Professor C. Pfluger
College of Engineering
219 Cullinane
Northeastern University
360 Huntington Avenue
Boston, MA 02115

West Village Residence Hall
450 Parker St.
Boston, MA 02115

Dear Professor Pfluger,

The attached document is our final report titled: Sustainable Mycoprotein Production via Hydrolysis and Fermentation of Coffee Waste. Our investigation into the broad topic of fermentation focused on addressing nutritional and sustainability deficits. We identified a dire need to produce protein for human consumption without traditional agriculture. In turn, we propose a novel process that ferments coffee silver skin (CSS), an agricultural waste product, to produce edible protein. Our goal is to reduce agriculture's environmental footprint through cost-competitive production and widespread adoption.

Our market analysis indicates we have a unique opportunity due to negligible raw material costs and a highly marketable origin. Our business-to-business model outsources complicated details (such as culinary style, consumer preferences, and seasonality) by selling protein directly to manufacturers. We propose a mid-size plant capable of processing 898 MT of CSS into 730 MT of protein slurry annually. This plant will require a capital investment of 27.4 million USD to yield 10.3% IRR and 26.5% ROI over 12 years.

We conducted both an experimental and computational proof of concept to validate the process. Our experiment successfully demonstrated the creation of reducible sugars with yields up to 52%. We used these results to justify a design pivot from an acidic to enzymatic hydrolysis. Similarly, a SuperPro simulation was built to investigate process viability. A techno-economic analysis found our initial plant scale (472 MT y^{-1}) was insufficient to meet our profitability threshold, which prompted us to increase the process scale.

We performed a safety analysis on both the actual experiment and the proposed process. Material hazards are mitigated using a HAZOP analysis, which includes a detailed control scheme. Inherently safer design was used to reduce potential implications on health and human safety.

Thank you for your time. We look forward to your feedback and insights.

Sincerely,

Team #3

Thomas Gnann

Andrew Larocque

Shreyas Ravichandar

Ian Wiatric

Firaz Zarrouk

SUSTAINABLE MYCOPROTEIN PRODUCTION VIA HYDROLYSIS AND FERMENTATION OF COFFEE WASTE

CHME 4703

Final Design Report

Detailed Report

Business Mentor: Christopher McLaughlin

Design Team
Thomas Gnann, Andrew Larocque
Shreyas Ravichandar, Ian Wiatric, Firas Zarrouk

April 23, 2024

Department of Chemical Engineering

College of Engineering, Northeastern University

Boston, MA 02115

Sustainable Mycoprotein Production via Hydrolysis and Fermentation of Coffee Waste

Thomas Gnann
Andrew Larocque
Shreyas Ravichandar
Ian Wiatric
Firas Zarrouk

Abstract

The global human population and associated carbon emissions continue to rise despite the dire need for plentiful and sustainable protein. Mycoprotein is one potential solution. Mycoprotein is typically produced by fermentation of pure sugar, but other industries are exploring the saccharification of waste products instead. A business that transforms an agricultural waste product, coffee silver skin (CSS), into edible mycoprotein is proposed. This novel process consists primarily of a delignification, hydrolysis, and fermentation reaction. Acidic hydrolysis was successfully demonstrated to produce reducible sugars with yields up to 52%. A process model was created in SuperPro to investigate the venture's financial viability. Based on this model, a plant capable of processing 898 MT of CSS into 730 MT of protein slurry annually is proposed. The final design includes a detailed safety analysis and control scheme. The plant would require a capital investment of $28.04 MM to yield a 26.5% return on investment over 12 years, making this project an attractive investment. A dramatic reduction in the environmental impact of agriculture is possible by realizing the cost-competitive production modeled here.

Keywords: mycoprotein, sustainability, fermentation, waste valorization

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2 Acknowledgments

We are very grateful for the enthusiastic support from our mentor Chris McLaughlin at Arcadis. Professor Courtney Pfluger of Northeastern University has been incredibly generous with her time and resources. We are also grateful to Dr. Honda for being immensely flexible and helpful with accessing the ChemE-ID studio. We appreciate the insightful discussions with Mike Smith at Renmatix and Professor Benjamin Woolston. Finally, we would like to acknowledge Tyler Wood at George Howell Roasters for supplying coffee silver skin.

3 Background

3.1 Project Need

The global population is projected to reach 9.7 billion by 2050 [1]. However, the need for sustainable and plentiful protein sources to feed this new generation remains woefully unmet. As of 2019, there were approximately 150 million cases of profound protein malnutrition. This includes 212,000 deaths per annum, primarily in sub-Saharan Africa and Southeast Asia [2]. Traditional animal husbandry is unlikely to meet this need due to its intensive water consumption, greenhouse gas (GHG) emissions (GGE), and land usage. For example, meat accounts for 57% of the GHGs produced by the entire food industry [3]. This is estimated to contribute up to 19.6% of total GHG warming [3]. Producing mycoprotein via microbial fermentation has emerged as a promising method to reduce carbon footprint while addressing global nutritional needs. Studies show that producing mycoprotein generates 48% of the GHG emissions when compared to beef production on a mass basis [4]. Therefore, replacing part of the animal-derived meat market with mycoprotein can significantly reduce GGE's compounding effects of the same areas most vulnerable to protein malnutrition [2]. Some successful commercialization ventures currently exist; however, industrial processes must be improved to increase cost competitiveness to drive widespread adoption. Therefore, the process designed aims to utilize agricultural waste to drive costs lower with the continued aim of reducing GGEs while improving upon the mycoprotein industry's availability to a wider socioeconomic scope.

3.2 Process Novelty & Rationale

Fermentation has been used for thousands of years to produce food and beverages through controlled microbial growth [5]. For this project, fungi continue to be important microbes that will be fermented with the goal of providing sufficient nutrient, carbon, and nitrogen sources to promote their growth. Growing and harvesting fungal mycelium, particularly filamentous fungi, can provide all the essential amino acids needed for human nutrition. This fungal product is referred to as 'mycoprotein' [4]. However, concerns regarding the nucleic acid content, mycotoxin production, and risk of inflammatory immune system responses (allergic reactions), made the approval process of new fungal strains time-consuming and expensive. A fungal strain known as *F. venenatum* was approved due to its low nucleic acid content and lack of adverse effects from potential toxins or allergens [6]. Due to the large and costly investment in the approval process, current companies, like Quorn, exclusively use *F. venenatum.*

Quorn is currently the commercial leader in the microbial protein industry and a top competitor in the broader alternative protein market [7]. Quorn ferments using commercial glucose syrup to feed their fungi [8]. However, sourcing solely glucose syrup increases the demand for monocultures, such as sugar cane, which is a large contributor to GGE and an inefficient use of arable land. Therefore, this process will differentiate itself both technically and commercially by utilizing a novel feedstock: coffee silver skin (CSS). CSS has gained recent attention resulting from research as a feedstock for the biofuel applications [9]. However, CSS has not been considered as a feedstock for the fermentation of edible proteins.

CSS is the sole waste product of the coffee roasting process, ergo it does not require purification for use as a feedstock. The highly fibrous composition of the material naturally lends itself to saccharification which can replace the commercial glucose syrup utilized by Quorn. Recent literature reports cellulose and

hemicellulose concentrations of 21% and 8% by mass, respectively [10]. These polymers are the principal source of fermentable sugars, such as glucose and xylose, which is suitable for the growth of mycoprotein.

The chosen feedstock is extremely abundant in the United States, making it conducive to rapid scaling and commercialization. Approximately one ton of CSS is produced per 120 tons of roasted coffee beans [11]. As of 2023, US coffee roasting capacity is nearly 1 million tons [12], implying approximately 8,300 tons of CSS waste is generated annually. The designed process will consume a small fraction of this available material. One of the largest coffee roasters in the United States, Starbucks, produces over 3 million pounds of coffee a year at just one of six facilities. They alone produce and discard more than 20,500 pounds of CSS every week [13]. Therefore, positioning a pilot-scale process in close proximity to one of these roasters leverages the well-developed supply chain infrastructure and makes logistics for CSS sourcing simpler as it would be locally shipped.

3.3 Intellectual Property Landscape

The intellectual property (IP) landscape for lignocellulose hydrolysis is rapidly expanding and relatively young; many patents are not set to expire within the decade [14]. Patents are generally awarded to inventions which improve chemical yield, reduce cost, or reduce reaction time. They are typically not awarded to specific feedstock materials, i.e., they cover any lignocellulosic material like CSS. For example, EP4271824A1 protects a pretreatment process which impregnates any cellulosic material with water reactive thiols to help decompose fibers and generate higher yields [15]. This patent would likely prohibit the use of water reactive thiols in CSS pretreatment. US8747561B2, a patent on Cellulose Hydrolysis with pH Adjustment also pertains to this project [16]. This patent is filed by Renmatix, a company that operates biorefineries in the United States for processing agricultural waste. This patent details desirable pH conditions, temperature, and pressure conditions for bioreactors that perform saccharification of cellulose at supercritical fluid conditions. The same company filed US8282738B2, which describes a novel process for the separation of lignin and cellulose from a biomass feed source [17]. These patents demonstrate the feasibility of high temperature and pressure hydrolysis for feeding bioreactors. However, they could also pose barriers to intellectually protecting the proposed system.

4 Business Plan

4.1 Mission and DEI Statement

The current state of the protein industry is accelerating the effects of greenhouse gas emission and compounding food insecurity among our world's most vulnerable populations. Traditional methods of high-quality protein production are environmentally destructive. Our mission is to produce a microbe-centered protein source using agricultural waste as a feedstock to reduce the environmental impact of protein production.

We value equity and inclusion across race, gender, religion, and experience to empower our employees. We believe a diverse group of engineers ultimately creates superior solutions, which are critically needed to address global inequity. We want our product to support a healthy and economical lifestyle for all customers, regardless of income, nationality, or race.

4.2 Overview

The core of the proposed venture is to produce and sell alternative protein. Through strategic partnerships, CSS will be acquired in bulk from coffee roasters at little or zero cost. This company will utilize an in-house novel technology to depolymerize the CSS, ferment resulting sugars, and output a protein slurry product. The novelty in this system is the design of a process that can efficiently break down the CSS into sugars that are optimal for fermentation, in both sugar content, protein profile, and other potential health benefits attractive to customers. The strategic advantage of the process is cheap feedstock that is traditionally wasted. The alternative protein can be marketed based on its low environmental impact, vegan-friendly nature, and cruelty-free origin to attract consumers.

A business-to-business (BTB) model will be adopted to create a streamlined distribution system. By outsourcing the task of product development and consumer marketing, the venture can focus exclusively on technological and business optimizations. The target market includes companies that outsource their protein production. These companies all have diverse consumer bases, ensuring that the product will reach a broad network of customers. This product could also be sold directly to smoothie bars and wellness cafes, who can market this coffee-based product as an attractive protein source, in line with global trends sustainable goals. The BTB customers will be encouraged to leverage the novel coffee-derived background of the protein material for their own marketing and sales purposes. Ideally, the protein produced will have a mild to nonexistent flavor profile which can be used as a blank canvas for culinary innovation.

4.3 Business Structure

The proposed venture will structure itself as a limited liability company (LLC). The high degree of flexibility to incorporate new partners while maintaining reduced legal exposure is beneficial for a startup company [18]. If members of this business decide to fund raise through public markets or venture capital, a legal advisor will be consulted to consider the switch to alternative structures like a corporation. Finally, LLCs allow for transfer of intellectual property between individuals developing the project and the company itself without taxation or legal maneuvering [18].

4.4 Market Analysis

The global mycoprotein industry currently involves a small number of private companies utilizing novel technology. The market is non-saturated, growing at a CAGR of 12.6% from 2022 to 2032, and is expected to reach $0.976 billion by the year 2032 [19] [19]. The rising trend of veganism and consumer climate awareness is expected to propel the market for alternative food proteins. The market is also influenced by the development of packaged foods, such as frozen products and snacks, which cater to the growing consumer preference for convenient yet health-conscious food options. Mycoprotein sales are likely to account for ~5.3% of the demand in the global meat substitute market, valued at USD $5.6 billion in 2022. The share of the alternative meat market is predicted to steadily increase in the next decade (Figure 1).

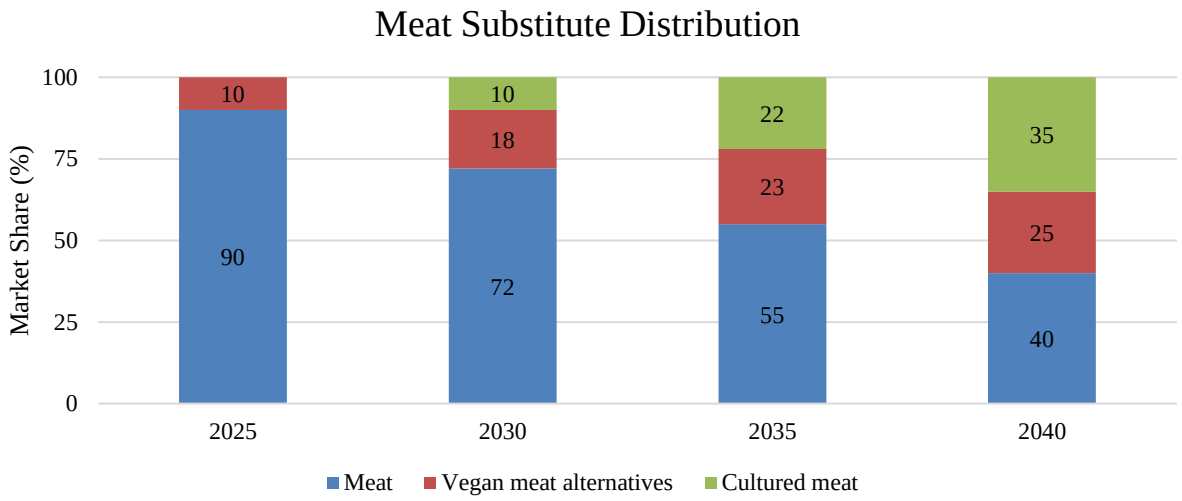

Figure 1 Predicted market share of major meat substitute products until 2040 **[20]**. The proposed plant exists in the vegan meat alternatives space, which benefits from a first-mover advantage but could be outpaced by cultured meat over a longer time horizon.

4.5 Market Competition

The competitive landscape is dynamic and growing because of process innovation, investment in R&D, and marketing strategies. Fermentation companies producing alternative proteins raised $1.69 billion in 2022 [21] [22]. The direct competitors in the mycoproteins industry include Marlow Foods Ltd., 3F Bio Ltd, MycoTechnology, Inc., General Mills, Beyond Meat Inc., Impossible Foods Inc., Quorn Foods, Marlow Foods Ltd., Kernel Mycofoods, and Atlast Food Co. All companies listed are privately owned so their costs of production, business information, and scale are not public information. The indirect competitors are meat producers and other biotechnology companies that produce protein alternatives such as plant-based meat.

4.5.1 Quorn

Quorn is the largest producer of mycoprotein in the direct-to-consumer market. According to a recent techno-economic analysis, mycoproteins were estimated to cost $3.55 per kg while processed Quorn-like products (PQP) cost approximately $4.03 per kilogram to produce. PQP includes the processing of mycoprotein slurry to a consumable product [23]. The retail prices of Quorn products range between $4.00 and $4.75 per item (0.2-0.35 kg per item). Therefore, it can be estimated that PQP yield margins of 10-30%. Quorn uses 155,000 L airlift bioreactors [24], capable of producing 5,000 burgers per hour. Assuming operation of 6,000 hr/yr, a single airlift bioreactor can produce 40 million patties per year, which yields $120 million in revenue per bioreactor. Quorn, sells directly to retailers in local supermarkets, so their production costs likely include distribution and product assembly. Specific information on the selling price of mycoproteins from large-scale producers is not offered to the public and is subject to quotes from companies.

4.5.2 Affiliated Markets

Because fermentable sugars are an intermediate product within the overall process, there is a potential revenue stream regarding industrial sale to biofuel producers. The global ethanol market was valued at $94.2 billion in 2022 and is expected to see CAGR of 5.1% over the subsequent decade [25]. This growth is driven by increased demand for clean energy alternatives, technological advancements in ethanol production, and legislative requirements for bioethanol in automotive fuel. This commercialization strategy merits its own rigorous analysis in another report.

Since this process requires the use of coffee silver-skin as feedstock, the enduring success of the proposed process is dependent on the coffee roasting industry. Fortunately, it is experiencing steady but modest grow. The global coffee roaster market is expected to reach USD 2.39 billion by 2032, expanding at a CAGR of 6.3% [26]. The most relevant coffee roaster, Starbucks, is likely to remain in good standing; 48% Americans report Starbucks as their favorite brand of coffee and the company maintains 40% of the coffee roasting market share [26].

4.6 Marketing Plan

The final product of the process is not a finished meal item, such as a patty or nugget. Instead, the final product is a protein slurry. This product will not be textured or flavored to replicate meat, but rather prioritize protein content. Therefore, it will be marketed to companies such as smoothie bars, vegan meat companies, and vegan restaurants. It will be used in their protein rich products to enhance the nutritional content. The name for this product will be “Fun Guy Protein” and it will be marketed as a “coffee-based” protein and would have great potential to be marketable at smoothie bars. The novel coffee silver skin aspect of this process employs a strategic advantage over competitors in the mycoprotein industry like a cheap feedstock and low environmental impact.

4.7 Operational Design

The location of the proposed plant is critical in ultimately determining the project’s feasibility and profitability. The town of York, Pennsylvania was selected because of its strategic location near a Starbucks roasting facility. According to Starbucks, this manufacturing plant processes 3 million pounds of coffee per week, creating about 25,000 pounds CSS over the same period [13].

Estimating the facility footprint is difficult given the nuances of equipment layout and piping design, which are outside the scope of this report. However, broad design considerations can still be discussed. The equipment layout will be based on operational affinities so processes which naturally conflict with each other are kept apart. For example, the combustible dust produced from CSS grinding should not be near air filtration and pump operation. Similarly, some operations benefit from proximity when frequent transfers between them are required. Their CSS waste could be acquired and transferred to the pilot site with minimal transportation costs.

4.7.1 Supply Chain

York is centrally located along the eastern seaboard which facilitates product export to key markets in New York, Philadelphia, Baltimore, and Washington. York is also 20 miles away from the Susquehanna River, which can be used to transport mass intensive materials and equipment at minor costs. As a state, Pennsylvania is attractive because of its talented work force, business friendly tax credits, and relatively inexpensive real estate. One downside to labor in Pennsylvania is the prevalence of unions and which can affect the associated labor costs for the process at scale [27].

The Business to Business (B2B) model will be used as a benchmark in planning an operation for the flow and storage of goods. The storage and inflow of 25,000 pounds of CSS will be handled in accordance with process scheduling and process consumables. This includes warehousing that facilitates dry conditions for the CSS. Storage must include cycle inventory, as well as safety inventory, a buffer against uncertainty – approximately 10% worth of yearly cycle inventory. The main mode of transportation involving flow of raw materials and processed mycoprotein includes trucks for shorter routes and ships for longer routes. These are subject to change, as the operation seeks to scale up or to reach different markets. Data logging and efficient supply chain information will be handled inhouse to ensure efficient coordination between production, inventory levels and transportation routes [28].

Throughput flexibility is essential for adapting the plant around the needs of the business model. Market fluctuations and time-sensitive opportunities will require the ability to dynamic scale production, up and down. To this end, the process has been partitioned into three distinct sections: preprocessing, core operations, and post-processing. At the personal level, these distinctions will allow for more specialized divisions of labor which better understand their intermediate product. At the organizational level, they enable easy identification of production bottlenecks. The current limiting step in the process is fermentation capacity, and as such, the facility should be built to allow for expansion with additional units.

5 Process Description

The proposed process has been segregated into three distinct phases of operation. The “Preprocessing” section prepares the raw CSS for hydrolysis and ends with the neutralized cellulosic mass. The second phase, “Core Operations”, features both the hydrolysis and fermentation reactors. Finally, the biomass effluent from the fermentation reactor goes through a brief “Post Processing” section before streams terminate. Despite differing capital allocations, all phases of the design contain critical operations which will determine the overall viability of the process.

There are several unit operations which will contain transient material to simulate realistic batch scheduling. This equipment is not described further herein. Their economic and scheduling impacts are accounted for later in the paragraph 0was to compare the feasibility of acidic and enzymatic hydrolysis of CSS. This comparison would be used to inform design decisions made for the final design solution. However, the team did not perform experiments on enzymatic hydrolysis due to enzyme shipping delays. The experimentation using acid hydrolysis was still informative for the decisions made about the final design solution.

5.1 Experiment Description

5.1.1 Drying and Grinding CSS

George Howell's roastery in Acton, MA graciously provided coffee silver skin. Approximately 5 lbs arrived in two vacuum sealed containers. Triplicate CSS samples weighing approximately 10 grams were dried in a convention gas-fired oven for two hours at 170 °F until a constant (± 0.01g) mass was reached. Each sample was ground in a Type F203 Krups Coffee Bean Grinder for one minute. Images were obtained using a Ziess Microscope, to observe the effect of the grinding and drying processing steps.

5.1.2 DNS Quantification and Calibration Curve

An analytical technique was needed quantify the sugars produced from acid hydrolysis. HPLC is a commonly used method for this goal, but was cost-prohibitive for this experiment. A spectroscopic assay using 3,5-Dinitrosalicylic acid (DNS) was found as an alternative to this method . The reaction in this assay is:

$$3,5-Dinitrosalicylic\ acid\ +\ Reducing\ sugar \rightarrow\ 3,-Aminonitrosalicylic\ acid\ +\ Oxidised\ sugar$$

The team ordered DNS from Sigma-Aldrich. Rochelle Salt, phenol, Sodium bisulfite, and Sodium hydroxide are typically used in combination with DNS for the reducing sugar quantification. The team determined that the critical chemical of these additives was sodium hydroxide. Other chemicals present were for additional stability and accuracy of the measurement. Food grade NaOH provided by the ChemE Capstone Lab was used for solution preparations. Rochelle Salts, phenol, and sodium bisulfite were not used in the assay due to cost restraints. The team prepared a calibration curve using d-glucose and the DNS quantification method. The DNS solution was prepared with 10 mg/mL of DNS and 16 mg/mL of NaOH in DI water. The DNS solution was then combined in a 20:1 ratio with glucose standards created. The solution was heated for 5 minutes at 80C using an air heater. Wavelengths were measured at 500, 520, 540, 560, and 580 wavelengths using a spectrophotometer. Absorbances at 500 are not presented because the instrument reading was overridden at these wavelengths. Values given as n/a in Table 2 overrode the maximum value of 2.5 AU. The values obtained at a wavelength of 580 nm were chosen to create a calibration curve because this was the only wavelength where the instrument did not override its absorbance maximum. The highest r-squared value was also obtained for this wavelength. While an r-squared value of 0.965 is not ideal for a calibration curve, the team was satisfied with this result, given the inability to obtain and use reagents that are typically used for this quantification method. Refer to Appendix A for Calibration Curve Data.

5.1.3 1st Stage of Hydrolysis

The team ordered 98 wt% Sulfuric Acid from Sigma Alrich to be used for the acid hydrolysis portion of this experiment. This solution was diluted down to a 72 wt% solution to be used for the 1st stage of acid hydrolysis. 3 grams of dried and ground CSS were placed into a 50 mL beaker with a Teflon stir bar. An important aspect of scale up economics and feasibility of acid hydrolysis is the ratio of acid that is used in comparison to the amount of biomass . The team chose to vary the ratio of sulfuric acid to biomass, from 6:1, 4:1, and 2:1 in weight percent ratios. These represent solutions A, B, and C. 72% wt acid was added to these 3 conditions in the appropriate volumes. The 1st Hydrolysis stage was performed at 30C for 30

minutes. Due to difficulty mixing the biomass acid slurry for 2:1 biomass loading ratio, an additional 3 mL of 72 wt% acid was added to the mixture. The biomass loading ratio of solution C therefore became approximately 3.33:1.

Figure 9. CSS before 1st Acid Hydrolysis Stage (Left) CSS during 1st Stage of Acid Hydrolysis (Right)

5.1.4 2nd Stage of Acid Hydrolysis

After the first hydrolysis stage, the slurry solutions of acid and biomass were transferred to 200 mL Erlenmeyer Flasks. They were then diluted with DI water with water until the volume reached 150 mL. The resulting Sulfuric acid weight percent of Solutions A, B, and C are 12.5%, 8.3% and 6.9%. The solutions were then heated at 80C for 3 hours.

5.1.5 Filtering and Sample Preparation

The final solutions were cooled down, neutralized, and then filtered through a coffee filter. The resulting solution was then stored in glass vials in the fridge.

5.1.6 Quantification

Quantification of the produced sugars was performed using the DNS method previously outlined. The team experimented with different ratios of sugar sample to DNS solution. The calibration curve was performed using a 1:20 ratio of sugar sample to DNS solution sample, but due to low concentrations of sugars in the sample, it was found that lower dilution rates were beneficial for measuring sugar content. A 1:1 ratio of DNS Solution to filtered, hydrolyzed CSS was used to perform the analysis. Despite qualitatively observing a darker solution for sample A, the absorbance values measured showed that this was the solution with the least sugars, and that Solution C had the highest yields. This conflicts with the hypothesis that higher ratios of acid to biomass loading should cause higher yields of sugar. Due to conflicting data, the team has decided not to draw any conclusions about the effects of different biomass loading ratios.

Table 2 Final results from the experimental proof of concept. The acid hydrolysis method tested was successful in producing measurable sugar concentrations but bucks the expected trend between biomass loading and yield.

	Absorbance	**Sugar concentration, pre-dilution**	**Sugar concentration, final**	**Total sugars produced**	**Estimated total sugars in CSS**	**Sugar yield**
Units	Au	mg/ml	mg/ml	g	g	%
Sample A	0.78	2.33	1.16	0.35	1.20	0.29
Sample B	0.75	2.22	1.11	0.33	1.20	0.28
Sample C	1.31	4.13	2.06	0.62	1.20	0.52

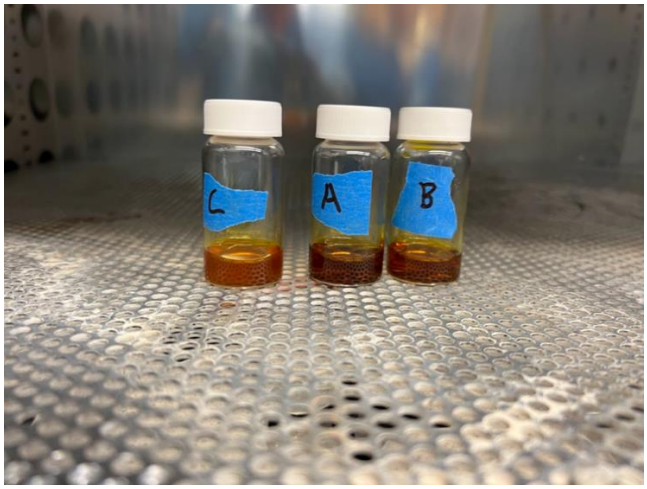

Figure 10 Hydrolyzed analyte after incubation with DNS reagent. In the presence of reducing sugars, a nitro group on the DNS reagent is converted to an amino group, resulting in a darker color. Therefore, the degree of coloration is expected to correlate with increased sugar concentration.

5.2 Results and Discussion

This experiment was successful in producing and measuring sugars from coffee silver skin by using acid hydrolysis. While no conclusions could be drawn from the different acid loading concentrations used, significant yields obtained suggest that coffee silver skin acid hydrolysis is a feasible means of obtaining sugar from the biomass source. However, due to the safety challenges and expenses required for using sulfuric acid at scale, as well as the byproducts expected from this reaction, the team does not believe that this method of hydrolysis is the best path forward for the project. Acid hydrolysis for sugar production may be first used for ethanol production. The increased importance of safety and product consistency for this project leads us to conclude that enzymatic hydrolysis will be the best path forward for mycoprotein production. This conclusion was further supported by encouraging data and economics from simulation proof of concept work performed in SuperPro.

5.3 Preprocessing Section

5.3.1 Drying

The literature typically reports the moisture content of coffee silver skin to lie between 2 – 7 % w/w [32], but some authors report as much as 35 % w/w [10]. Therefore, a drying step is needed to maintain process consistency and reduce equipment volume. Benchtop-scale literature analysis recommends drying between 40 °C [9] and 50 °C [10] [33] until mass remains constant. Given the proposed scale of the pilot plant, this procedure will need to be adapted to a rotary-style drying unit. Additionally, this unit operation may be adapted to save energy by using combustible waste or heat generated elsewhere in the process. The proposed plant will dry until the target composition described in Figure 2 is reached.

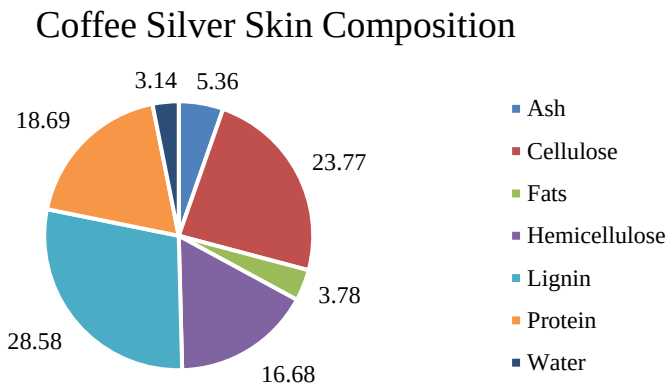

Figure 2 Coffee silver skin composition determined by Ballesteros and colleagues **[34]**. This composition was used to create a stock mixture in the SuperPro environment to define the feedstock. Component properties copied from the SuperPro V13 database, accessed using a view-only license.

5.3.2 Grinding

Industrial grinding will be used to control the feedstock's surface area, another key process parameter. Subsequent unit operations (delignification and enzymatic hydrolysis) are surface-based reactions. Increasing the available substrate area will reduce reaction time, and by extension, increase unit throughput and decrease capital cost in the long term. The literature has identified particle sizes between 500 – 1000 µm to be sufficient for good reaction yield [10] [33] [9]. SuperPro's grinding unit will be used to model the energy consumption and process time required to achieve this threshold.

5.3.3 Delignification

Delignification serves a similar purpose to grinding–increase reaction area–but on a molecular scale. The metabolically relevant sugars stored in cellulose and hemicellulose polymers are typically compact and inaccessible because of their lignin binding (Figure 3). Lignin is a robust polymer which will not meaningfully degrade under the previous thermo-mechanical conditions. A dedicated unit operation is needed to remove lignin. The literature indicates agitation with 5% w/v solution of NaOH is a robust and

reliable method [10]. A continuously stirred reactor (CSTR) filled with basic solution at 12% biomass loading, 121 ºC, and 2 atm will be modeled to account for this operation.

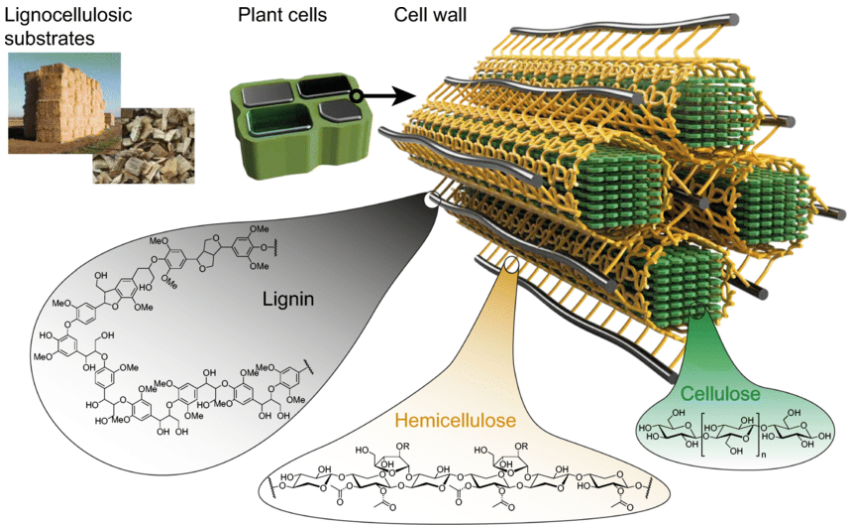

Figure 3 Macromolecular diagram of typical cellulosic biomass, like coffee silver skin **[35]**. The tight, ordered packing of each polymer results in high chemical stability and low reaction area. A delignification operation will degrade lignin fibers enough to facilitate hydrolysis of the fermentable sugars.

5.3.4 Neutralization

The intermediate product must be neutralized before proceeding to the hydrolysis reactions. This will be accomplished using a 50% solution of sulfuric acid in a stainless-steel batch reactor [33]. The salts resulting from neutralization, such as Na_2SO_4, are expected to be highly soluble and have minimal impact on downstream processing.

5.4 Core Operations

5.4.1 Hydrolysis

Hydrolysis is the core operation which transforms the low-value biomass waste into usable metabolic fuel. Dissolution of the glycosidic ether linkage between saccharide monomers produces water as the only byproduct (Figure 4). There are two leading methods for performing biomass hydrolysis: acidic and enzymatic techniques. Acidic hydrolysis involves a three-way trade off between cost, yield, and time [36]. Highly concentrated acid and elevated temperatures afford great reaction yield and speed, at a substantial expense. This method carries additional risk of transforming sugar monomers into toxic byproducts like furfural. Dilute acid conditions result in a low yield and cost. Acid hydrolysis requires careful tuning of parameters to become a cost-competitive process for any agricultural saccharification process.

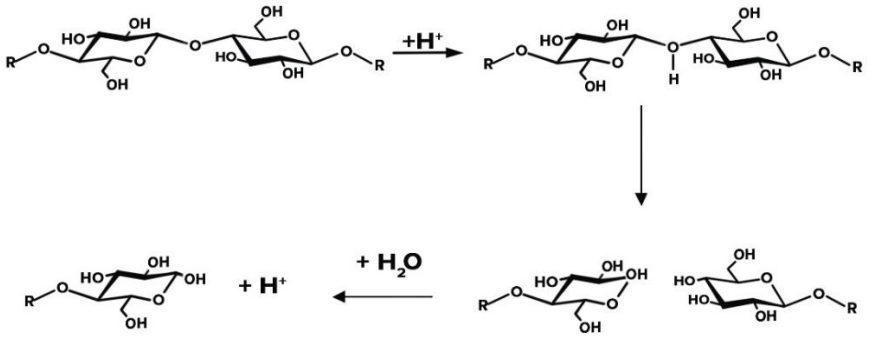

Figure 4 General hydrolysis reaction for cellulose into glucose. The reaction is catalyzed by a proton, which ultimately replenishes the catalyst and generates a singular water molecule. The scheme for hydrolysis of hemicellulose is nearly identical.

Enzymatic hydrolysis is the only viable alternative to using concentrated acid. Traditionally, cellulase and hemicellulose naturally found in symbiotic bacteria were cultured and harvested for industrial use. Currently, proprietary blends of genetically engineered enzymes, such as Cellic Ctec3 from Novozyme, can degrade a wider variety of substrates at higher rates. Progress in protein engineering has continued to improve product selectivity, cost, and stability. This process would assume the one-time use of enzyme solution at 100 g/l and 5.4% w/w loading with respect to initial biomass, although cutting edge research indicates enzyme reuse may be possible through fixed scaffolds. The choice of hydrolysis method contains technical nuance which are further discussed in Section 5.6.

5.4.2 Screw Pressing

Successful solution hydrolysis will result in a monophasic aqueous solution with an insoluble biomass fraction. The desired products, soluble sugars, are in the aqueous phase. The solid fraction is composed of undegraded organic polymers, oils, and ash. The separation of these phases will be modeled with a screw press operation in SuperPro. This equipment requires relatively large capital investment compared to comparable operations like decanting, but in turn, rewards with superior yields.

5.4.3 Fermentation

Fermentation is the sole bioprocess operation in the proposed process, but it is possibly the most critical for value generation and profitability. Both the bacteria and fungi domains have viable microorganisms for industrial fermentation. The metabolic requirements of potential candidates are an important point to guide microorganism selection. Naturally occurring strains of both fungi and bacteria tend to thrive best on six carbon sugars, such as glucose, galactose, and mannose. Some bacteria have secondary metabolic pathways which are capable of pentose metabolism, but it is typically suppressed when hexoses are abundant. Furthermore, the bacteria which possess this pathway almost exclusively produce substantial volumes of chemical waste such as ethanol or methane. Considering cellulose and hemicellulose have near equal abundance in coffee silver skin, an ideal microbe would be able to digest both pentoses and hexoses simultaneously to maximize biomass produced per unit feedstock.

Typically, bacteria are employed when simple chemical products like ethanol, hydrogen gas, or methane are desired [37]. Their ability to develop under purely anerobic conditions simplifies the sterilization processes and risk of contamination. Their plasmid genome also simplifies potential genetic modifications. However, bacteria are typically less effective for generating edible protein-biomass because of their high nucleic acid content and small cell size [38].

Fungi has a distinct advantage over bacterial fermentation in this regard. Both filamentous and non-filamentous strains boast larger cell mass and higher protein content. They are naturally resistant to a variety of endotoxins which can be produced inadvertently in industrial steps like acid hydrolysis. For these reasons, filamentous fungi are the only microbe to succeed commercially at generating edible food products. Existing commercial mycoprotein facilities implement airlift fermentation units, which can enable high mass transfer with minimal shear stress. Experimental findings indicate hydrolysis and fermentation could be performed concurrently. Co-fermentation allows the continual removal of sugar substrates as they are produced by the enzymes, pushing the kinetic equilibrium forward to prevent product-driven inhibition. Although this technological development is promising, it has not achieved a sufficient technological readiness level to implement at the pilot scale. The techno-economic feasibility of co-fermentation remains to be determined.

5.5 Post Processing Section

5.5.1 Pasteurization

A heat treatment step is necessary to arrest microbe growth in a controlled fashion. Temporary heating activates RNases which degrade nucleic acid into its constituent nucleotides. Reducing the nucleic acid content of the final product to less than 2% w/w is recommended by the WHO for safe human consumption [39]. The literature suggests this can be accomplished by a simple temperature elevation to approximately 68 °C for 30 minutes in a batch or continuous manner [40]. This operation will be modeled as a pasteurization unit in SuperPro, enabled with a reflux to reduce heat losses.

5.5.2 Filtration

The concentration of edible biomass in the product stream will be relatively dilute, in some cases (< 10%). This fraction must be concentrated to be nutritionally useful and efficient to distribute before it can be sold. A rotary vacuum filtration operation was chosen to achieve the target purity (75%) in the final solid product while the filtrate was discarded.

5.6 Key Operational Decisions

Two operations merit further description due to their outsized impact on the plant design. The first operational decision is the choice to use either enzymes or acid for hydrolysis. The second decision concerns the feed method, which limits what microbes are viable for downstream fermentation. Both decisions were analyzed in the context of one another, and a decision matrix was used to guide the final selection.

5.6.1 Hydrolysis Method

The first decision concerns the method of hydrolysis used to break the prepared polymers into sugars. The most environmentally friendly and innovative method (Figure 5) uses enzymes to saccharify either purified or mixed biopolymers. The soluble sugars produced, whether they have five or six carbons, are then separated.

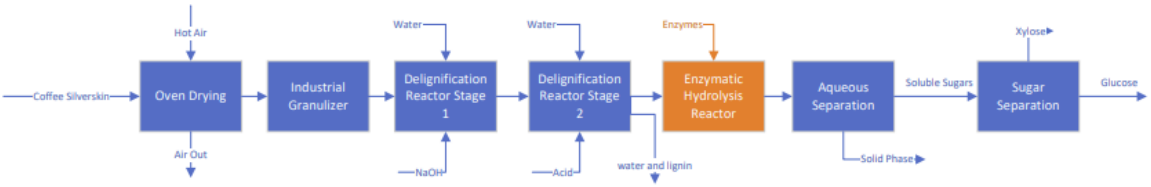

Figure 5 BFD Proposal 1A which highlights the enzymatic hydrolysis of cellulosic biomass.

The alternative, proposal 1B, leverages a cruder yet effective method (Figure 6) to forcibly break glycosidic linkages using a high pressure, high temperature acid solution. The downstream products undergo the same separation as proposal 1A.

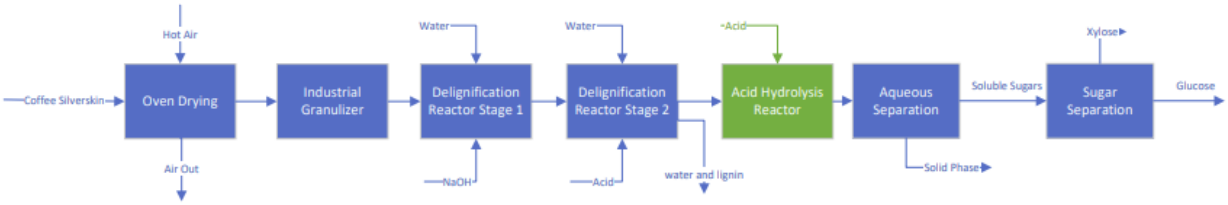

Figure 6 BFD Proposal 1B highlights the alternative use of acid to hydrolyze cellulosic biomass.

The decision to choose enzymatic or acid hydrolysis will depend on conversion efficiencies, capital equipment costs, operational costs, and the presence of undesired byproducts. These tradeoffs are elaborated on in Section 4.

5.6.2 Fermentation Feeds

The second process decision requires consideration of both phase one and two of the process. In proposal 2A/2B, hemicellulose and cellulose are deliberately separated prior to performing hydrolysis. This would result in exclusive hydrolysis of cellulose, yielding only glucose for later fermentation. The alternative plans do not have this separation step.

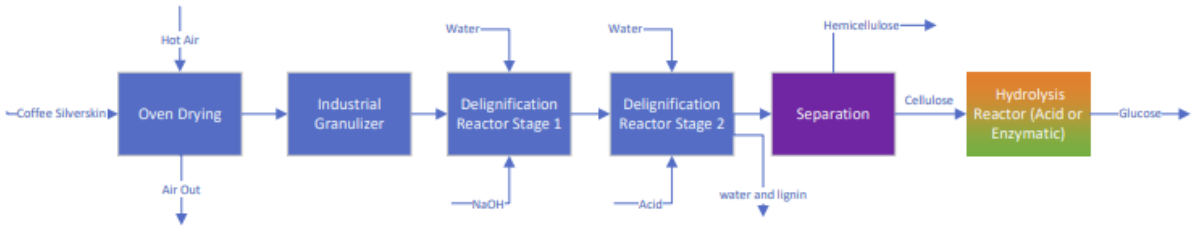

Figure 7 BFD Proposal 2A/2B which highlights the alternative plan to separate hemicellulose before the hydrolysis operation.

Proposal 3A/B performs hydrolysis on a combination of hemicellulose and cellulose, generating both glucose and xylose. The sugars remain mixed for subsequent operations. This proposal is desirable if the microbe strain can grow efficiently on both sugar types, thereby reducing overall waste and feed requirements.

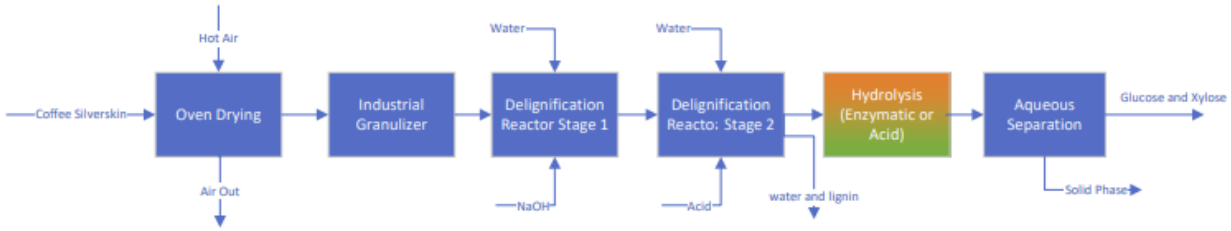

Figure 8 BFD Proposal 3A/3B depicts the default plan to execute hydrolysis without separating hemicellulose before hydrolysis.

The decision to pursue either of these proposals depends on the separation efficiencies that can be achieved between the polymers (hemicellulose and cellulose) compared to their respective monomers (xylose and glucose). Varied efficiency when performing hydrolysis on isolated cellulose will also be considered. Finally, it is worth noting that this design decision can be made independently of the hydrolytic method discussed previously (hence 2*A/B* and 3*A/B).*

5.6.3 Decision Matrix

A preliminary decision matrix was made to summarize the tradeoffs between key design decisions. Values are weighted low to high, from less desirable to desirable. Scores are multiplied by weighing factors and a total score is calculated. This analysis suggests that enzymatic hydrolysis, and a non-separated fermentation feed is the best course of action. Because of the early stage of this decision matrix, the group continued to investigate the feasibility of acid versus enzymatic hydrolysis through proof-of-concept experiments.

Table 1 Preliminary weighted decision matrix which compares design alternatives with respect to hydrolysis and separation method. The enzymatic and non-separated design case is most favored due to the relative advantage in critical categories such as environmental and conversion.

		Acid Hydrolysis		Enzymatic Hydrolysis	
Factor	**Weight**	**Hemi Separated**	**Non Separated**	**Hemi Separated**	**Non Separated**
Speed	6	5	3	3	2
Complexity	4	3	6	6	9
Capital Investment	3	2	2	7	7
Operating Costs	6	7	7	3	3
Conversion (xylose)	7	0	4	0	4
Conversion (glucose)	9	6	4	6	4
Environmental	5	2	2	8	8
Total Score		**154**	**164**	**175**	**191**

6 Experimental Proof of Concept

6.1 Experimental Overview

The team's initial experimental objective was to compare the feasibility of acidic and enzymatic hydrolysis of CSS. This comparison would be used to inform design decisions made for the final design solution. However, the team did not perform experiments on enzymatic hydrolysis due to enzyme shipping delays. The experimentation using acid hydrolysis was still informative for the decisions made about the final design solution.

6.2 Experiment Description

6.2.1 Drying and Grinding CSS

George Howell's roastery in Acton, MA graciously provided coffee silver skin. Approximately 5 lbs arrived in two vacuum sealed containers. Triplicate CSS samples weighing approximately 10 grams were dried in a convention gas-fired oven for two hours at 170 °F until a constant (± 0.01g) mass was reached. Each sample was ground in a Type F203 Krups Coffee Bean Grinder for one minute. Images were obtained using a Ziess Microscope, to observe the effect of the grinding and drying processing steps.

6.2.2 DNS Quantification and Calibration Curve

An analytical technique was needed quantify the sugars produced from acid hydrolysis. HPLC is a commonly used method for this goal, but was cost-prohibitive for this experiment. A spectroscopic assay

using 3,5-Dinitrosalicylic acid (DNS) was found as an alternative to this method [29]. The reaction in this assay is:

$$3,5-Dinitrosalicylic\ acid\ +\ Reducing\ sugar \rightarrow\ 3,-Aminonitrosalicylic\ acid\ +\ Oxidised\ sugar$$ [30]

The team ordered DNS from Sigma-Aldrich. Rochelle Salt, phenol, Sodium bisulfite, and Sodium hydroxide are typically used in combination with DNS for the reducing sugar quantification. The team determined that the critical chemical of these additives was sodium hydroxide. Other chemicals present were for additional stability and accuracy of the measurement. Food grade NaOH provided by the ChemE Capstone Lab was used for solution preparations. Rochelle Salts, phenol, and sodium bisulfite were not used in the assay due to cost restraints. The team prepared a calibration curve using d-glucose and the DNS quantification method. The DNS solution was prepared with 10 mg/mL of DNS and 16 mg/mL of NaOH in DI water. The DNS solution was then combined in a 20:1 ratio with glucose standards created. The solution was heated for 5 minutes at 80C using an air heater. Wavelengths were measured at 500, 520, 540, 560, and 580 wavelengths using a spectrophotometer. Absorbances at 500 are not presented because the instrument reading was overridden at these wavelengths. Values given as n/a in Table 2 overrode the maximum value of 2.5 AU. The values obtained at a wavelength of 580 nm were chosen to create a calibration curve because this was the only wavelength where the instrument did not override its absorbance maximum. The highest r-squared value was also obtained for this wavelength. While an r-squared value of 0.965 is not ideal for a calibration curve, the team was satisfied with this result, given the inability to obtain and use reagents that are typically used for this quantification method. Refer to Appendix A for Calibration Curve Data.

6.2.3 1st Stage of Hydrolysis

The team ordered 98 wt% Sulfuric Acid from Sigma Alrich to be used for the acid hydrolysis portion of this experiment. This solution was diluted down to a 72 wt% solution to be used for the 1st stage of acid hydrolysis. 3 grams of dried and ground CSS were placed into a 50 mL beaker with a Teflon stir bar. An important aspect of scale up economics and feasibility of acid hydrolysis is the ratio of acid that is used in comparison to the amount of biomass [31]. The team chose to vary the ratio of sulfuric acid to biomass, from 6:1, 4:1, and 2:1 in weight percent ratios. These represent solutions A, B, and C. 72% wt acid was added to these 3 conditions in the appropriate volumes. The 1st Hydrolysis stage was performed at 30C for 30 minutes. Due to difficulty mixing the biomass acid slurry for 2:1 biomass loading ratio, an additional 3

mL of 72 wt% acid was added to the mixture. The biomass loading ratio of solution C therefore became approximately 3.33:1.

Figure 9. CSS before 1st Acid Hydrolysis Stage (Left) CSS during 1st Stage of Acid Hydrolysis (Right)

6.2.4 2nd Stage of Acid Hydrolysis

After the first hydrolysis stage, the slurry solutions of acid and biomass were transferred to 200 mL Erlenmeyer Flasks. They were then diluted with DI water with water until the volume reached 150 mL. The resulting Sulfuric acid weight percent of Solutions A, B, and C are 12.5%, 8.3% and 6.9%. The solutions were then heated at 80C for 3 hours.

6.2.5 Filtering and Sample Preparation

The final solutions were cooled down, neutralized, and then filtered through a coffee filter. The resulting solution was then stored in glass vials in the fridge.

6.2.6 Quantification

Quantification of the produced sugars was performed using the DNS method previously outlined. The team experimented with different ratios of sugar sample to DNS solution. The calibration curve was performed using a 1:20 ratio of sugar sample to DNS solution sample, but due to low concentrations of sugars in the sample, it was found that lower dilution rates were beneficial for measuring sugar content. A 1:1 ratio of DNS Solution to filtered, hydrolyzed CSS was used to perform the analysis. Despite qualitatively observing a darker solution for sample A, the absorbance values measured showed that this was the solution with the least sugars, and that Solution C had the highest yields. This conflicts with the hypothesis that higher ratios of acid to biomass loading should cause higher yields of sugar. Due to conflicting data, the team has decided not to draw any conclusions about the effects of different biomass loading ratios.

Table 2 Final results from the experimental proof of concept. The acid hydrolysis method tested was successful in producing measurable sugar concentrations but bucks the expected trend between biomass loading and yield.

	Absorbance	**Sugar concentration, pre-dilution**	**Sugar concentration, final**	**Total sugars produced**	**Estimated total sugars in CSS**	**Sugar yield**
Units	Au	mg/ml	mg/ml	g	g	%
Sample A	0.78	2.33	1.16	0.35	1.20	0.29
Sample B	0.75	2.22	1.11	0.33	1.20	0.28
Sample C	1.31	4.13	2.06	0.62	1.20	0.52

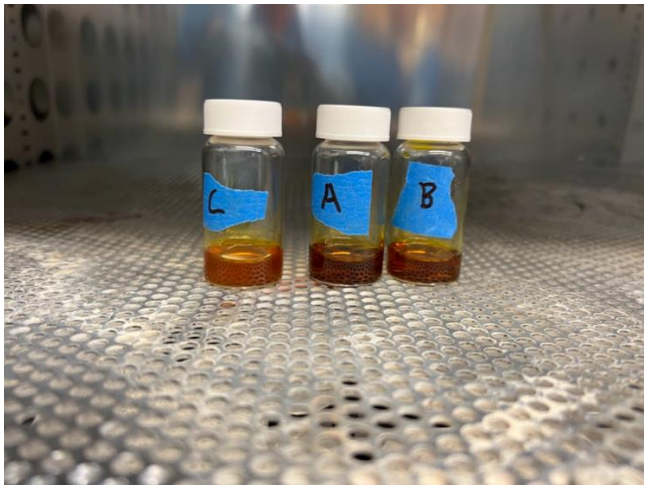

Figure 10 Hydrolyzed analyte after incubation with DNS reagent. In the presence of reducing sugars, a nitro group on the DNS reagent is converted to an amino group, resulting in a darker color. Therefore, the degree of coloration is expected to correlate with increased sugar concentration.

6.3 Results and Discussion

This experiment was successful in producing and measuring sugars from coffee silver skin by using acid hydrolysis. While no conclusions could be drawn from the different acid loading concentrations used, significant yields obtained suggest that coffee silver skin acid hydrolysis is a feasible means of obtaining sugar from the biomass source. However, due to the safety challenges and expenses required for using sulfuric acid at scale, as well as the byproducts expected from this reaction, the team does not believe that this method of hydrolysis is the best path forward for the project. Acid hydrolysis for sugar production may be first used for ethanol production. The increased importance of safety and product consistency for this project leads us to conclude that enzymatic hydrolysis will be the best path forward for mycoprotein production. This conclusion was further supported by encouraging data and economics from simulation proof of concept work performed in SuperPro.

7 Final Design Solution

7.1 Overview of Final Design Solution

A techno-economic analysis of the proposed plant was conducted using the SuperPro V12.3 platform. An initial estimate was conducted using a feedstock throughput of 20,000 lbs per week. However, this was ultimately scaled to 43,546 lbs per week to leverage a better profit margin at greater scales. Careful equipment scheduling resulted in a process batch time of 6.43 weeks, with an hourly processing rate of approximately 280 lbs. The equipment sizing throughout the plant was dynamically calculated to accommodate the materials necessary to process the target CSS volume. The stoichiometric ratios and conversion efficiency which determine the intermediate streams can be found in Appendix B – SuperPro Analysis.

The plant design has several interesting features because of the varied unit operations. For example, the overall batch time of 6.43 weeks is chiefly determined by the 6.00 week cycle time of the airlift fermentation reactor. This operation is modeled as a semi-batch process, where material is fed daily to sustain the microbes which convert the saccharified biomass into edible protein. The remaining 0.43 weeks is almost entirely dedicated to preprocessing enough material to begin fermentation. This protracted, semi-batch operation style results in relatively high vacancy times throughout the simulation, which has implications that are further discussed herein.

The cellulosic hydrolysis method was a major design consideration that was investigated using several methods. A value-weighted decision matrix (Table 1) was performed during the base case design, which indicated that enzymatic hydrolysis is a holistically superior choice. This finding is corroborated by the experimental results of acidic hydrolysis. As a result, the final plant design reflects a decision to use the enzymatic method instead of acidic hydrolysis.

Process yield and safety were the key considerations which drove the decision to operate with enzymatic hydrolysis. Safety concerns regarding the high pressure, temperature, and concentration of acid, which were predicted in the literature, were affirmed experimentally in the proof of concept (see section 0). Additional considerations which are outside this project's scope also support the decision. For example, the furfural side product generated during acid hydrolysis can inhibit fungal growth and microbe productivity. Furthermore, increased maintenance costs from corrosion with reaction vessels corrosion would undermine long-term plant profitability.

Furthermore, it was chosen to avoid a separation step of cellulose and hemicellulose as it would result in higher equipment and operating costs. Additionally, due to the fungal fermentation methods, the desirable microbe strain can grow efficiently on both sugar types and separation is not necessary to achieve a high conversion in the airlift fermenter.

7.2 Process Flow Diagram

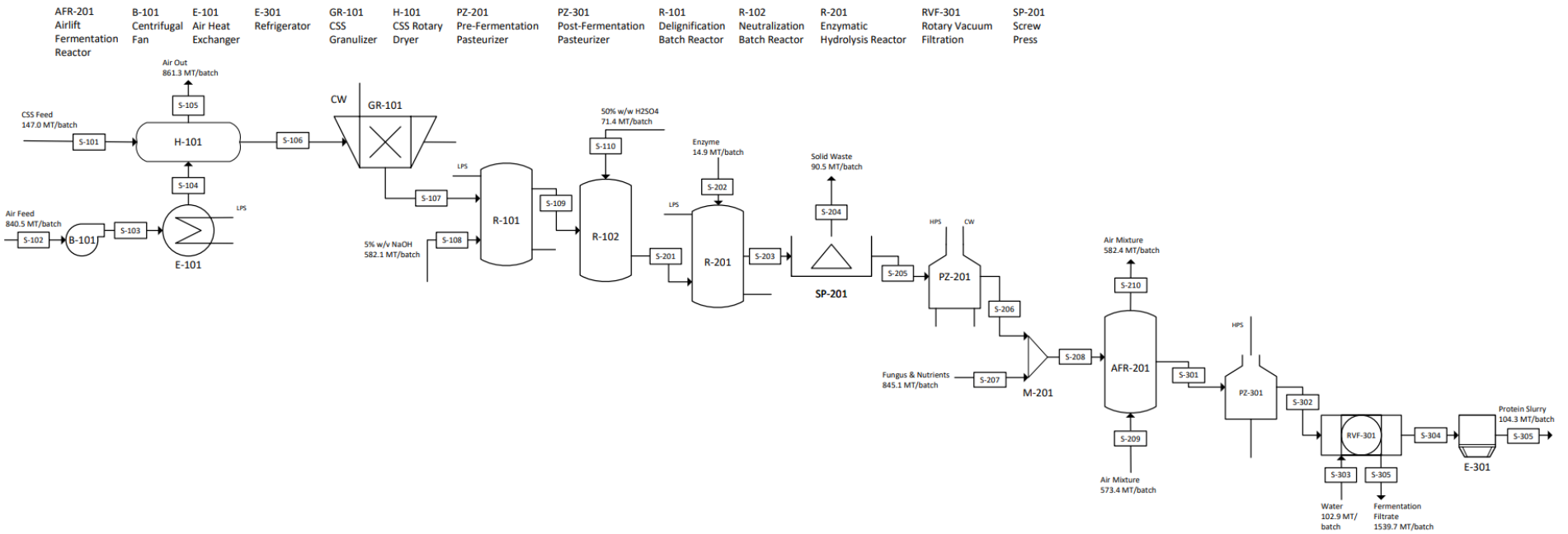

Figure 11. Process Flow Diagram (PFD) of mycoprotein production plant. The stream numbers used to label the PFD differ from both the P&ID and simulation diagrams in order to simplify the overall process. System-wide process inputs and outputs are labeled with mass flowrates and limited composition data

7.3 Material Balances

A system-wide, batch-based material balance was used to better understand the process's material dependencies. The overall mass flowrate through the system was approximately 2,991.1 MT. A mass discrepancy of 0.005% (144.4 kg) affirms material balances were compiled correctly to obey conservation of mass. Certain simplifying assumptions were applied to better understand the data. For example, vapor emissions from non-key reaction vessels were excluded. Additionally, individual components of CSS were consolidated into a single input category ("CSS"), but not at the output. All streams which contributed to the mass balance are classified by their status, purpose, and flowrate in Table 3. A complete list of streams, which includes intermediates, is detailed exhaustively in Section 16.2.

The material balance is dominated by a handful of species: H_2O, O_2, and N_2 constitute ~93% of the total mass flow in and out (Figure 12). The volume of air used does not impact plant economics, but the process is sensitive to the industrial prices of water. This analysis assumes water is priced at \$3.071 m^{-3}, as per the York industrial rate [41]. The plant may stand to benefit significantly from a water reclamation unit which processes the final filtrate.

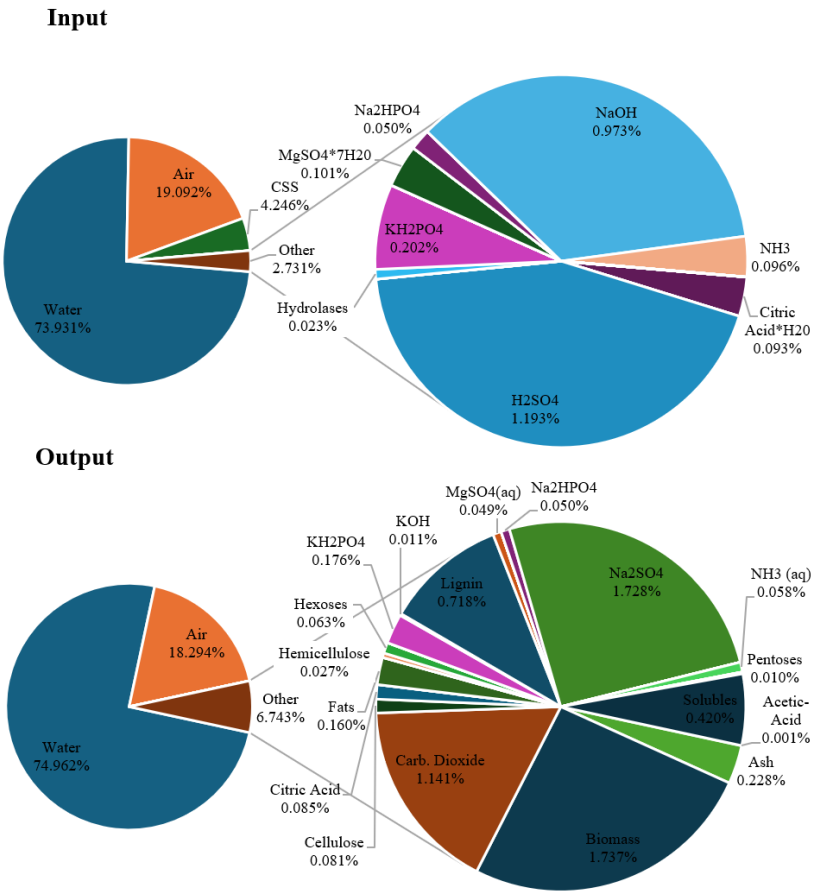

Figure 12 System-wide material balance of the mycoprotein plant modeled in SuperPro. Air and water dominate both as inputs and products from throughout the system which highlight the potential of a water recovery module. An expanded view of the "other" fraction shows the main reagents are acid and base, while the effluent is a mix of neutralized salts, biomass, and carbon dioxide.

The remaining species have been expanded from the "other" section to visualize their relative contributions (Figure 12). The non-water and non-air input fraction is mostly (79%) composed of the acid and base used to manipulate process pH. Consequently, the output shows a large portion of salt produced during neutralization (Na_2SO_4). Sodium sulfate is highly soluble and considered nontoxic. The output is otherwise composed primarily of effluent CO_2 from aerobic digestion and product protein (listed as biomass). It is worth noting the final product, a protein slurry, is being sold as a 3:1 ratio of biomass and water. Therefore, the true mass fraction of final product is somewhat higher than the fraction shown in Figure 12.

Table 3 Mass flowrates of process streams relevant to the material balance shown in Figure 12. All mass values are compared on a metric ton scale for a batch basis.

Stream	Type	Name	Flowrate (MT/batch)
S-101	Inlet	CSS Feed	127.0
S-105	Inlet	NaOH Feed	582.1
S-106	Outlet	Delignification Vent	0.9474
S-108	Inlet	H2SO4 Feed	71.37
S-201	Inlet	Enzyme Feed	6.848
S-204	Outlet	Screw Press Cake	90.12
S-207	Inlet	Sulphate Dilution	32.98
S-208	Inlet	Sulphate Salt Feed	7.549
S-210	Inlet	Phosphate Dilution	28.52
S-211	Inlet	Phosphate Salt Feed	5.807
S-213	Inlet	Fermentation Dilution	778.0
S-218	Inlet	Ammonia Feed	2.867
S-219	Inlet	Air Feed	0.5706
S-223	Outlet	Fermentation Vent	0.5952
S-302	Inlet	Filter Wash	102.9
S-303	Outlet	Protein Stream	104.2
S-304	Outlet	Filtrate Stream	1527

7.4 Equipment Information

Most equipment sizing was calculated dynamically in SuperPro to adjust around CSS input. Expanded information regarding equipment pricing and size is available in the appendix (Section 16.5 - Equipment Costing & Sizing).

7.4.1 Storage Units

A total of five storage tanks will be needed for this process. Three receiver 40,800 L tanks will be used to store the ground CSS, costing $250,000. A storage tank is needed to store the neutralized product before being transferred into the hydrolysis reactor. This tank will have a volume of 60,000 L for a price of $199,000. A blending tank will be used to agitate and store the pasteurized product with additional salts and water before each daily transfer into the airlift fermenter. This tank will be able to hold approximately

40,000 L and cost $267,000. Each of these units are essential for containing intermediate product volumes to reduce batch time and make realistic scheduling estimates. The total cost of the storage units is $1,216,000.

7.4.2 Grinders

A grinder is needed to process the CSS delivery as described in section 5.3.2. One singular grinding unit, labeled GR-101 in SuperPro, with a manually fixed capacity of 2,000 lb/h is planned for this purpose. Design mode was deliberately used for this unit to align material throughput with the subsequent delignification cycle time. Because delignification only requires 2,000 lbs to begin, a relatively small throughput can still enable downstream production to start within 1 hour, while the remaining portion is processed over a protracted period (140 hours). The price of this equipment will total $85,000.

7.4.3 Compressors

A centrifugal compressor is used to compress the air through a sterilization filter, which is then mixed with an ammonia feed stream to sustain fermentation in the airlift reactor. This unit has a capacity of 44.52 kW and a price of $80,000.

7.4.4 Filtration Units

Three distinct separation units are needed throughout the process. A screw press, labeled SP-201, processes the enzymatic hydrolysis product to remove the solid waste and maximize sugar product. This unit costs $676,000 and has a capacity of 28,407 kg/h. Additionally, an air filtration unit is needed to sterilize the air feed before it is and mixed with the ammonia feedstock. This unit can process 480,000 L/h and has a price of $8,000. Finally, to separate the pasteurized protein product from the other byproducts of the airlift fermenter, an 80 m^2 rotary vacuum filter, RVF-301, is used. This unit costs $245,000, bringing the total filter equipment cost to $929,000.

7.4.5 Pasteurizers

Pasteurization is another essential step whose importance is described in section 5.4.2. Pasteurization is performed before fermentation, to protect the microbes, and after, to protect customers. These pasteurizers, PZ-201 and PZ-301, have a capacity of 24,176 L/h and 1,500 L/h, respectively. The total cost of pasteurizing units amounts to $480,000, with PZ-201 making up most of the cost at $455,000 due to the high processing volume.

7.4.6 Reactors

Four reactors are utilized in the process for delignification, neutralization, hydrolysis, and fermentation. The delignification reactor, R-101, carries out four reactions: delignification, hemicellulose hydrolysis, cellulose hydrolysis, and protein degradation (Appendix B). To achieve high conversion of these reactions, the reactor is sized at 11,000 L and will cost $199,000. A neutralizer, R-102, is used to blend 50% H2SO4 with the product of the delignification reactor to neutralize the product. This tank will be able to hold around 31,000 L and cost $163,000 to achieve the desired throughput.

The enzymatic hydrolysis reactor, R-201, carries out three reactions: hemicellulose hydrolysis, cellulose hydrolysis, and enzyme(hydrolases) inactivation (Appendix B). This reactor is sized at 6.31 m^3 and will cost $467,000 to achieve the desired throughput. The airlift fermenter, AFR-201, carries out nine reactions (Appendix B). This reactor is sized at 12,500 L and will cost $105,000 to output the desired amount of product. Overall, the total reactor cost amounts to $934,00 to bring the total equipment cost of this plant to $3,602,000.

8 Unit Control and Instrumentation

The P&ID for the entire process from CSS to protein slurry is divided into 3 parts: pre-processing, Section (Section 18.1), core processes (Section 18.2), and post-processing (Section 18.3). Due to the use of high and low pressure steam (HPS and LPS), the temperature and pressure limits of associated equipment are disclosed in Appendix D, Section 18, to ensure safe operations. Valves and feedback controllers are implemented throughout the three P&ID sections to facilitate safe and efficient operations. Additional information on valve types and limitations are discussed in Section 8.1 and tables 15-21 of Appendix D, Section 18.

8.1 Process Control Considerations

The implementation of process safety and control elements in the P&ID was categorized by pressure, level, flow, concentration, and temperature control schemes. Each element was measured in the same simplified feedback system with a sensor-transmitter, controller, and transducer to affect control valves. Pressure was regulated when HPS or LPS were used to control process temperature. The manipulated valve was placed at the inlet of the steam and operates as a fail-close (FC) valve to prevent steam from overheating units during failure. Additionally, pressure release valves, which would vent to the immediate surroundings of the unit, are in place as a secondary response if the pressure became uncontrollable. Level was used to ensure tanks would not overflow by controlling either the inlet or outlet valves to tanks or reactors. For the inlet case, those valves would be FC while the outlet case would be fail-open (FO) to ensure the tanks do not overflow with material which would be more hazardous than a controlled loss of product. Flow and concentration sensors are utilized for introducing reagents into unit operations. Therefore, all of those valves will be FC to ensure reagents are not being introduced during failure which could result in hazardous conditions. Finally, temperature is controlled in both heating and cooling applications and controls the inlet valves for both cases working fluids. For heating control, the valves are FC to prevent unit overheating. While cooling control is FO as further cooling of a system during failure far outweighs the consequence of wasted cooling water.

9 Safety, Health, and Environmental Considerations

Safety and risk assessments to produce mycoprotein will require attention, because of stringent food safety regulations required during the production process. The safety impacts, operational personnel, and the environment directly affect the health and wellbeing of consumers. Therefore, implementing process safety and control elements into the plant's P&ID plays an integral role in achieving this.

9.1 Process Safety

To ensure safe processing of materials used in the process, Safety Data Sheets (SDS) of all chemical components were analyzed based on relevant safety measures. These are highlighted in the SDS summary (Table 17). All unit operations are run at appropriate temperatures to ensure safety of the product, equipment, and operators. The pipe materials used are selected based on compatibility with chemicals and assessed individually based on their respective SDS, to avoid degradation.

9.2 Product Safety

The final protein slurry needs to have a high degree of consistency to ensure consumer safety and protect Fun Guy Protein from liability. All material entering the reactors is pasteurized to reduce potential microbial contaminants. Another pasteurization step following the airlift reactor is used to activate RNases which degrade the nucleic acid content of the mycoprotein. The final nucleic acid content of the product will be tested using PCR to ensure it is no higher than 2% w/w. The final product will be tested to guarantee pH is between 5 and 8, to preserve taste and culinary flexibility.

9.3 Hazard and Operability Analysis

A hazard and operability analysis (HAZOP) was performed on the most critical unit operations of the process (Table 4). A deviation from the normal operation of each step was identified and characterized. For example, if the delignification reactor exceeds the required temperature a detonation event could occur from gas accumulation. To that end, safeguards like pressure relief valves, feedback control systems, and manual gauges have been implemented. Operators will be equipped with necessary personal protective equipment (PPE) to minimize health implications. In the event of a containment loss, emergency response protocols are required to mitigate potential harm to employees and equipment. To mount an effective emergency response, the facility will require automated systems for real-time monitoring, safety mechanisms to mitigate equipment failure, and a workforce that is trained to respond to deviations.

Table 4 - HAZOP analysis of potential deviation events from standard process operation.

Unit Procedure	Parameter	Guide Word	Cause	Consequence	Safeguards	Actions
Delignification	NaOH Concentation	More	Overfeeding of NaOH to Delgnification Batch Reactor, due to operator error	Damage to equipment due to corrosion, safety risk to personell, potential for hazardous reaction to organic metal	Automated dosing system, alarms for high concentrations, emergency neutrilization process	Implement real-time monitoring of NaOH concentration with automated adjustments, regular training for operators on manual intervention procedures.
Neutrilization	Acid Addition for pH	Less	Insufficient acid dosing, incorrect pH measurement	Incomplete neutralization leading to high pH wastewater, environmental compliance issues	Redundant pH measurement systems, automated feedback control for acid dosing	Conduct regular calibration of pH meters, establish a protocol for manual pH adjustment and re-testing.
Enzymatic Hydrolosis	Enzyme Concentratio	Less	Incorrect dosing, activity loss in enzymes	Inefficient hydrolysis, lower yield of fermentable sugars	Regular activity checks on enzymes, calibration of dosing equipment	Develop a protocol for enzyme activity assessment, adjust process parameters based on enzyme performance.
Fermentation	Microbial Contamination	As well as	Inadequate sterilization, breach in process containment	Contamination of the product, safety risks to consumers, product loss	Sterilization of feedstock and equipment, monitoring of microbial culture health	Enhance sterilization protocols, implement rapid detection methods for microbial contamination, develop contingency plans for contaminated batches.

10 Plant Economics

SuperPro was used to itemize and account for all equipment, utility, and material costs. The raw SuperPro analysis can be found in Appendix E – SuperPro Reports. Based on these investment and operating estimates, a discounted cash flow (DCF) analysis was conducted in excel (Appendix F – Internal Economic Analysis).

10.1 Initial Investment

The total initial investment required is build the proposed plant is projected to be $28,041,000. This includes broad, facility-wide costs such as construction, installation, and instrumentation. An up-front royalty for the use of an engineered microbe was estimated to cost $500,000. This estimate does not incorporate potential startup costs or royalties for using proprietary enzymes. The total facility cost is distributed somewhat evenly across the subcategories, with construction constituting the largest fraction at 20% (Figure 13). Excluding engineering, construction, contractors, and contingency yields a direct cost of $14,758,000. The Direct Fixed Capital Cost (DFC) for this operation sums to $27,155,000.

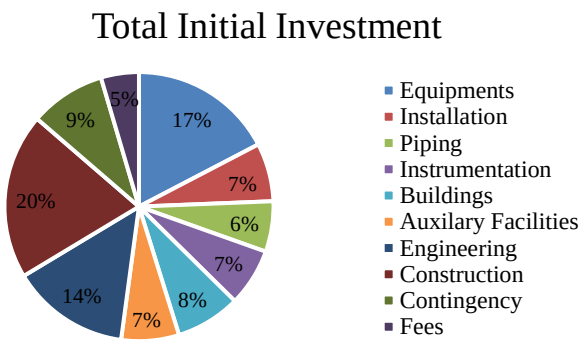

Figure 13 Total investment cost calculated using SuperPro. Each contribution is calculated as a percentage of equipment purchase cost, based on industry data.

10.2 Capital Costs

The project's capital costs represent the actual equipment needed to conduct each unit operation. The individual price of each unit is derived from an internal model of SuperPro industry data. The largest equipment cost is a set of three 41-liter large vessel tanks. These are followed by industrial pasteurizers, and two large scale stirred reactors of which can handle 6,250 liter and 11,019 liters (Figure 14).

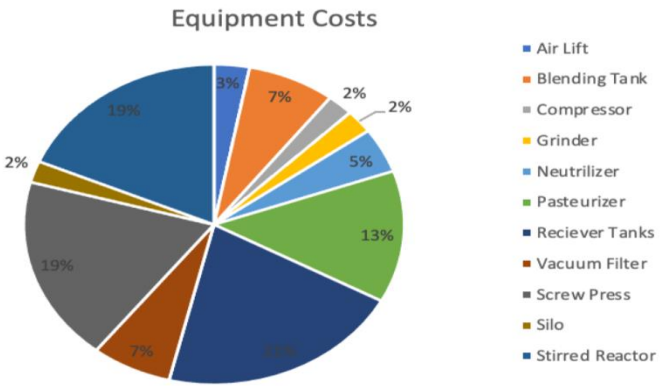

Figure 14 Breakdown of equipment purchase costs by unit operation. Despite being the most critical step of the proposed process, the airlift fermenter, is only 3% of the purchase cost.

10.3 Operating Costs

Operating costs were tabulated according to SuperPro material balance, labor costs, quality control, and utilities. Of the operating costs, the largest contributors include raw materials, labor, and facility dependent costs. The total operating costs summed to $9,283,000 per annum, and $12.72 per unit of product. Calculations are outlined in section 9 & 10.

10.4 Revenue

To provide an accurate forecast of this operations revenue, an in-depth market analysis was conducted. Protein powder retail prices were pooled and averaged $44 per kg of whey protein and $39 per kg. Quorn retail prices on the other hand, were averaged to $9.5 per kg. To stay competitive yet maintain profitability, it was decided to price this operations mycoproteins at $20 per kg. This provides room for companies that package, flavor, and distribute received mycoproteins to make adequate margins. Overall, this makes the product attractive to direct customers, which will allow this product to enter markets adequately. With the market price of $20 per kg of mycoprotein and a total output of 729,733 kg/year, the forecasted Annual Recurring Revenue (ARR) sums to $14,594,660.

10.5 Income Statement

The income statement in Table 4 shows Fun Guy Protein's projected income statement for 2024. All cost and revenue values are calculated by SuperPro. Fun Guy protein is projected to generate around $14,595.000 in revenue and a net income of $3,884,741 in 2024. This income statement assumes that all product that is produced is sold at the price of $20.

Table 5. Fun Guy Protein 2024 Income Statement

Fun Guy Protein		
Income Statement		
4/23/24		
		Current Period
		2024
REVENUES		
Protein Product	$	14,595,000.00
Other Revenue		-
TOTAL REVENUES	**$**	**14,595,000.00**
COST OF GOODS SOLD		
Raw Materials	$	1,237,000.00
Labor	$	2,315,000.00
Facility	$	5,101,111.00
Quality	$	347,000.00
Waste Treatment	$	20,000.00
TOTAL COST OF GOODS SOLD	$	9,020,111.00
GROSS PROFIT	$	5,574,889.00
OPERATING EXPENSES		
Std Power	$	103,045.00
Steam	$	23,692.00
Cooling Water	$	3,496.00
Chilled Water	$	102,203.00
Low Pressure Steam	$	20,889.00
TOTAL OPERATING EXPENSES	**$**	**253,325.00**
	$	-
OPERATING PROFIT (LOSS)	**$**	**5,321,564.00**
	$	-
INTEREST (INCOME), EXPENSE & TAXES	**$**	**5,321,564.00**
Interest Expense		7%
Income Tax Expense	$	1,064,312.80
TOTAL INTEREST (INCOME), EXPENSE & TAXES	**$**	**1,436,822.28**
NET INCOME	**$**	**3,884,741.72**

10.6 Financial Performance

To assess projected financials, gross profit was calculated as the difference between revenue and operating costs. This yields a yearly cash flow of $5,311,160. Fixed Capital is first in into the operation in year one and year two. Working capital, start-up costs and royalties are injected into the operation in year three. A Discounted Cash Flow (DCF) model was utilized to calculate the Net Present Value (NPV) over the course of a 12-year valuation period (Table 19).

The calculated NPV is $7,780,000. The calculated Return on Investment (ROI) is 26.5% with an Internal Rate of Return (IRR) of 10.3%. The forecasted gross margin is 38% and is calculated by the difference between Cost of Goods Sold (COGS) and Revenue. These metrics highlight the promising financial performance, whereby comparing it to industry standards make it an attractive investment.

10.7 Utilities and Energy Efficiency

The utilities needed in this plant are electricity, steam (high and low pressure), and cooling water (cool and chilled). The total consumption of these utilities amounts to $253,324, which only amounts to 2.73% of the total plant operating costs. Electricity and chilled water each constitute approximately 40% of the total (Figure 15). Consequently, the most expensive unit operation to maintain is the air compression unit used

to feed the airlift fermenter. Further process optimizations, such as a water recovery unit, would likely shift the balance of costs away from materials and onto utilities.

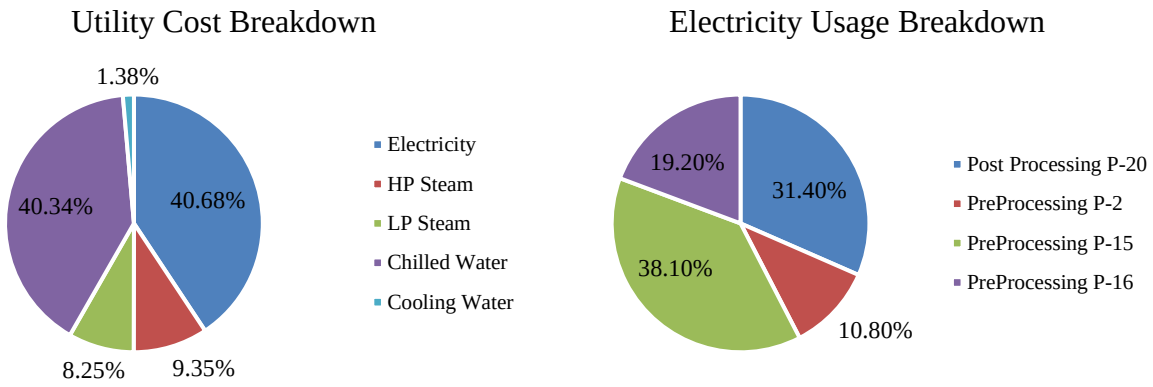

Figure 15 (Left) Cost distribution of all utilities in the plant, which is dominated by cooling water and electricity. (Right) Cost breakdown for electricity usage by equipment which indicates gas compression, P-15, is the most demanding piece of equipment. Operations with less than 0.02% of the total are omitted for clarity.

11 Societal Impact

Increasing popularization of mycoprotein and its potential to take over a portion of the protein market can have a large impact on different facets of a local and global society. From a public health perspective, mycoprotein is regarded as having high protein quality, low saturated fat, and high fiber content [4]. This makes mycoprotein a healthier alternative to many traditional protein sources. When a life-cycle analysis of mycoprotein and beef was performed it was found that transitioning to mycoprotein resulted in a 48% reduction to GGEs. Alleviating the need for natural resources used in beef production will create more availability for wildlife and habitats, and act as a buffer for an expected increase in population sizes.

Culturally, the fermentation of fungi has been implemented for thousands of years and fungi alone has been consumed for longer, however, the production of mycoprotein was first proposed in the mid to late 20th century [6]. Therefore, the relatively recent invention of this protein source could conflict with culturally accepted foods for consumption. However, because mycoprotein is classified and can be identified by consumers as a type of fungus, it can more easily be assimilated into the palates of new consumers. Also, food is an important cornerstone to many social and cultural activities, therefore, due to the vegan-friendly nature of mycoprotein, people with a wide range of dietary restrictions can eat this product together. This makes mycoprotein an inclusive product.

With all the societal benefits of mycoprotein, this process utilizes CSS as the carbon source for mycoprotein production. This delineates the process's product from the industry standard of predominantly using glucose syrups as a feedstock. Using glucose syrup drives the demand for monoculture, thus promoting an inefficient use of arable land and large proponent of GGEs and pollution. Therefore, utilizing CSS relieves the economic burden of waste removal into landfills, in addition to adding value to a waste product.

12 Project Summary

12.1 Context

There is a dire need to produce protein for human consumption without using traditional agriculture. The current meat industry accounts for up to 19.6% of greenhouse gas warming [3]. As the global economy continues to industrialize, this reliance is expected to increasingly stifle objectives related to mitigating the climate crisis. Mycoprotein production already exists at a commercial scale, but it currently relies on industrial glucose feedstocks and operates in a limited market space.

12.2 Core Results

Fun Guy Protein is a business venture which seeks to challenge these standards and capitalize on a growing market. Although this group did not conceive waste valorization, the proposed process is novel in its application to the challenge of mycoprotein. The process was developed by using both experimental and computational proofs of concept. Experimentally, the initial stages of preprocessing and hydrolysis were conducted at benchtop scale. A low-cost analytical assay was applied to quantify the concentration of reducing sugars. Yields ranging from 29-52% demonstrated concept viability, but sample concentration deviated from the expected trend. This data ultimately informed the decision to investigate enzymatic hydrolysis during the scale-up analysis, rather than the acidic method.

The digital process model was designed to encapsulate the CSS valorization process from nearly end-to-end. This includes raw material delivery all the way up to refrigeration and packaging. The flexible model design allowed the investigation of plant profitability at various operational scales. Ultimately, the initial CSS throughput of 455 MT was scaled to 898 MT to make the design viable. This plant will require a capital investment of 28.04 million USD to yield 10.3% IRR and 26.5% ROI. The project succeeded in demonstrating the viability of this technology to create a cost-competitive product.

12.3 Future Work

There are typically many unforeseen barriers to commercialization in process scale-up. The plant proposed in the present report is six orders of magnitude larger than the benchtop demonstrations. It is expected that realizing this plant will require intensive research in smaller plant designs which bridge the gap between the grams and metric tons.

There are several potential improvements which could improve yield and profitability of the overall process. For example, a hydrolytic enzyme cocktail could specifically be optimized for the hydrolysis of coffee silver skin. This cocktail might improve the yields and process time of the enzymatic hydrolysis steps, leading to other downstream benefits. Similarly, the batch-limiting airlift fermentation step in this process could be expanded to include multiple staggered reactors. This would reduce equipment downtime and improve total throughput.

This analysis assumed the strain of *Fusarium venenatum* efficiently metabolizes both five and six carbon sugars simultaneously. This allowed for a higher mycoprotein yield and elimination of a hemicellulose separation step. Strains typically consume one or the other, and organisms which are omnivorous only do

so with partial efficiency and a strong preference. Significant work will need to be done to engineer microbes which can leverage the full biomass content of agricultural waste.

13 Project Management

To ensure a successful project, steps were taken to design the process, analyze the economics of the plant, and optimize the unit operations to ensure maximum profitability. Plans for this process were communicated to the team and responsibilities were delegated. The team followed the production schedule dictated by the Gantt chart in Figure 16.

Team 3 - Capstone Project Schedule

NEU

Project Lead:
Project Start Date: 1/8/2024 (Monday)
Display Week: 1

WBS	Task	Lead	Predecessor	Start	End	Cal. Days	% Done	Work Days
1	**Research**	[Name]						
1.1	Market Analysis pt 1	Shreyas		Mon 1/08/24	Wed 1/10/24	3	100%	3
1.2	Market Analysis pt 2	Firas		Mon 1/08/24	Wed 1/10/24	3	100%	3
1.3	Background pt 1	Ian		Mon 1/08/24	Wed 1/10/24	3	100%	3
1.4	Background pt 2	Thomas		Mon 1/08/24	Wed 1/10/24	3	100%	3
1.5	Background pt 3	Andrew		Mon 1/08/24	Wed 1/10/24	3	100%	3
1.6	Problem Statement	Ian		Mon 1/08/24	Wed 1/10/24	3	100%	3
1.7	Feasability memo	Shreyas		Mon 1/08/24	Wed 1/10/24	3	100%	3
1.8	Feasability presentation	Andrew		Mon 1/08/24	Wed 1/10/24	3	100%	3
1.9	memo 1	Firas		Mon 1/08/24	Mon 1/15/24	7	100%	6
2	**Team Charter**							
2.1	Team Effectiveness Q's	Firas		Mon 1/08/24	Mon 1/15/24	8	100%	6
2.2	Mentor-team effectiveness	Andrew		Mon 1/08/24	Sun 1/14/24	7	100%	5
2.3	gantt chart	Thomas		Mon 1/08/24	Sun 1/14/24	7	100%	5
2.4	team-building	Shreyas		Mon 1/08/24	Sun 1/14/24	7	100%	5
3	**Business Plan + Base Case Design**							
3.1	**Abstract**			Mon 1/15/24	Sun 2/04/24	21	100%	
3.2	**Background**			Mon 1/15/24	Sun 1/21/24	7	100%	
3.2.1	Need of Project	Shreyas/Thomas		Mon 1/15/24	Sun 1/21/24	7	100%	
3.2.2	Key Novel Ideas	Shreyas/Thomas		Mon 1/15/24	Sun 1/21/24	7	100%	
3.2.3	Competitor IP	Shreyas/Thomas		Mon 1/15/24	Sun 1/21/24	7	100%	
3.3	**Business Plan**			Mon 1/15/24	Sun 2/04/24	21	100%	
3.3.1	Vision/Mission Statement	Shreyas/Thomas		Mon 1/15/24	Sun 2/04/24	21	100%	
3.3.2	Market Analysis	Firas		Mon 1/15/24	Sun 2/04/24	21	100%	
3.3.3	Glucose product analysis	Firas		Mon 1/15/24	Sun 2/04/24	21	100%	
3.3.4	projected econ. eval.	Firas		Mon 1/15/24	Sun 2/04/24	21	100%	
3.4	**Engineering Design Process**			Mon 1/15/24	Sun 2/04/24	21	100%	
3.4.1	Multiple Preliminary Designs	Ian/Andrew		Mon 1/15/24	Sun 2/04/24	21	100%	
3.4.1.1	business specs	Ian/Andrew		Mon 1/15/24	Sun 2/04/24	21	100%	
3.4.1.2	throughput specs	Ian/Andrew		Mon 1/15/24	Sun 2/04/24	21	100%	
3.4.1.3	quality specs	Ian/Andrew		Mon 1/15/24	Sun 2/04/24	21	100%	
3.4.2	Identify/Justify final design solution	Ian/Andrew		Mon 1/15/24	Sun 2/04/24	21	50%	
3.5	**Written Communication/Formatting**			Mon 1/15/24	Sun 2/04/24	21	100%	
3.5.1	Formatting			Mon 1/15/24	Sun 2/04/24	21	100%	
3.5.2	Organization, spell-check, etc			Mon 1/15/24	Sun 2/04/24	21	100%	
0.1	*[Insert Rows above this one, then Hide or Delete this row]*							
1	**Detailed Design Report**							
1.1	Experiments	Team		Fri 3/1/24	Wed 4/3/24	32	0%	4
1.2	Simulations/Mass Balances	Ian/Shreyas		Fri 3/1/24	Wed 4/3/24	32	0%	5
1.3	PFD & PID	Andrew/Thomas		Fri 3/1/24	Wed 4/3/24	32	0%	4
1.4	Safety	Firas		Fri 3/1/24	Wed 4/3/24	32		
1	**Detailed Design Poster**							
1.1	Task			Sun 2/04/24	Wed 4/03/24	60	0%	43
2	**Final Report**			Wed 4/03/24	Tue 4/23/24			

Week 8 | Week 9 | Week 10 | Week 11

Figure 16 Gantt chart used to specify individual responsibilities throughout the semester deliverable timeline.

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15 Appendix A - Experimental Protocols

Figure 17 Unprocessed CSS (Left) and Dried and Ground CSS (Right)

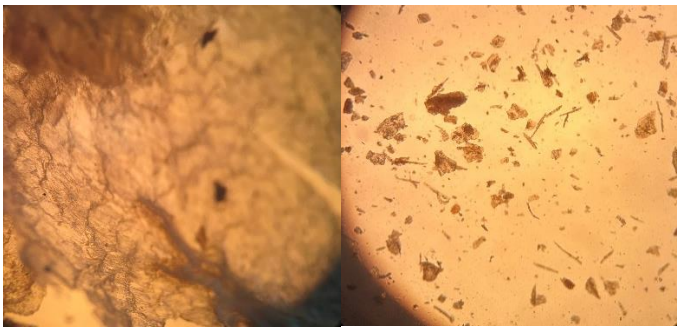


Figure 18 4x Magnified Unprocessed CSS (Left) and Dried and Ground Coffee CSS (Right)

15.1 Sugar Quantification and Assay Calibration

Table 6 - Glucose standards used to create a UV-Vis calibration curve for quantifying hydrolyzed sugars.

Sample (#)	Target Concentration (mg/mL)	Actual Concentration (mg/mL)
1	64	63.92
2	32	31.96
3	16	15.98
4	8	7.99
5	4	3.995
6	1	0.999

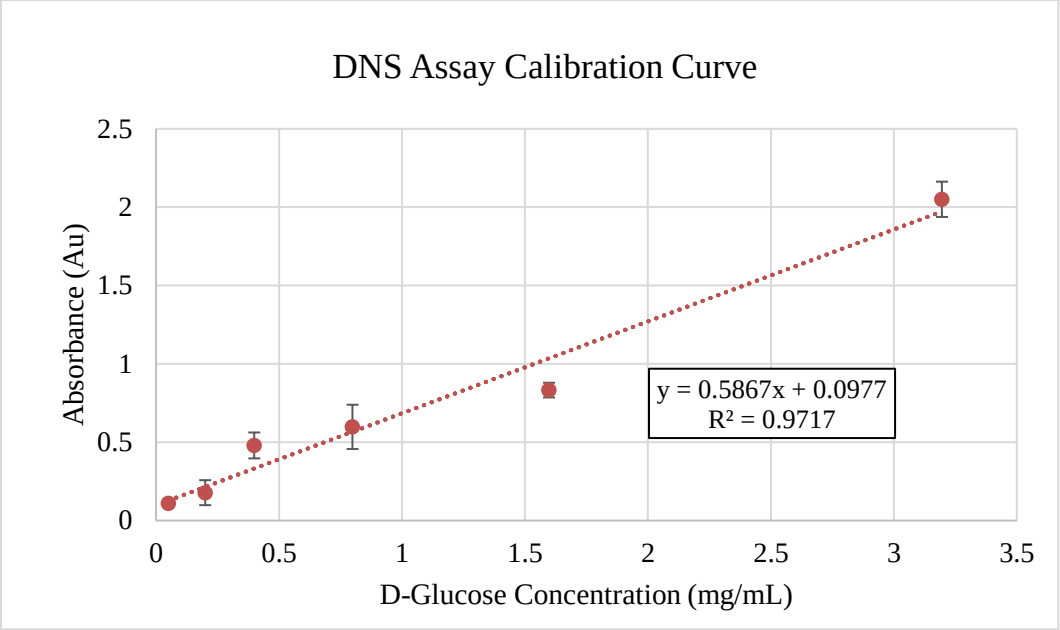


Figure 19: Calibration data generated using solutions of D-Glucose in deionized water. A 10 mg/ml DNS solution was loaded at 1:20 volumetric ratio with the standard, as per literature recommendation **[30]**.

Table 7 Absorbance Values Collected for Samples and D-Glucose Calibration Curve

		Absorbance (Au) at wavelength λ				**Concentration (mg/mL)**
Sample	Replicate	520 nm	540 nm	560 nm	580 nm	-
1	A	-	-	-	1.922	3.196
1	B	-	-	-	2.096	3.196
1	C	-	-	-	2.133	3.196
2	A	-	-	1.624	0.848	1.598
2	B	-	-	1.512	0.780	1.598
2	C	-	-	1.689	0.870	1.598
3	A	-	2.482	1.320	0.698	0.799
3	B	-	2.004	0.989	0.498	0.799
4	B	-	1.593	0.789	0.421	0.400
4	C	-	1.957	0.993	0.538	0.400
5	A	1.473	0.373	0.156	0.086	0.200
5	B	2.200	0.855	0.411	0.227	0.200
5	C	1.633	0.512	0.290	0.221	0.200
6	A	1.557	0.443	0.196	0.110	0.050
6	C	1.510	0.334	0.126	0.072	0.050
7	A	2.333	0.963	0.474	0.270	0.000

Figure 20. 2nd Stage of Acid Hydrolysis

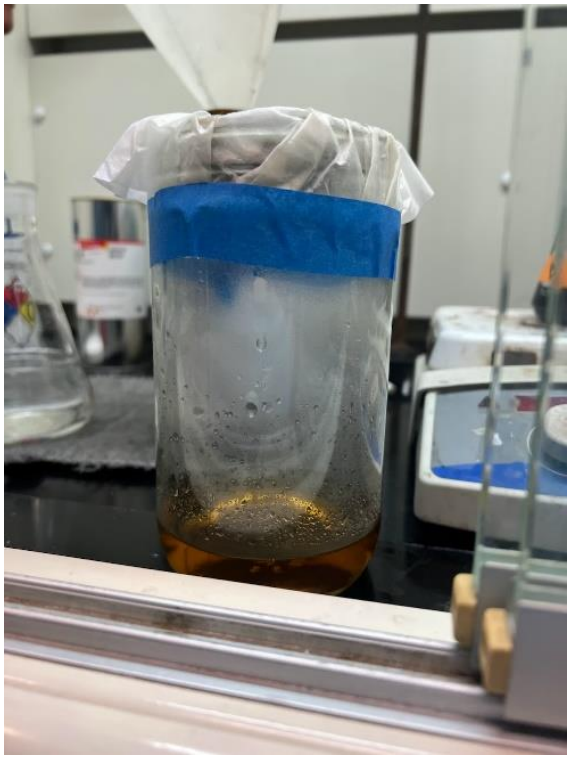


Figure 21. Filtering Prior to Quantification

16 Appendix B – SuperPro Analysis

16.1 SuperPro Simulation Diagram

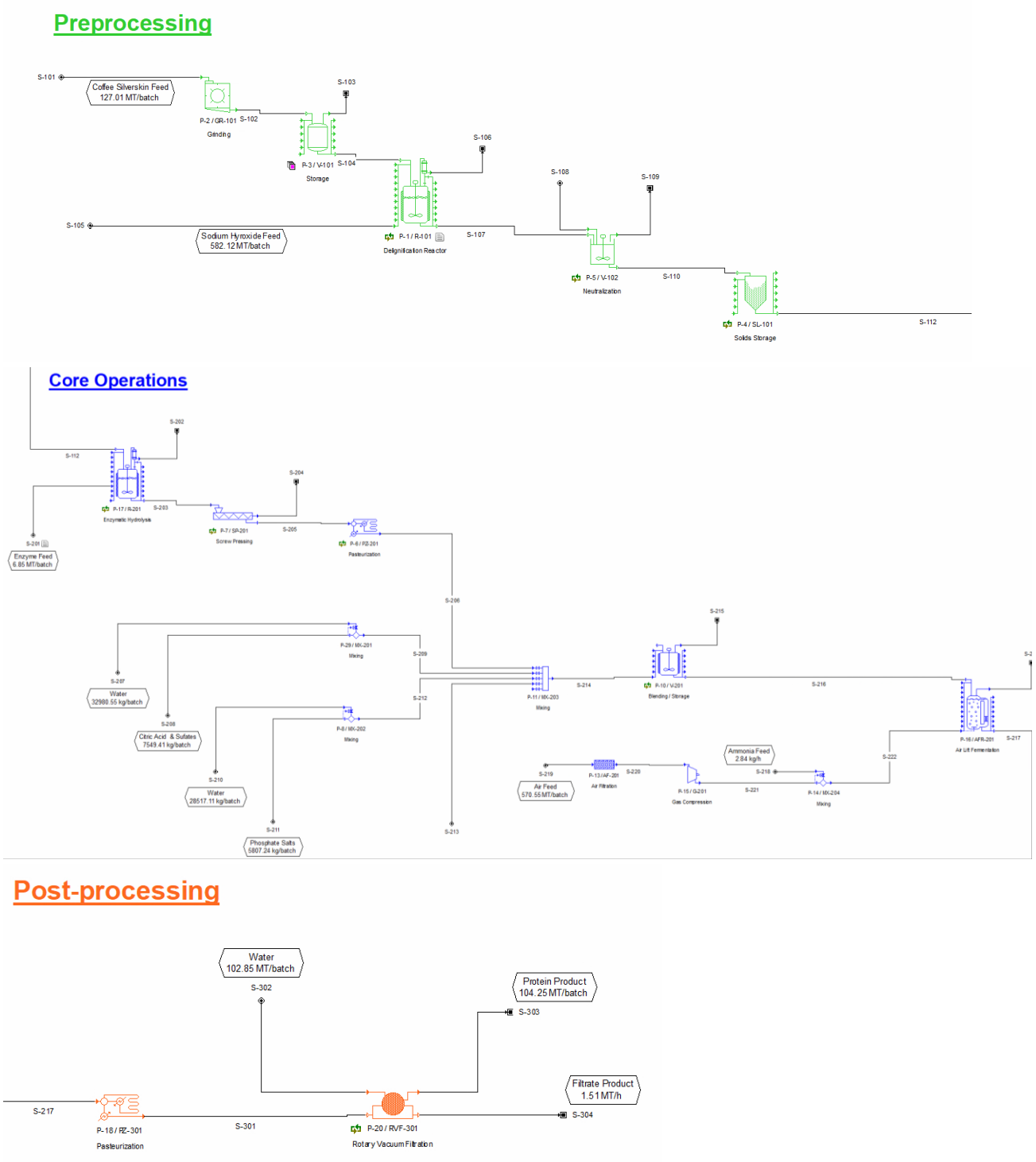


Figure 22 Simulation diagram featuring the preprocessing, core operations, and post processing sections in green, blue, and orange respectively.

16.2 Stream Compositions

Table 8 Stream tables for the preprocessing section, which include species composition and thermophysical state. The stream names correlate to SuperPro simulation diagram in section 16.1, but not necessarily the PFD or P&ID.

Stream Information	**Stream Name**	**Units**	**S-101**	**S-102**	**S-104**	**S-105**	**S-106**	**S-107**	**S-108**	**S-110**	**S-112**
	Source	-	INPUT	P-2	P-3	INPUT	P-1	P-1	INPUT	P-5	P-4
	Destination	-	P-2	P-3	P-1	P-1	OUTPUT	P-5	P-5	P-4	P-17
Stream Properties	Activity	U/ml	-	-	-	-	-	-	-	-	-
	Temperature	°C	25.00	25.00	25.00	25.00	8.00	8.00	25.00	75.92	75.90
	Pressure	bar	1.01	1.01	1.01	1.01	2.00	2.00	1.01	1.01	1.01
	Density	g/L	65.00	1,152.76	1,152.76	1.10	1.79	1,021.27	1,395.10	1,042.32	1,042.32
	DS Flow	kg-ds/batch	118,217.0	118,217.0	118,217.0	29,106.00	-	148,307.7	-	119,201.7	119,201.7
	Aqueous Flow	kg-aq/batch	8,788.80	8,788.80	8,788.80	553,014.0	947.44	560,664.4	71,368.17	661,138.6	661,138.6
	DS	%	93.08	93.08	93.08	5.00	-	20.92	-	15.28	15.28
	Total Enthalpy	kW-h	1,124.84	1,124.84	1,124.84	16,578.67	131.07	57,060.32	1,392.56	58,626.42	58,612.36
	Specific	kcal/kg	7.62	7.62	7.62	24.50	119.03	69.25	16.79	64.64	64.63
	Heat Capacity	kcal/kg-°C	0.30	0.30	0.30	0.98	0.28	0.87	0.67	0.85	0.85
Component Flowrates	Acetic-Acid	kg/batch	-	-	-	-	0.01	39.22	-	39.22	39.22
	Ash	kg/batch	6,807.51	6,807.51	6,807.51	-	-	6,807.51	-	6,807.51	6,807.51
	Biomass	kg/batch	-	-	-	-	-	41,527.10	-	41,527.10	41,527.10
	Carb. Dioxide	kg/batch	-	-	-	-	-	-	-	-	-
	Cellulose	kg/batch	30,189.29	30,189.29	30,189.29	-	-	24,151.43	-	24,151.43	24,151.43
	Citric Acid	kg/batch	-	-	-	-	-	-	-	-	-
	Citric	kg/batch	-	-	-	-	-	-	-	-	-
	Fats	kg/batch	4,800.82	4,800.82	4,800.82	-	-	4,800.82	-	4,800.82	4,800.82
	H2SO4	kg/batch	-	-	-	-	-	-	35,684.09	-	-
	Hemicellulose	kg/batch	21,184.57	21,184.57	21,184.57	-	-	3,177.69	-	3,177.69	3,177.69
	Hexoses	kg/batch	-	-	-	-	-	7,238.35	-	7,238.35	7,238.35
	Hydrolases	kg/batch	-	-	-	-	-	-	-	-	-
	KH2PO4	kg/batch	-	-	-	-	-	-	-	-	-
	KOH	kg/batch	-	-	-	-	-	-	-	-	-
	Lignin	kg/batch	36,298.27	36,298.27	36,298.27	-	-	21,469.07	-	21,469.07	21,469.07
	MgSO4(aq)	kg/batch	-	-	-	-	-	-	-	-	-
	MgSO4*7H20	kg/batch	-	-	-	-	-	-	-	-	-
	N2	kg/batch	-	-	-	-	608.96	-	-	-	-
	Na2HPO4	kg/batch	-	-	-	-	-	-	-	-	-
	Na2SO4	kg/batch	-	-	-	-	-	-	-	51,680.49	51,680.49
	NaOH	kg/batch	-	-	-	29,106.00	-	29,106.00	-	-	-
	NH3	kg/batch	-	-	-	-	-	-	-	-	-
	NH3 (aq)	kg/batch	-	-	-	-	-	-	-	-	-
	O2	kg/batch	-	-	-	-	184.87	-	-	-	-
	Pentoses	kg/batch	-	-	-	-	-	2,961.92	-	2,961.92	2,961.92
	Proteins	kg/batch	23,737.39	23,737.39	23,737.39	-	-	-	-	-	-
	Solubles	kg/batch	-	-	-	-	-	11,868.70	-	11,868.70	11,868.70
	Water	kg/batch	3,987.98	3,987.98	3,987.98	553,014.0	153.61	555,824.4	35,684.09	604,618.1	604,618.1
	Mass Total	kg/batch	127,005.8	127,005.8	127,005.8	582,120.0	947.44	708,972.2	71,368.17	780,340.4	780,340.4
	Volume Total	L/batch	195,393.6	110,175.3	110,175.3	529,200.0	529,146.9	694,206.6	51,156.31	748,659.1	748,654.2

Table 9 Stream tables for the core operations section, which include species composition and thermophysical state. The stream names correlate to SuperPro simulation diagram in section 16.1, but not necessarily the PFD or P&ID.

Stream Information	**Stream Name**	**Units**	**S-201**	**S-203**	**S-204**	**S-205**	**S-206**	**S-207**	**S-208**	**S-209**	**S-210**	**S-211**
	Source	-	INPUT	P-17	P-7	P-7	P-6	INPUT	INPUT	P-29	INPUT	INPUT
	Destination	-	P-17	P-7	OUTPUT	P-6	P-11	P-29	P-29	P-11	P-8	P-8
Stream Properties	Activity	U/ml	-	-	-	-	-	-	-	-	-	-
	Temperature	°C	25.00	74.84	74.84	74.84	3.00	25.00	25.00	25.00	25.00	25.00
	Pressure	bar	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
	Density	g/L	994.70	1,048.34	1,103.80	1,041.57	1,057.70	994.70	1,644.42	1,073.73	994.70	2,521.60
	DS Flow	kg-ds/batch	-	122,566.5	54,727.36	67,839.15	67,839.15	-	1,509.88	1,509.88	-	-
	Aqueous Flow	kg-aq/batch	6,848.00	664,621.8	35,391.46	629,230.4	629,230.4	32,980.55	6,039.53	39,020.07	28,517.11	5,807.24
	DS	%	-	15.57	60.73	9.73	9.73	-	2.00	3.73	-	-
	Total Enthalpy	kW-h	199.84	59,001.08	4,199.02	54,802.06	21,952.04	962.44	36.38	998.82	832.19	37.33
	Specific	kcal/kg	25.11	64.49	40.09	67.64	27.10	25.11	4.15	21.20	25.11	5.53
	Heat Capacity	kcal/kg-°C	1.00	0.87	0.54	0.91	0.90	1.00	0.17	0.84	1.00	0.22
Component Flowrates	Acetic-Acid	kg/batch	-	39.22	1.96	37.26	37.26	-	-	-	-	-
	Ash	kg/batch	-	6,807.51	6,807.51	-	20,763.55	-	-	-	-	-
	Biomass	kg/batch	-	41,527.10	20,763.55	20,763.55	-	-	-	-	-	-
	Carb. Dioxide	kg/batch	-	-	-	-	-	-	-	-	-	-
	Cellulose	kg/batch	-	2,415.14	2,415.14	-	-	-	-	-	-	-
	Citric Acid	kg/batch	-	-	-	-	-	-	-	-	-	-
	Citric	kg/batch	-	-	-	-	-	-	-	-	-	2,787.47
	Fats	kg/batch	-	4,800.82	2,400.41	2,400.41	2,400.41	-	-	-	-	-
	H2SO4	kg/batch	-	-	-	-	-	-	-	-	-	-
	Hemicellulose	kg/batch	-	794.42	794.42	-	-	-	-	-	-	-
	Hexoses	kg/batch	-	31,786.99	1,589.35	30,197.64	30,197.64	-	-	-	-	-
	Hydrolases	kg/batch	684.80	-	-	-	-	-	-	-	-	-
	KH2PO4	kg/batch	-	-	-	-	-	-	6,039.53	6,039.53	-	-
	KOH	kg/batch	-	-	-	-	-	-	-	-	-	-
	Lignin	kg/batch	-	21,469.07	21,469.07	-	-	-	-	-	-	-
	MgSO4(aq)	kg/batch	-	-	-	-	-	-	-	-	-	-
	MgSO4*7H20	kg/batch	-	-	-	-	-	-	-	-	-	3,019.76
	N2	kg/batch	-	-	-	-	-	-	-	-	-	-
	Na2HPO4	kg/batch	-	-	-	-	-	-	1,509.88	1,509.88	-	-
	Na2SO4	kg/batch	-	51,680.49	2,584.02	49,096.46	49,096.46	-	-	-	-	-
	NaOH	kg/batch	-	-	-	-	-	-	-	-	-	-
	NH3	kg/batch	-	-	-	-	-	-	-	-	-	-
	NH3 (aq)	kg/batch	-	-	-	-	-	-	-	-	-	-
	O2	kg/batch	-	-	-	-	-	-	-	-	-	-
	Pentoses	kg/batch	-	5,212.78	260.64	4,952.14	4,952.14	-	-	-	-	-
	Proteins	kg/batch	-	-	-	-	-	-	-	-	-	-
	Solubles	kg/batch	-	12,553.50	627.67	11,925.82	11,925.82	-	-	-	-	-
	Water	kg/batch	6,163.20	608,101.3	30,405.07	577,696.2	577,696.2	32,980.55	-	32,980.55	28,517.11	-
	Mass Total	kg/batch	6,848.00	787,188.4	90,118.82	697,069.5	697,069.5	32,980.55	7,549.41	40,529.96	28,517.11	5,807.24
	Volume Total	L/batch	6,884.46	750,889.9	81,643.85	669,246.1	659,042.1	33,156.13	4,590.92	37,747.05	28,668.94	2,303.00

Table 10 Additional stream tables for the core operations section, which include species composition and thermophysical state. The stream names correlate to SuperPro simulation diagram in section 16.1, but not necessarily the PFD or P&ID.

Stream Information	**Stream Name**	**Units**	**S-212**	**S-213**	**S-214**	**S-216**	**S-217**	**S-218**	**S-219**	**S-220**	**S-221**	**S-222**	**S-223**
	Source	-	P-8	INPUT	P-11	P-10	P-16	INPUT	INPUT	P-13	P-15	P-14	P-16
	Destination	-	P-11	P-11	P-10	P-16	P-18	P-14	P-13	P-15	P-14	P-16	OUTPUT
Stream Properties	Activity	U/ml	-	-	-	-	-	-	-	-	-	-	-
	Temperature	°C	25.00	25.00	27.14	27.14	3.00	25.00	25.00	25.00	4.00	39.85	3.00
	Pressure	bar	1.01	1.01	1.01	10.21	1.01	1.01	1.01	1.01	6.01	1.01	1.01
	Density	g/L	1,108.24	994.70	1,026.49	1,026.49	454.35	0.70	1.18	1.18	6.66	1.12	1.16
	DS Flow	kg-ds/batch	-	-	69,349.03	69,349.03	47,408.20	-	-	-	-	-	31,726.11
	Aqueous Flow	kg-aq/batch	34,324.35	777,953.3	1,480,528.	1,480,528.	1,480,688.	2,867.09	570,551.2	570,551.2	570,551.2	573,418.3	563,472.5
	DS	%	-	-	4.47	4.47	3.10	-	-	-	-	-	5.33
	Total Enthalpy	kW-h	869.52	22,702.33	46,522.71	46,521.52	50,946.93	41.19	4,011.54	4,011.54	6,420.01	6,461.20	15,967.83
	Specific	kcal/kg	21.80	25.11	25.83	25.83	28.69	12.36	6.05	6.05	9.68	9.70	23.08
	Heat Capacity	kcal/kg-°C	0.87	1.00	0.95	0.95	0.95	0.50	0.24	0.24	0.24	0.24	0.25
Component Flowrates	Acetic-Acid	kg/batch	-	-	37.26	37.26	37.01	-	-	-	-	-	0.26
	Ash	kg/batch	-	-	20,763.55	-	-	-	-	-	-	-	-
	Biomass	kg/batch	-	-	-	20,763.55	31,203.03	-	-	-	-	-	-
	Carb. Dioxide	kg/batch	-	-	-	-	2,417.97	-	-	-	-	-	31,726.11
	Cellulose	kg/batch	-	-	-	-	-	-	-	-	-	-	-
	Citric Acid	kg/batch	-	-	-	2,787.47	2,548.51	-	-	-	-	-	-
	Citric	kg/batch	2,787.47	-	2,787.47	-	-	-	-	-	-	-	-
	Fats	kg/batch	-	-	2,400.41	2,400.41	2,400.41	-	-	-	-	-	-
	H2SO4	kg/batch	-	-	-	-	-	-	-	-	-	-	-
	Hemicellulose	kg/batch	-	-	-	-	-	-	-	-	-	-	-
	Hexoses	kg/batch	-	-	30,197.64	30,197.64	301.98	-	-	-	-	-	-
	Hydrolases	kg/batch	-	-	-	6,039.53	-	-	-	-	-	-	-
	KH2PO4	kg/batch	-	-	6,039.53	-	5,251.89	-	-	-	-	-	-
	KOH	kg/batch	-	-	-	-	324.75	-	-	-	-	-	-
	Lignin	kg/batch	-	-	-	-	-	-	-	-	-	-	-
	MgSO4(aq)	kg/batch	-	-	-	-	1,474.44	-	-	-	-	-	-
	MgSO4*7H20	kg/batch	3,019.76	-	3,019.76	3,019.76	-	-	-	-	-	-	-
	N2	kg/batch	-	-	-	-	-	-	437,680.0	437,680.0	437,680.0	437,680.0	437,680.0
	Na2HPO4	kg/batch	-	-	1,509.88	1,509.88	1,509.88	-	-	-	-	-	-
	Na2SO4	kg/batch	-	-	49,096.46	49,096.46	49,096.46	-	-	-	-	-	-
	NaOH	kg/batch	-	-	-	-	-	-	-	-	-	-	-
	NH3	kg/batch	-	-	-	-	-	2,867.09	-	-	-	2,867.09	-
	NH3 (aq)	kg/batch	-	-	-	-	342.02	-	-	-	-	-	1,397.61
	O2	kg/batch	-	-	-	-	-	-	132,871.1	132,871.1	132,871.1	132,871.1	108,787.3
	Pentoses	kg/batch	-	-	4,952.14	4,952.14	49.52	-	-	-	-	-	-
	Proteins	kg/batch	-	-	-	-	-	-	-	-	-	-	-
	Solubles	kg/batch	-	-	11,925.82	11,925.82	11,925.82	-	-	-	-	-	-
	Water	kg/batch	28,517.11	777,953.3	1,417,147.	1,417,147.	1,419,213.	-	-	-	-	-	15,607.38
	Mass Total	kg/batch	34,324.35	777,953.3	1,549,877.	1,549,877.	1,528,096.	2,867.09	570,551.2	570,551.2	570,551.2	573,418.3	595,198.7
	Volume Total	L/batch	30,971.94	782,095.0	1,509,880.	1,509,879.	3,363,296.	4,118,850.	483,837,1	483,837,1	85,629,65	512,251,6	514,753,6

Table 11 Stream tables for the post processing section, which include species composition and thermophysical state. The stream names correlate to SuperPro simulation diagram in section 16.1, but not necessarily the PFD or P&ID.

Stream Information	**Stream Name**	**Units**	**S-301**	**S-302**	**S-303**	**S-304**
	Source	-	P-18	INPUT	P-20	P-20
	Destination	-	P-20	P-20	OUTPUT	OUTPUT
Stream Properties	Activity	U/ml	-	-	-	-
	Temperature	°C	3.00	25.00	25.54	29.91
	Pressure	bar	1.01	1.01	1.01	1.01
	Density	g/L	454.35	994.70	1,008.06	454.00
	DS Flow	kg-ds/batch	47,408.20	-	26,522.58	20,885.62
	Aqueous Flow	kg-aq/batch	1,480,688.	102,850.8	77,724.99	1,505,814.
	DS	%	3.10	-	25.44	1.37
	Total Enthalpy	kW-h	50,946.93	3,001.41	2,599.71	51,348.63
	Specific	kcal/kg	28.69	25.11	21.46	28.94
	Heat Capacity	kcal/kg-°C	0.95	1.00	0.84	0.96
Component Flowrates	Acetic-Acid	kg/batch	37.01	-	-	37.01
	Ash	kg/batch	-	-	-	-
	Biomass	kg/batch	31,203.03	-	26,522.58	4,680.46
	Carb. Dioxide	kg/batch	2,417.97	-	-	2,417.97
	Cellulose	kg/batch	-	-	-	-
	Citric Acid	kg/batch	2,548.51	-	-	2,548.51
	Citric	kg/batch	-	-	-	-
	Fats	kg/batch	2,400.41	-	-	2,400.41
	H2SO4	kg/batch	-	-	-	-
	Hemicellulose	kg/batch	-	-	-	-
	Hexoses	kg/batch	301.98	-	-	301.98
	Hydrolases	kg/batch	-	-	-	-
	KH2PO4	kg/batch	5,251.89	-	-	5,251.89
	KOH	kg/batch	324.75	-	-	324.75
	Lignin	kg/batch	-	-	-	-
	MgSO4(aq)	kg/batch	1,474.44	-	-	1,474.44
	MgSO4*7H20	kg/batch	-	-	-	-
	N2	kg/batch	-	-	-	-
	Na2HPO4	kg/batch	1,509.88	-	-	1,509.88
	Na2SO4	kg/batch	49,096.46	-	-	49,096.46
	NaOH	kg/batch	-	-	-	-
	NH3	kg/batch	-	-	-	-
	NH3 (aq)	kg/batch	342.02	-	-	342.02
	O2	kg/batch	-	-	-	-
	Pentoses	kg/batch	49.52	-	-	49.52
	Proteins	kg/batch	-	-	-	-
	Solubles	kg/batch	11,925.82	-	-	11,925.82
	Water	kg/batch	1,419,213.	102,850.8	77,724.99	1,444,339.
	Mass Total	kg/batch	1,528,096.	102,850.8	104,247.5	1,526,700.
	Volume Total	L/batch	3,363,296.	103,398.3	103,413.9	3,362,770.

16.3 Reaction Information

Table 12 - Delignification Reactions

Reaction Name	Stoichiometric Equation	Basis
Delignification	180 Hemicellulose + 180 Lignin → 360 Biomass	Mass
Hemicellulose Hydrolysis	162 Hemicellulose + 18 Water → 27 Hexoses + 151 Pentoses	Mass
Cellulose Hydrolysis	162 Cellulose + 18 Water → 180 Hexoses	Mass
Protein Degradation	50 Proteins → 25 Biomass + 25 Solubles	Mass

Table 13 - Enzymatic Hydrolysis Reactions

Reaction Name	Stoichiometric Equation	Basis
Hemicellulose Hydrolysis	162 Hemicellulose + 18 Water → 27 Hexoses + 153 Pentoses	Mass
Cellulose Hydrolysis	162 Cellulose + 18 Water → 180 Hexoses	Mass
Hydrolase Inactivation	100 Hydrolases → 100 Solubles	Mass

Table 14 - Fermentation Reactions

Reaction Name	Stochiometric Equation	Basis
NH3 Solubilization	1 NH3 → 1 NH3(aq)	Molar
Na_2HPO_4 Dissociation	1 Na_2HPO_4 + 2 H_2O → 1 H_3PO_4 + 2NaOH	Molar
KH_2PO_4 Dissociation	1 KH_2PO_4 +1 H_2O → 1 H_3PO_4 + 1 KOH	Molar
Fungal Growth via Hexoses	1.63 H_3PO_4 (l/s) + 3.24 NH_3 (aq)(g) + 69.21 O_2 + 100 Hexoses (l/s) → 30 Biomass(l/s) + 98.12 CO_2(g) + 45.96 H_2O (l/s)	Mass
Fungal Growth via Pentoses	1.63 H_3PO_4 (l/s) + 3.24 NH_3 (aq)(g) + 69.21 O_2 + 100 Pentoses (l/s) → 30 Biomass(l/s) + 98.12 CO_2(g) + 45.96 H_2O (l/s)	Mass
Citric Acid Dissolution	210.14 Citric Acid*H_2O → 192.13 Citric Acid + 18.02 H_2O	Mass
$MgSO_4$ Crystal Dissolution	246.51 $MgSO_4$*7H_2O → 120.36 $MgSO_4$(aq) + 126.15 H_2O	Mass
Reverse Dissociation of Na_2HPO_4	1 H_3PO_4 + 2 NaOH → 1 Na_2HPO_4 + 2 H_2O	Molar
Reverse Dissociation of KH_2PO_4	1 H_3PO_4 + 1 KOH → 1 KH_2PO_4 + 1 H_2O	Molar

16.4 Material Balance

Table 15 System-wide mass balance on of the SuperPro simulation on a batch basis. The total discrepancy between input and output is 286 kg, or 0.01% of the total flowrate ,which can likely be attributed to rounding error.

Component	Input (kg/batch)	Output (kg/batch)
Water	2,211,382	2,242,449
Air	571,059	547,261
CSS	127,006	0
Acetic-Acid	0	39
Ash	0	6,808
Biomass	0	51,967
Carb. Dioxide	0	34,144
Cellulose	0	2,415
Citric Acid	0	2,549
Citric Acid*H20	2,787	0
Fats	0	4,801
H2SO4	35,684	0
Hemicellulose	0	794
Hexoses	0	1,891
Hydrolases	685	0
KH2PO4	6,040	5,252
KOH	0	325
Lignin	0	21,469
MgSO4(aq)	0	1,474
MgSO4*7H20	3,020	0
N2	0	0
Na2HPO4	1,510	1,510
Na2SO4	0	51,680
NaOH	29,106	0
NH3	2,867	0
NH3 (aq)	0	1,740
O2	0	0
Pentoses	0	310
Solubles	0	12,554
Total	**2,991,146**	**2,991,432**

16.5 Equipment Costing & Sizing

Table 16 Equipment Sizing and Pricing Summary

Name	Type	Units	Capacity		Unit Price ($/Unit)	Total Price ($)
AF-201	Air Filter	1	479,997.19	L/h	8,000	8,000
AFR-201	Airlift Fermenter	1	12,482.47	L	105,000	105,000
V-201	Blending Tank	1	39,943.92	L	267,000	267,000
G-201	Centrifugal Compressor	1	44.52	kW	80,000	80,000
GR-101	Grinder	1	2,000.01	lb/h	85,000	85,000
MX-204	Mixer	1	567.74	kg/h	0	0
MX-203	Mixer	1	1,534.53	kg/h	0	0
MX-201	Mixer	1	40.13	kg/h	0	0
MX-202	Mixer	1	33.99	kg/h	0	0
V-102	Neutralizer	1	30,991.37	L	163,000	163,000
PZ-301	Pasteurizer	1	1,485.14	L/h	25,000	25,000
PZ-201	Pasteurizer	1	23,901.65	L/h	454,000	454,000
V-101	Receiver Tank	3	40,805.68	L	250,000	750,000
RVF-301	Rotary Vacuum Filter	1	80.00	m2	245,000	245,000
SP-201	Screw Press	1	28,113.87	kg/h	670,000	670,000
SL-101	Silo	1	59,417.01	L	78,000	78,000
R-101	Stirred Reactor	1	11,019.15	L	199,000	199,000
R-201	Stirred Reactor	1	6.25	m3	467,000	467,000

17 Appendix C – Safety Analysis

Table 17 - SDS summary for all chemicals found in the process. Chemicals that require most attention are Anhydrous Ammonia, Sodium Hydroxide, Sulfuric Acid, Acetic Acid.

Chemical Name	Chemical Formula	Molecular Weight	State at 25°C and 1 Atm	Boiling Point, °C	Melting Point, °C	Flashpoint, °C	LFL(Lower Flammable Limit),%	(UFL) Upper Flammable Limit, %	(AIT) Auto Ignition Temperature, °C	Flammability (Red)	Health Hazard (Blue)	Reactivity (yellow)	Lethal Dose$_{(ALD50)}$ or LC$_{50}$	ΔG (kJ/g)
Acetic Acid	CH3COOH	60.05	Liquid	118	16	40	4	16	427	Can ignite at room temperatures	Skin Burns, Eye damage	Reacts with bases, OA and RA	3330	-389.9
Carbon Dioxide	CO2	44.01	Gas	78.5	NA	NA	NA	NA	NA	Not Flammable	Respiratory Distresss, acidosis in extreme cases	Inert	NA	394.4
Citric Acid	C6H807	192.1	Solid	Decomposes	153	NA	NA	NA	NA	Not Flammable	Skin irritations	Reacts with bases	3000	NA
Sulfuric Acid	H2SO4	98.08	Liquid	337	10	NA	NA	NA	NA	Not Flammable	Corrosive to skin and eyes	Highly reactive with water, bases, metals and organic materials	NA	-744.5
Monopotassium phosphate	KH2PO4	136.1	Solid	Decomposes	252.6	NA	NA	NA	NA	Not Flammable	NA	Stable	4640	NA
Potassium Hydroxide	KOH	56.11	Solid	1320	360	NA	NA	NA	NA	Not Flammable	Corrosive to skin and eyes	Highly reactive with acids	NA	-424.4
Magnesium Sulfate	MgSO4	120.4	Solid	Decomposes	1124	NA	NA	NA	NA	Not Flammable	NA	Non-reactive	4000	-1287
Nitrogen	N2	28.01	Gas	-195.8	-210	NA	NA	NA	NA	Not Flammable	NA	Non-reactive	NA	0
Sodium Sulfate Anhydrous	Na2SO4	142	Solid	Decomposes	884	NA	NA	NA	NA	Not Flammable	NA	Non-reactive	5000	-1387
Disodium Phosphate	Na2HPO4	142	Solid	Decomposes	35	NA	NA	NA	NA	Not Flammable	NA	Reacts with acids	2000	NA
Sodium Hydroxide	NaOH	40	Solid	1388	318	NA	NA	NA	NA	Not Flammable	Highly corrosive to skin and eyes	Highly reactive with acids and certain metals		-379.1
Anhydrious Ammonia	NH3	17.03	Gas	-33.34	-77.7	NA	15	28	651	Flammable under specific conditions	Irritating and corrosive to skin and eyes	Reacts with acids	2000 [ppm/ 4hr]	-16.5

18 Appendix D: P&IDs and Equipment Descriptions

18.1 P&ID Section 1: Pre-Processing Steps

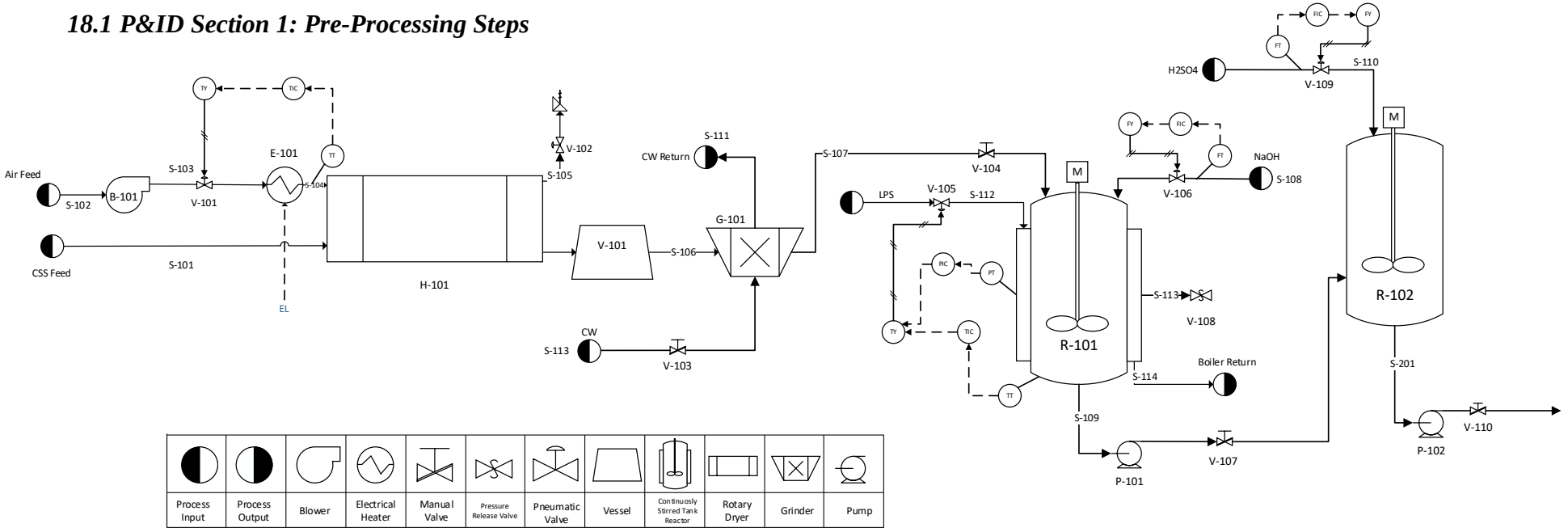


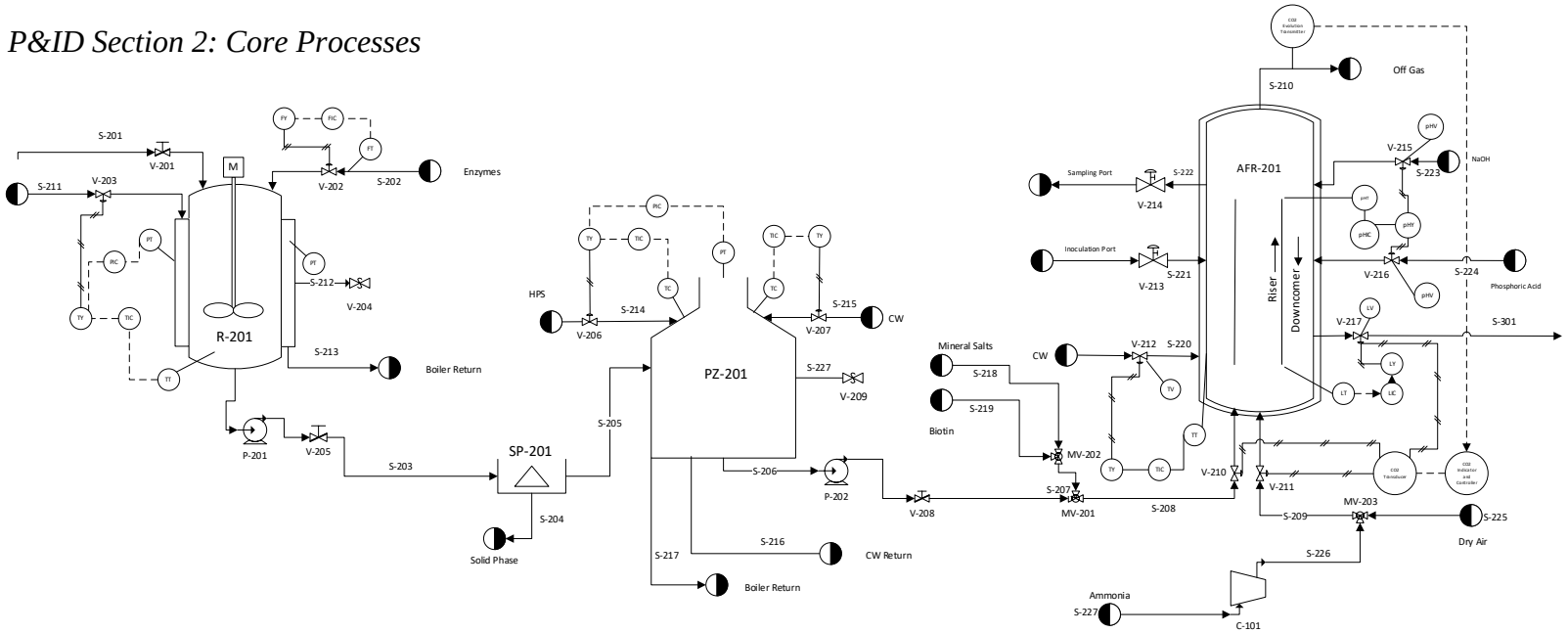
Table 15: Pre-Processing Equipment

Equipment	Limits
B-101	155 cfm, 50 C, 1 atm
E-101	95 C, 1 atm
H-101	95 C, 1 atm
G-101	100 C, 1 atm
R-101	150 C, 35 psig
R-102	100 C, 1 atm
P-101	90 C, 69 psi
P-102	90 C, 69 psi

Table 16: Pre-processing Valves

Valves	Description
V-101	FC, pneumatic diaphragm valve
V-102	FO, diaphragm valve
V-103	Manual valve
V-104	Manual valve
V-105	FC, pneumatic diaphragm valve
V-106	FC, pneumatic diaphragm valve
V-107	Manual valve
V-108	Pressure Release Valve, 27 psig
V-109	FC, pneumatic diaphragm valve
V-110	Manual valve

18.2 *P&ID Section 2: Core Processes*



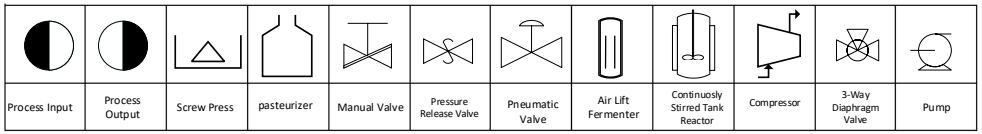


Table 17: **Core Processes Equipment**

Equipment	Limits
R-201	150 C, 35 psig
P-201	90 C, 69 psi
SP-201	N/A
PZ-201	480 psi, 226 C
P-202	90 C, 69 psi
C-101	50 C, 150 psi
AFR-201	80 C, 1 atm

Table 18: Core Processes Valves

Valves	Description
V-201	FC, Diaphragm
V-202	FC, Pneumatic, Diaphragm
V-203	FC, Pneumatic, Diaphragm
V-204	Pressure Release Valve, 27 psig
V-205	Diaphragm Valve
V-206	FC, Pneumatic, Diaphragm
V-207	FC, Pneumatic, Diaphragm
V-208	Manual
V-209	Pressure Release Valve, 420 psi
V-210	FC, Pneumatic, Diaphragm

Table 19: Core Processes Valves

Valves	Description
V-211	FC, Pneumatic, Diaphragm
V-212	FC, Pneumatic, Diaphragm
V-213	Manual Valve
V-214	Manual Valve
V-215	FC, Pneumatic, Diaphragm
V-216	FC, Pneumatic, Diaphragm
V-217	FC, Pneumatic, Diaphragm
MV-201	Mixing Valve, Manual
MV-202	Mixing Valve, Manual
MV-203	Mixing Valve, Manual

18.3 *P&ID Section 3: Post-Processing*

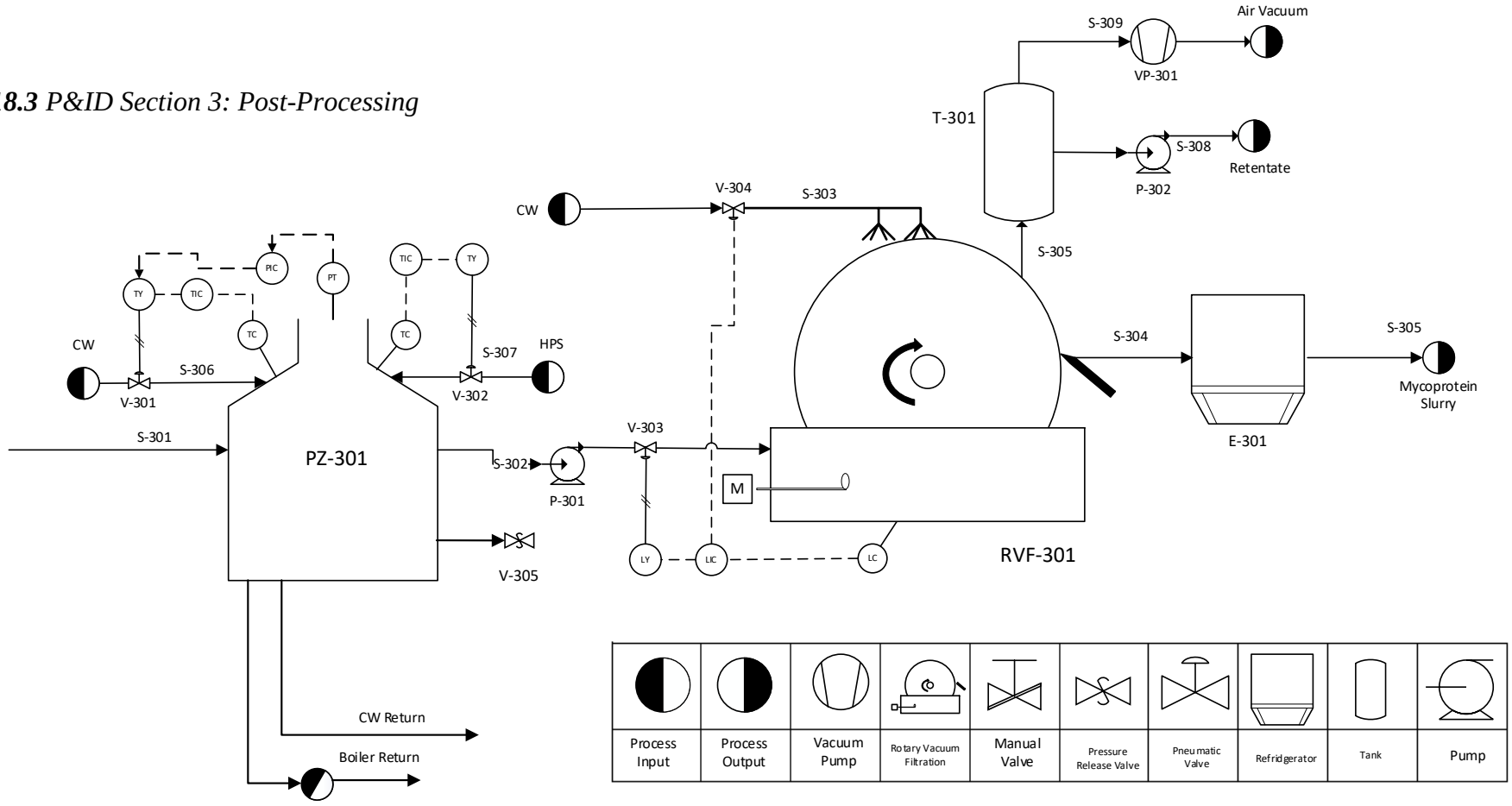


Table 20: Post Processing Equipment

Equipment	Limits
PZ-301	480 psi, 226 C
P-301	90 C, 69 psi
P-302	90 C, 69 psi
VP-302	30 C, 685 mmHg
RVF-301	30 C, 1 atm
E-301	5 C, 1 atm

Table 21: Post Processing Valves

	Description
V-301	FC, Pneumatic, Diaphragm
V-302	FC, Pneumatic, Diaphragm
V-303	FC, Pneumatic, Diaphragm
V-304	FC, Pneumatic, Diaphragm
V-305	Pressure Release Valve, 420 psi

19 Appendix E – SuperPro Reports

See attached documet "Appendix E" which contains the economic evaluation report, equipment report, itemized cost report, and materials & streams report.

20 Appendix F – Internal Economic Analysis

Table 18 Economic assumption used when calculating the plant outlook using a discounted cash flow analysis.

ROI	26.5%
IRR	10.3%
Profit	$ 5.31
FC	$ 27.15
WC	$ 2.24
Startup	$ 1.86
Royalties	$ 0.50
Tax	$ 0.24
C. of C.	$ 0.07

Table 19 Discounted cash flow (DCF) of the mycoprotein plant. A 5-year MACRS tax schedule is used to maximize the time value of initial profits. Startup cost, royalties, and working capital are invested in year 3.

Year	Capital Investment (MM)	Working Capital (MM)	Income (MM)	Profit (MM)	5-yr MACRS (%)	Dep. Charge (MM)	Taxable Income (MM)	Taxes Due (MM)	After Tax CF (MM)	Present Value (MM)	NPV (MM)
1	-13.58		0.00	-13.58		0.00	-13.58	0.00	-13.58	-13.58	-13.58
2	-13.58		0.00	-13.58		0.00	-13.58	0.00	-13.58	-12.69	-26.26
3		2.24	5.31	7.55	20.00	5.43	2.12	0.00	7.55	6.60	-19.67
4			5.31	5.31	32.00	8.69	-3.38	-0.51	4.80	3.92	-15.75
5			5.31	5.31	19.20	5.21	0.10	0.81	6.12	4.67	-11.07
6			5.31	5.31	11.52	3.13	2.18	-0.02	5.29	3.77	-7.30
7			5.31	5.31	11.52	3.13	2.18	-0.52	4.79	3.19	-4.11
8			5.31	5.31	5.76	1.56	3.75	-0.52	4.79	2.98	-1.13
9			5.31	5.31		0.00	5.31	-0.90	4.41	2.57	1.44
10			5.31	5.31		0.00	5.31	-1.27	4.04	2.196	3.63
11			5.31	5.31		0.00	5.31	-1.27	4.04	2.05	5.68
12		0.38	5.31	5.69		0.00	5.69	-1.27	4.42	2.10	7.78