contaminated with salt solution. Washing with IPA and water removes the salts and leaves a wet protein behind. Initially, flocculation in the laboratory could maintain high purity only when using volumes of a few milliliters, but this has since been rectified. This process has now been verified in volumes of up to several liters (Unpublished Data). The implementation of this technique will undoubtedly save costs for large-scale production, but issues with purity at this scale may need to be addressed.

After washing in IPA, and water, the protein is dried using lyophilization. The economic benefit of spray drying as an alternative to lyophilization has been considered, and though it represents a 10% reduction in sub-process costs, it only gives a 0.04% reduction in the overall cost of production. At low production yields, the drying stage does
not contribute much to cost. Removing the drying sub-process altogether (Case 1f) results in a 0.4\% overall cost reduction.

The techniques described have the ability to be implemented together without interference, and the collective effect is significant. Their combined effect (Case 1g) results in a 55\% decrease in the levelized cost bringing the minimum product sale price to $344 kg\(^{-1}\). Most of these methods are feasible for large application, with some additional testing necessary. These identified methods of process optimization have substantial impact on price, but further optimization of protein yield will be required for the delivery of an economically viable product.

4.3-2 \(N^{th}\) Plant Analysis

Optimization and fine tuning will occur with the construction of additional plants. Experience curves have been developed to quantify and estimate the benefits of building established technologies. A facility that employs previous design gains from the knowledge and optimization learned from earlier plants and will have a decreased capital cost. This reduction in capital costs observed from a pioneer plant to the \(N^{th}\) plant has been quantified and estimated. The benefits come from lower contingency costs, reduced engineering, and a less conservative design [65]. The 17\(^{th}\) plant is typically considered a mature plant, and with this input a 22\% reduction in TOC and a 3.8\% reduction in sale price is observed over Case 1. However, the building of additional plants will likely not only result in lower capital costs, but in improvements in other areas such as increased protein expression.
4.3-3 Case 2

Combining all of the advantages discussed above, with the addition of increased protein expression, represents the optimistic scenario, Case 2. This scenario represents an optimized production facility, using the more economical processing options (Case 1g), and is the 17th production facility of its kind with a protein yield of 10 g l\(^{-1}\). All of these inputs combined (Case 2) result in a production capacity of 3,550 tons per year and a sale price of $23 kg\(^{-1}\). This is a 97% reduction in sale price from Case 1. As expected, the product price is dramatically impacted by the protein production level. Process optimization also has an important impact, but not at the same level as protein expression. A large fraction of this total cost, 52%, is attributed to material purchasing. This is far reduced from Case 1, but still significant. At this price spider silk would be a much more competitive commodity in the material industry and not limited to high valued products such as medical implants and would be much more competitive with other high strength materials. A complete list of Case 2 assumptions is included in Table 2.

Though immediate spider silk production would have a relatively high price, a pathway to support the economical production of synthetic spider silk is realizable. Feasibility will largely be determined by end product use. A variety of product options have been identified and include items from biomedical applications to a carbon fiber precursor. Under the postulation of inexpensive manufacturing, spider silk carbon fiber could be used for vehicle weight reduction. However, for spider silk from \textit{E. coli} to be economical for vehicle lightweighting as a carbon fiber pre-cursor, protein content would have to be between 25-40 g l\(^{-1}\), which is a significant synthetic biology challenge.
Table 2: Case 2 (Optimized) vs. Case 1(Pioneer) plant modeling assumptions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basic Input</th>
<th>Pioneer</th>
<th>Optimized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein expression (g/l)</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Nth plant</td>
<td>1</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Minimal media</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Glycerol replacement</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Heat induction</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Skip first centrifuge</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Flocculation</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No Drying</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

Applications using small amounts silk are better suited for economic viability. Due to its hypoallergenic properties and high strength, a likely candidate is the medical industry where only a few grams of spider silk might be used to make a replacement ligament, tendon, or other implant. The diverse properties of synthetic spider silk make it a promising material for a variety of applications.

4.3-4 Protein Expression

As the technology is further developed in large-scale applications genetic optimization for greater protein expression will likely occur and have the greatest impact on improving sustainability. The benefit of increased protein expression is illustrated in the sensitivity analysis previously discussed. The estimate of the sale price is inversely proportional to
Increasing protein expression doesn’t require additional materials, equipment, or labor for most of the processing steps. Effectively, these inputs, and the process flows are independent. For example, a 10X increase in protein expression results in only 0.9% increase in operational costs. The only modeled processes directly affected by increased protein expression are drying and product formation, which are inexpensive compared to the other processes.

Increasing protein expression is not only the most beneficial method of optimization, but is expected to advance through research and development. *E. coli* are commercially used to produce more than 150 recombinant proteins [68]. The techniques used for production and purification have been highly developed. The limit on protein production has been explored in *E. coli*. Increased yield can come through increased dry cell weight (DCW- grams of *E. coli* per liter of media) or genetic optimization for greater expression. Laboratory fermentations typically result in a DCW of 45 g l⁻¹ (Unpublished Data).

Other work with glycerol fed *E. coli* has had very high levels of cell concentrations, as much as 174 g l⁻¹ of DCW [66]. Recombinant proteins have been known to occupy as much as 20% of the cell volume [69]. Assuming that both high protein expression and cell concentrations are possible, then reaching a protein content of 25 g l⁻¹ would reduce the cost of production by 96% below the baseline, Case 1, to $32 kg⁻¹. Since the inception of the work, RSSPs levels have constantly been increasing. In comparison to other recombinant proteins, expression levels for spider silk have remained relatively low. This challenge is largely thought to be due to the size of the protein which is expected to limit
the final yield compared to what has been achieved with other proteins. Economic
modeling shows this to be the most valuable area where advancements can be made.

![Graph showing product sale price as a function of protein expression in E. coli for baseline scenario, Case 1, and optimized scenario, Case 2.]

**Figure 7: Product sale price as a function of protein expression in E. coli for the baseline scenario, Case 1, and the optimized scenario, Case 2.**

4.4 Alternative Production Platforms

Alternative protein production platforms were evaluated through the modular
construction of the engineering process model. Transgenic goats and genetically modified alfalfa represent promising alternative platforms for protein production. Expression of RSSPs in transgenic goats has been ongoing for more than a decade and was originally selected as goats are excellent producers of whey protein [13]. Alfalfa can also produce significant protein in its leaves with minimal inputs. Modeling of these production pathways was done through substituting representative models for the fermentation and
harvesting sub-processes required for *E. coli* with the corresponding protein production platform of goats and alfalfa. The downstream processing steps for protein purification and spinning were used directly from *E. coli* process model as these methods are agnostic to the source of protein production.

### 4.4.1 Goat Silk Economics

In stark contrast to the streamlined continuous flow system of *E. coli*, goat milking and farming operations require significant labor, Figure 8. Goat farming operations have a greater impact than purification, drying, and product formation with respect to the overall cost of production. In addition to the large cost associated with building and maintaining the farm, high costs are associated with the gradual installation of goats. Transgenic goats have to be implanted into a host goat, grown, and let mature for a year. Additional generations can be born from transgenic goats, but this takes time. A nine year growth period is required with annual loan installments to cover the cost of growing the herd, which increases costs substantially. A large portion of the estimated sale price is directly related to farming and milking. These costs were verified by considering only the cost of farming and milking for traditional milk production. Under this assumption, a sale price of $3.31 gallon$^{-1}$ was obtained, which is reasonable in comparison to an estimate of $3.40 gallon$^{-1}$ made by the University of California [70]. After the milk is collected its processing differs from that of *E. coli*. Purification of the milk is achieved using tangential flow filtration in contrast to affinity chromatography or flocculation.
Figure 8: Synthetic spider silk sale price estimates from various facilities and levels of optimization. Case 1, 3a, and 4a represent pioneer E. coli, goat, and alfalfa production plant results. Case 2, 3b, and 4b represent optimized E. coli, goat, and alfalfa production plant results.

In spite of the different processing methods, the spread for operational and capital costs is similar to that of E. coli. Goat pioneer plant results indicate a sale price of $730 kg\(^{-1}\). This high cost is due to the relatively small protein content in the milk, 1.5 g l\(^{-1}\). Though goat milk contains significant protein, not all of the protein from the transgenic goats is expressed as RSSPs. Costly commodities, such as arginine, make material costs a dominating factor in purification. The large processing volumes and low yields result in elevated costs. However, if genetic optimization of the goats could allow them to produce half as much MaSp as they do naturally with whey then goat MaSP production would be 13 times higher than the value used for the pioneer plant estimate. This expression level
combined with being the 17th plant and removing the drying sub-process results in sale price of $54 kg\(^{-1}\).

This price does not include the end of life considerations of the goats. The euthanasia of the transgenic laboratory goats is costly. Modeling work assumes the goats can be sold into the food market at the end of silk production life, assuming FDA approval, but if this were not the case, then it would be an additional expense.

4.4-2 Alfalfa Silk Economics

Alfalfa is one of the main agricultural products of the U.S. Alfalfa farming requires less labor than goat farming operations, and seeds for farms can be generated at a much quicker rate. Much of the cost for alfalfa processing comes from the harvesting stage, referring to the stage where the protein is extracted from the plant matter and not the alfalfa removed from the field. This sub-process utilizes heptane, acetone, and chlorophyllase to separate the protein from the bio-waste. Modeling work assumes these solvents could be recovered with an efficiency of 90%. With farming, alfalfa protein harvesting, and the additional remaining sub-processes included a pioneer plant sale price estimate of $207 kg\(^{-1}\) is obtained. This estimate is significantly lower than that of goats and *E. coli*, while still remaining higher than what is expected for synthetic spider silks to be commercially viable for base community markets. Still, laboratory work is preliminary, and protein expression has been limited and inconsistent. There are also additional challenges in the area of social acceptance. Research and work with genetically modified organisms have encountered significant resistance and this may stop large-scale operations based on genetically
modified alfalfa [71-73]. Assuming this can be overcome, the economics favor this protein production platform. Increasing the available protein content in the leaves seems highly feasible based on the level of natural protein production in the plant. Assuming that 50% of the crude alfalfa protein could be expressed as MaSp protein, corresponding to a 30X increase in RSSPs expression, combined with being the 17th plant and no drying, results in a sale price of $9.22 kg\(^{-1}\), Figure 8. Modeling work shows additional research needs to be done to verify the potential of alfalfa.

4.5 Life Cycle Assessment

The rising concerns of global warming has caused an increased interest in commercial production emissions. The emissions and environmental impact of the system was quantified through the metric of GWP. The net GHGs were determined leveraging the inputs and outputs of the engineering process model with LCI data. The results for the pioneer plant emissions are 572 kg CO\(_2\) eq. kg\(^{-1}\), Figure 9. Material consumption and process electricity account for 98% of the total emissions as expected. Similar to the economics, the majority of the emissions are directly related to the first three process: fermentation, harvesting, and purification. Electrical energy, glucose, and urea represent the largest contributors to the emissions at 213, 161, and 147 kg CO\(_2\) eq. kg\(^{-1}\). Energy recovery is integrated into the process model, which reduces most of the heating requirements to a third of their original amount, resulting in the low natural gas consumption.

Alternative scenarios were considered which focused on reducing the environmental impact of the system. Potential reductions in emissions are possible through co-product utilization and optimization. It is theorized that the waste from fermentation and
would be able to be used as a basic fertilizer. The left over bio-waste, urea, and glucose would represent a credit as it would replace the need for farmers to use traditional fertilizers. Additionally, the use of glycerol as the media carbon source represents a free carbon source as it is expected to be a waste product from the biofuel industry. Assuming the urea emissions could be removed as a result of co-product allocation, the emissions would be 24% lower, at 474 kg CO₂ eq. kg⁻¹.

Another method of decreasing the emissions per unit mass is to increase the production through improved protein expression. The net effect of going from Case 1, at 1.0 g l⁻¹ of protein expression, to Case 2 at 10 g l⁻¹ of protein expression results in a 90% reduction of emissions to 55 kg CO₂ eq. kg⁻¹. In spite of the optimization and increased yield, the reduced emissions of 55 kg CO₂ eq. kg⁻¹ are still higher than that of many other high strength materials. Linear low-density polyethylene and poly propylene have emissions of 1.62 kg CO₂ eq. kg⁻¹ and 1.59 kg CO₂ eq. kg⁻¹ [75]. Average steel has an emissions of 1.36 kg CO₂ eq. kg⁻¹ steel [39]. When compared with carbon fiber the emissions for silk are not quite as
substantial. Emissions for the PAN carbon fiber are 31 kg CO$_2$ eq. kg$^{-1}$ [76]. There are additional benefits that an optimized plant would experience, such as reduced energy demand, which cannot be fully quantified at this stage of analysis.
CHAPTER V
CONCLUSIONS

This work directly evaluated the economic viability of the production of synthetic spider silk proteins through *E. coli*, transgenic goats, and alfalfa. Experimental data was used to validate sub-process models that are integrated into an engineering system model. The engineering system model serves as the foundation for sustainability assessment through TEA and LCA. Through modeling estimates of $23 \text{ kg}^{-1}$, $54 \text{ kg}^{-1}$, and $9.22 \text{ kg}^{-1}$ were obtained for silk from optimized *E. coli*, goats and alfalfa production facilities, respectively. Emissions from *E. coli* pioneer and optimized plants are higher than anticipated at 572 kg CO$_2$ eq. kg$^{-1}$ and 55 kg CO$_2$ eq. kg$^{-1}$ product, respectively. Each of the protein production methods evaluated have inherent advantages and disadvantages. Alfalfa represents the most promising scenario due to established cultivation techniques, but has challenges with social acceptance. Transgenic organisms also require containment, which is much easier with *E. coli* than with alfalfa or even goats. *E. coli* can also be grown in large volumes in short periods of time, but its methods are material intensive. Purification of goat milk seems to use less harsh products and its processing methods may be more socially acceptable. Optimization through processing techniques and increased protein expression will be required before any of the three protein production methods can become feasible for large scale production. Additionally, as global warming awareness increases an eventual national carbon tax is conceivable. This would further affect the
economics as emissions are high. Further work to advance spider silk products should focus on the key model inputs identified though the sensitivity analysis, namely increased protein production. Increasing protein output sets off the high cost and emissions weight that are tied with low yields. Higher yields will not only increase the economic viability, but will hopefully increase social acceptance. Under this assumption it is likely that spider silk based products will start to enter the market in at least some industries. Additional market penetration is probably upon achieving higher levels of process optimization and protein expression
REFERENCES


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APPENDICES
Appendix A: Table of standard economic design case assumptions

*Table 3: Standard economic design case assumptions for techno-economic modeling*

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Installation time</td>
<td>3</td>
<td>Years</td>
</tr>
<tr>
<td>Plant financing debt</td>
<td>60</td>
<td>% of total capital cost</td>
</tr>
<tr>
<td>Loan term</td>
<td>10</td>
<td>Years</td>
</tr>
<tr>
<td>Interest rate on debt financing</td>
<td>10</td>
<td>%</td>
</tr>
<tr>
<td>Internal rate of return</td>
<td>10</td>
<td>%</td>
</tr>
<tr>
<td>Plant life</td>
<td>30</td>
<td>Years</td>
</tr>
<tr>
<td>Use tax</td>
<td>6.44</td>
<td>%, 2016 national average [77]</td>
</tr>
<tr>
<td>Income tax</td>
<td>35</td>
<td>%</td>
</tr>
<tr>
<td>Working capital</td>
<td>5</td>
<td>%</td>
</tr>
<tr>
<td>Depreciation Period</td>
<td>5</td>
<td>Years, MACRS schedule [55]</td>
</tr>
</tbody>
</table>
## Appendix B: Pricing for Equipment for the Baseline Synthetic Spider Silk Manufacturing Via. E. coli

**Table 4: Equipment purchase price estimates and averages for modeled equipment for the baseline E. coli production scenario, Case 1.**

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Aspen Estimate</th>
<th>Commercial Comparison</th>
<th>Loh et al. estimate</th>
<th>BOM &amp; construction Estimate</th>
<th>Quote or Other Estimate</th>
<th>Averaged Estimate</th>
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</thead>
<tbody>
<tr>
<td>Affinity Column (Shell Only)</td>
<td>-</td>
<td>-</td>
<td>$30,305</td>
<td>-</td>
<td>$33,947</td>
<td>32,105</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>$7,395.2</td>
<td>$7,395.2</td>
<td></td>
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<tr>
<td>Affinity Column Shell &amp; Resin</td>
<td></td>
<td>58</td>
<td></td>
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<tr>
<td>Air Compressor</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bioreactor-(1,250 liters)</td>
<td>$63,000</td>
<td>$12,000</td>
<td>$95,778</td>
<td>$49,357</td>
<td>$74,907</td>
<td>$59,149</td>
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<tr>
<td>Bioreactor-(25,000 liters)</td>
<td>$347,000</td>
<td>$209,000</td>
<td>$277,327</td>
<td>$227,459</td>
<td>$367,024</td>
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<td>Centrifuge</td>
<td>$170,000</td>
<td>$156,000</td>
<td>$272,753</td>
<td>-</td>
<td>-</td>
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<td>Distillation Column</td>
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<td>$89,345</td>
<td>-</td>
<td>-</td>
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<td>Filtration Unit</td>
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<tr>
<td>Fiber Extruder/stretcher</td>
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<td>$77,000</td>
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<td>-</td>
<td>$73,260</td>
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<tr>
<td>Genetic Maintenance Equipment</td>
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<td>-</td>
<td></td>
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<tr>
<td>Homogenizer</td>
<td></td>
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<td>-</td>
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</tr>
<tr>
<td>Lyophilizer</td>
<td></td>
<td>138,600</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Microwave Heater/mixer</td>
<td></td>
<td>$138,600</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixing Tank (10,000 liters)</td>
<td>$77,000</td>
<td>$41,000</td>
<td>$114,362</td>
<td>$33,442</td>
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<td>$25,800</td>
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<td>Mixing Tank (2,000 liters)</td>
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<td>$11,600</td>
<td>$53,750</td>
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<tr>
<td>Mixing Tank (200 liters)</td>
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<td>$14,096</td>
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<tr>
<td>Office Equipment</td>
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<td>-</td>
<td>-</td>
<td>$169,100</td>
<td>-</td>
<td>$169,100</td>
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<tr>
<td>Pump- Centrifugal (120,000 l/h)</td>
<td>$5,100</td>
<td>$3,458</td>
<td>$15,724</td>
<td>-</td>
<td>-</td>
<td>$8,103</td>
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<tr>
<td>Pump- Inline (10,000 l/h)</td>
<td>$3,800</td>
<td>$600</td>
<td>$5,718</td>
<td>-</td>
<td>-</td>
<td>$3,372</td>
</tr>
<tr>
<td>Pump- Inline (1,000 l/h)</td>
<td></td>
<td>$250</td>
<td>$5,146</td>
<td>-</td>
<td>-</td>
<td>$2,698</td>
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<tr>
<td>Storage Tank – 500,000 liters</td>
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<td>$162,000</td>
<td>$126,084</td>
<td>$183,789</td>
<td>-</td>
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<tr>
<td>Storage Tank – 50,000 liters</td>
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<td>$30,020</td>
<td>$48,935</td>
<td>-</td>
<td>$35,651</td>
</tr>
<tr>
<td>Storage Tank – 5,000 liters</td>
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<td>$14,000</td>
<td>$14,800</td>
<td>-</td>
<td>-</td>
<td>$14,400</td>
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<tr>
<td>Water boiler (4.2 MW)</td>
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<td>$220,000</td>
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<td>Water purifier</td>
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<td>$127,000</td>
<td>$60,040</td>
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<td>$93,520</td>
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& Shell & tube heat exchanger (68 m^2) $37,000