NTNU
Norwegian University of Science and Technology

Faculty of Natural Sciences and Technology
Department of Chemical Engineering

TKP4171 PROCESS DESIGN PROJECT

Title: Techno-Economic Analysis of Fish Feed Protein Additive made from Fungal SCP using Starch from Potato Processing Waste	**Keyword (3-4):** SCP, TEA, fish feed, potato processing waste
Written by: Group H. Lars Føleide, Aleksander Mittet, Jonas Mæhlum and Andreas Wold	**Work period:** Spring 2023
Supervisor: Per Bruheim	**Number of pages:** 38 **Main report:** 28 **Appendix:** 9

Summary:
This project focuses on designing, modeling, and conducting a techno-economic analysis (TEA) for a process that produces fungal single-cell protein (SCP) from potato processing waste (PPW) as a fish feed protein additive. The proposed starch-based process is compared to a traditional molasses-based process in terms of profitability and design.

The objective is to produce 20,000 metric tons of dry *Fusarium venenatum* biomass. Starch from PPW emerges as the most viable carbon source, with pre-processing steps converting starch into glucose. To meet production goals, six continuous airlift reactors are required, operating for 8000 hours annually. Utilizing the black box model and Monod kinetics, the necessary PPW and minerals for biomass growth are calculated, resulting in 21,645 metric tons of dry biomass. A downstream process is designed to achieve a 90% dry mass concentration, ultimately yielding 24,050 metric tons of product.

Conclusions and recommendations:
The TEA revealed that the starch-based process is more economically viable than the molasses-based process, with an NPV of -$6.6 million compared to -$14.9 million. The starch-based process achieved an ROI of 229% and an IRR of 9.3%. The sensitivity analysis identified electricity, investment cost, and product price as the most influential factors on profitability. Additionally, the PPW process displayed a lower environmental impact than the molasses process due to reduced transportation and utilization of a local waste stream.

Recommendations include emphasizing SCP sustainability, investigating energy-efficient water removal methods, and exploring batch or fed-batch reactor operations to minimize water consumption. The findings indicate that potato waste starch holds potential as a sustainable feedstock for SCP production. Future research should focus on process optimization, developing strategies to increase product price, engaging with industry stakeholders, and conducting comprehensive environmental impact assessments to further promote this sustainable protein additive within the aquaculture industry.

Date and signature: April 25th 2023

NTNU

Kunnskap for en bedre verden

TKP4171 Design of Sustainable Chemical and Biochemical processes

Techno-Economic Analysis of Fish Feed Protein Additive made from Fungal SCP using Starch from Potato Processing Waste

Authors:

Group H

Lars Føleide, Aleksander Mittet, Jonas Mæhlum and Andreas Wold

Trondheim
Sem Sælands vei 10
Spring 2023

Summary

This project presents the design, modeling, and techno-economic analysis (TEA) of a process for producing fungal single-cell protein (SCP) from potato processing waste (PPW), intended for use as a fish feed protein additive. The proposed starch-based process was compared to a traditional molasses-based process in terms of profitability and process design.

The design basis for the process was to produce 20,000 metric tons of dry *Fusarium venenatum* biomass. To make the process more sustainable, the use of different carbon sources was examined, and starch from PPW was found to be the most viable option. To make PPW into a suitable carbon source, a pre-processing step was designed to convert the starch to glucose. To meet the required production goal, it was calculated that 6 continuous airlift reactors operating each at a total of 8000 hours per year were needed. The amount of PPW and minerals needed for biomass growth were calculated using the black box model and Monod kinetics, assuming a biomass yield of 54% and steady-state conditions. This resulted in an SCP production of 21,645 metric tons of dry biomass. A downstream process was designed to remove excess water, and to achieve a dry mass concentration of 90%, making the total weight of the product 24,050 metric tons.

The techno-economic analysis demonstrated that the starch-based process is more economically viable than the molasses-based process, with a Net Present Value (NPV) of -$6.6 million compared to -$14.9 million for the molasses-based process. The Return on Investment (ROI) for the starch-based process was found to be 229%, and the Internal Rate of Return (IRR) was 9.3%. The sensitivity analysis revealed that the most influential factors on profitability were electricity costs and product price. The sensitivity analysis highlighted that the NPV of the PPW plant is sensitive to changes in the product price and investment cost, with electricity being the most sensitive operational cost.

Table of Contents

1 Introduction

1.1 Global Protein Demand and the Role of Norwegian Salmon Industry

The Food and Agriculture Organization estimates that to accommodate the predicted world population of 9.3 billion by 2050, food production needs to increase by 60%[1]. This growing population will require more protein to meet dietary demands. Fish is an excellent protein source, with protein accounting for 18-50% of fish feed's nutritional content[2]. The aquaculture industry must explore novel and sustainable feed protein sources to meet this increasing demand.

Given the current state of fish supply, it is crucial to decrease wild catches, as one-third of marine stocks were overfished in 2015[3]. In order to alleviate pressure on wild catches, the aquaculture industry needs to more than double its production to accommodate a projected 58% increase in fish consumption between 2010 and 2050. Presently, commercial fish feeds are unsustainable due to their reliance on fishmeal and fish oil, both sourced from wild-caught fish. The existing demand for fishmeal and fish oil surpasses supply, resulting in overfishing and the depletion of wild fish stocks[4].

In 2020, the Norwegian salmon industry harvested 1.4 million metric tons of Atlantic salmon, resulting in an export value of NOK 70.1 billion[5]. One challenge the Norwegian salmon industry faces is the reliance on imported ingredients in fish feed. In 2020, 92% of fish feed ingredients were imported[6]. Vegetable protein sources made up 41% of salmon feed that year, with soy protein concentrate accounting for 413,611 metric tons.

1.2 Current Challenges in Aquaculture Feed Production

The use of soy protein as an alternative to fishmeal and fish oil has been attempted, but it has led to negative environmental impacts such as deforestation, greenhouse gas emissions, water pollution, and widespread pesticide use[4]. Furthermore, fish may struggle to digest soy-based feeds, which can cause long-term damage to their digestive systems[4]. In response to these challenges, the Norwegian government has set a goal to source all aquaculture feed from sustainable sources by 2030[6], emphasizing the need for novel and eco-friendly protein sources like Single Cell Protein (SCP).

1.3 Single Cell Protein from *Fusarium venenatum*: A Sustainable Alternative

SCP refers to the microbial biomass produced through the fermentation of carbon sources by microorganisms such as fungi, yeast, bacteria, and algae[7]. In recent years, there has been an increasing demand for high-quality protein in aquaculture, especially in the production of fish feed. SCP can be used as a sustainable and cost-effective alternative source of protein for animal feed, human food, and other industrial applications. Even though SCP generally contains less protein (30-80%)[8] than soy protein used in fish feed (65%)[9], there are several advantages of using SCP. These include lower land and water requirements, reduced greenhouse gas emissions, and the utilization of waste streams as substrates for fermentation[10]. Sustainability can be further improved by localizing an SCP fermentation plant near a source of waste streams. Additionally, SCP is a source of essential amino acids such as methionine, threonine, and lysine[8].

The filamentous fungus *Fusarium venenatum* is recognized as a valuable source for single-cell protein (SCP) production due to its relatively high protein content (44% on a weight basis[11]) and safety for human consumption[12]. Since 1985, *Fusarium venenatum* has been successfully employed in the industrial-scale production of mycoprotein, a food ingredient derived from the fungus' mycelium, under the brand name Quorn[12]. Mycoprotein is used in a variety of meat-free food alternatives, showcasing its versatility and potential as a sustainable protein source.

Nonetheless, the cost of producing mycoprotein for human consumption remains high, limiting

its application in animal feed production [12]. The current Quorn fermentation process relies on highly refined glucose syrup (also called molasses) derived from starch as a carbon source. This creates challenges related to price and supply, as glucose syrup is in high demand for various fermentation processes, leading to increased costs. Additionally, the production logistics depend heavily on timely deliveries of glucose syrup via heated tankers, making the entire fermentation process susceptible to disruptions in the supply chain [12].

This project aims to develop a bioprocess for the production of SCP using *Fusarium venenatum*, specifically for use in fish feed production. The goal is to design a bioprocess plant capable of producing 20,000 metric tons of *Fusarium* mycoprotein annually, with a focus on cost-effectiveness and sustainability. The use of alternative carbon sources such as potato processing waste (starch) and whey will be explored to reduce production costs and minimize waste. By providing a sustainable and cost-effective protein source for aquaculture, this project aims to contribute to the growth and development of the industry in a more environmentally friendly and economically feasible manner.

1.4 Selection of Carbon Source

Carbon sources play a critical role in single-cell protein (SCP) production, as they significantly influence the yield, quality, and composition of the final product. A wide range of carbon sources has been investigated for SCP production using *F. venenatum*, such as glucose, fructose, sucrose, and molasses, which are byproducts of sugar production and contain high levels of fermentable sugars [13]. Molasses, in particular, has been recognized as an ideal source due to their efficiency in SCP production [14]. However, the focus of this study is on identifying more sustainable and cost-effective alternatives.

1.4.1 Potato Processing Waste

Potato processing waste (PPW) is a by-product of the potato processing industry, consisting of peelings, pulp, and starch. PPW has been reported to contain high levels of carbohydrates, making it a potentially attractive source of carbon for SCP production [15]. For example, the solid fraction of potato peel waste consists of 30 - 85% starch, 24 - 65% fiber, and 6.2 - 18.6% protein [15]. Potato pulp contains substantial amounts of fiber (cellulose, hemicellulose, and pectin), contributing to the overall nutritional value of PPW as a substrate for SCP production.

Utilizing PPW for SCP production can offer a sustainable solution for disposing of waste generated by the potato processing industry while simultaneously producing valuable protein sources. Fungi such as *Aspergillus niger* and *Scytalidium acidophilum* have demonstrated the ability to ferment potato starch, converting it into microbial biomass [15;16].

Although *F. venenatum* has not been shown to grow well on PPW-based media, alternative strategies could improve its growth on this substrate. One approach is to add cellulolytic enzymes, such as α-amylase and glucoamylase, to convert the starch into glucose monomers that can be easily assimilated by *F. venenatum*. Another option is to grow it in co-culture with a more effective amylolytic organism, which could help break down the complex starches and improve the overall conversion efficiency of PPW into SCP [17]. These strategies have the potential to enhance the utilization of PPW for SCP production, contributing to a more sustainable and circular bioeconomy.

1.4.2 Whey

Whey is a by-product of cheese and casein production, consisting of 4-5.5% lactose and 0.6-0.65% protein [18]. The most significant advantage of whey is its protein content, providing a nitrogen source for the process. Although there are no direct studies on *F. venenatum* growth on whey, the *Fusarium* species *Fusarium moniliforme* and *Fusarium oxysporum* have been reported to ferment lactose. For the latter, the reported yield on lactose (0.44 gg^{-1}) was lower than glucose (0.48

gg^{-1})[19]. Using whey as a carbon source for SCP production can offer a sustainable solution for disposing of whey generated by the dairy industry[20].

It is assumed that *F. venenatum* possesses some beta-galactosidase activity, so adding additional enzymes might be necessary to achieve sufficient yields for commercial SCP production. The largest cheese factory in Norway, TINE Jæren, generates approximately 28,000 metric tons of whey per year, with a production cost of around 0.5 NOK/liter and a transport cost of 0.5 NOK/liter using tank trucks[21]. The price of whey varies greatly depending on its dry matter content, ranging from 1-2 NOK/liter.

Norway produces approximately 900,000 tons of whey per year, as described in the email correspondence with Tine in Appendix E. Whey is an important by-product of the dairy industry due to its nutritional value. However, whey is not utilized for further lactose production, so its disposal poses a significant economic and environmental challenge[22]. One potential solution is to locate a fermentation plant beside a cheese factory to utilize the whey produced.

1.4.3 Other Carbon Sources

Lignocellulose represents a potential carbon source for fermentation, as it is an abundant source of biopolymer that could be utilized for fermentation. However, there are significant challenges associated with the process of converting lignocellulose into fermentable sugars. This process is complex and energy-intensive, making it expensive. Additionally, the yield of fermentable sugars from lignocellulose is lower than that from starch[23].

Additional carbon sources, such as fruit and vegetable waste, brewer's spent grain, and pea-processing waste, were investigated, but they were found to be insufficient in quantity to serve as a feasible carbon source for meeting the production goal of 20,000 metric tons of SCP.

1.5 Chosen Carbon Source: Potato Processing Waste

Selecting a suitable carbon source from waste streams for microbial growth requires the consideration of various factors, such as nontoxicity, abundance, renewability, non-exotic nature, and affordability[14]. Additionally, cost-efficiency, availability, and limited pre-processing requirements are important aspects. The ideal waste material should be a low-value resource that might otherwise be underutilized or discarded, while also promoting rapid growth and high-quality biomass production in microorganisms[24].

Considering these factors, we have selected potato processing waste (PPW) as our carbon source for several compelling reasons. Firstly, utilizing PPW for SCP production offers a sustainable solution to addressing the environmental challenges related to waste disposal in the potato processing industry[15]. While whey is already employed in the production of products such as brown cheese and whey powder in Norway[25], PPW currently has no established uses and is typically discarded. Moreover, incorporating waste materials into SCP production can potentially lower overall production costs, as waste-derived carbon sources are generally more cost-effective than alternatives like whey[26;27]. Since whey is a byproduct, it would be more expensive than the waste product PPW. Additionally, the low lactose content of whey would make it necessary to buy more raw materials in order to meet the carbon source requirements. Consequently, by focusing on PPW as a carbon source, we not only address sustainability concerns but also emphasize its greater economic viability through a Techno-Economic Analysis (TEA).

1.6 Project Objectives and Scope

The primary objective of this project is to design a bioprocess plant capable of producing 20,000 metric tons of *F. venenatum* mycoprotein annually, using an alternative carbon source and comparing the plant to a traditional molasses-based plant. The plant will utilize potato processing waste as the main carbon source for SCP production.

In order to assess the economic feasibility of the proposed bioprocess plant and estimate the time required to recoup the initial investment, a Techno-Economic Analysis (TEA) will be conducted. The TEA will consider key financial metrics such as Net Present Value (NPV), Return on Investment (ROI), Internal Rate of Return (IRR), and payback period. These metrics will provide a thorough assessment of the plant's economic performance, allowing for informed decision-making regarding the project's viability and potential profitability.

By providing a sustainable and cost-effective protein source for aquaculture, this project aims to contribute to the growth and development of the industry in a more environmentally friendly and economically feasible manner.

1.7 Potential Impact on the Aquaculture Industry

The successful development of a bioprocess plant using *Fusarium venenatum* to produce SCP from potato processing waste and whey could have far-reaching impacts on the aquaculture industry. By providing a sustainable, cost-effective, and high-quality protein source, this project could contribute to reducing the reliance on fishmeal and soy protein, thereby mitigating overfishing, deforestation, and other negative environmental impacts associated with current feed production methods. This would align with the Norwegian government's sustainability goals and support the global demand for environmentally responsible protein sources.

Furthermore, the localization of an SCP production plant near sources of potato processing waste and whey could lead to a more circular and localized bioeconomy, reducing transportation costs and greenhouse gas emissions associated with importing raw materials for fish feed production. This could also create new opportunities for collaboration between the potato processing, dairy, and aquaculture industries, fostering innovation and further advancements in sustainable aquaculture feed production.

2 Design Basis

2.1 Feedstock and Enzymatic Hydrolysis

The design basis for the process plant focuses on the production of mycoprotein for SCP fish feed, utilizing starch-rich PPW as the primary carbon source for fermentation. It is assumed that the PPW only contains starch and water. The raw material comprises a 90% starch slurry [28], which will be processed into glucose through enzymatic hydrolysis. In addition to the carbon source, the fermentation broth necessitates a nitrogen source, essential minerals such as potassium, sodium, and magnesium, an oxygen supply for the fermentation process, and appropriate acids and bases for starch pre-processing. Ammonium sulfate was chosen for this process as it serves as a common nitrogen source for SCP production, offering a highly accessible form of nitrogen for microorganisms that can be easily assimilated for biomass production [29]. In comparison to other nitrogen sources like urea or ammonium nitrate, ammonium sulfate has demonstrated superior results in terms of biomass yields and protein content in various SCP production processes [29].

The enzymatic hydrolysis of starch was preferred over acid hydrolysis due to its ability to produce higher DE (dextrose equivalent) values, which indicates the chain length of dextrins and their potential for fermentation [30]. Additionally, enzymatic hydrolysis has been found to have higher starch conversion efficiency compared to acid hydrolysis [31]. Acid hydrolysis, on the other hand, can cause damage to the equipment, leading to increased costs in the long run.

2.2 Production Target and Product Specifications

The process plant's design aims for an annual production target of 20,000 metric tons of dried fungal SCP. For human consumption, Quorn products containing *Fusarium venenatum* SCP have

a protein content of approximately 44% on weight basis [11]. The final product is dried fungal SCP with a solids fraction of 90% and 10% moisture.

3 Process Description

The fungal SCP production process using PPW is comprised of three primary stages: starch pre-processing, inoculum preparation and fermentation in reactors, and downstream processing. A comprehensive process flow diagram is illustrated in Figure 1, while Table 1 provides details on all major equipment utilized throughout the process. The starch pre-processing stage is based on the chapter *Starch-Processing Enzymes* from the book *Enzymes in Food Technology* [30]. The design of the inoculum preparation, fermentation, and downstream processing stages draws significant inspiration from the Quorn production process [32;33], with the addition of a vacuum filter and spray dryer to further increase the biomass concentration.

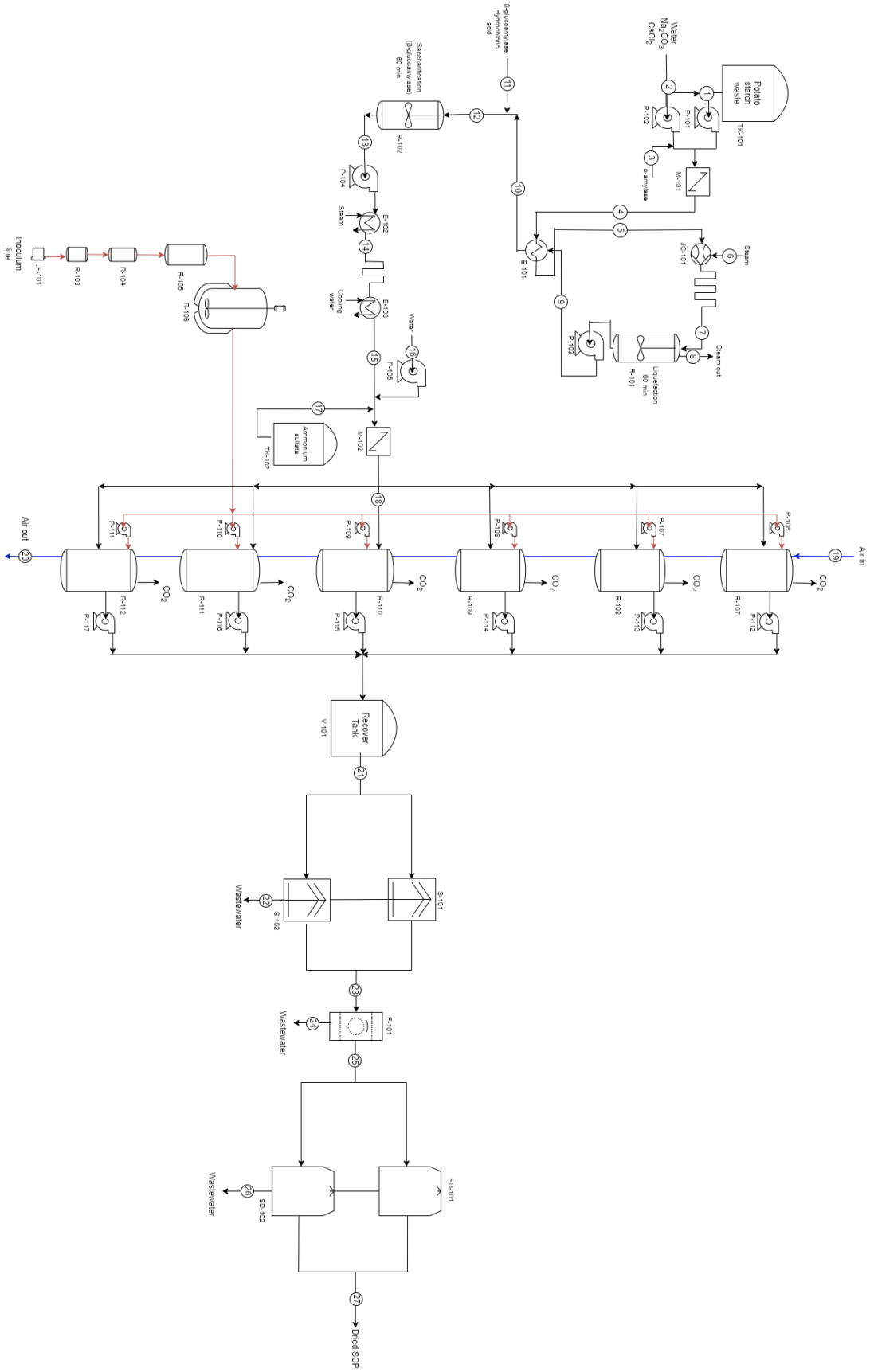

Figure 1: Process flow diagram for production of fungal SCP from starch-rich potato processing waste. Red lines indicate the inoculum pipeline, while blue lines indicate the air stream. The compressors and air filters related to the air stream are excluded from the process flow diagram for better readability. The glucose and mineral flow to the inoculum line was neglected to improve readability, and because these flows are not continuous.

Table 1: Equipment sizes and quantities for the SCP production process. Equipment names correspond to those found in Figure 1.

Equipment	Name	Size	Units	Amount
Pre-processing				
Storage tank	TK-101	1 000	$[m^3]$	1
Pump	P-101	1.2	$[L/s]$	1
Pump	P-102	2.7	$[L/s]$	1
Mixer	M-101	4	$[L/s]$	1
Heat exchanger (U-tube shell and tube)	E-101	16.5	$[m^2]$	1
Jet cooker	JC-101	20	$[m^3]$	1
CSTR tank (jacked agitator)	R-101	40	$[m^3]$	1
Pump	P-103	4	$[L/s]$	1
CSTR tank	R-102	40	$[m^3]$	1
Pump	P-104	4	$[L/s]$	1
Heat exchanger	E-102	13	$[m^2]$	1
Heat exchanger	E-103	11	$[m^2]$	1
Pump	P-105	35	$[L/s]$	1
Mixer	M-102	38	$[L/s]$	1
Inoculum and reactors				
Lab flask	LF-101	0.002	$[m^3]$	1
0.02 m^3 tank	R-103	0.02	$[m^3]$	1
0.2 m^3 tank	R-104	0.2	$[m^3]$	1
2 m^3 tank	R-105	2	$[m^3]$	1
20 m^3 tank	R-106	20	$[m^3]$	1
Reactor	R-107 to R-112	200	$[m^3]$	6
Storage tank	TK-102	10.14	$[m^3]$	1
Pump	P-106 to P-111	0.1	$[L/s]$	6
Compressor	C-101 to C-106	1 250	$[m^3/h]$	6
Air filter	F-101 to F-106	36.00	$[m^2]$	6
Compressor for airlift	C-107 to C-112	4 200	$[m^3/h]$	6
Air filter for airlift	F-107 to F-112	32.00	$[m^2]$	6
Pump	P-112 to P-117	6.5	$[L/s]$	6
Downstream				
Recover tank (cone roof)	V-101	4 000	$[m^3]$	1
Centrifuge	S-101 to S-102	0.69	$[m]$	2
Filter	F-101	23	$[m^2]$	1
Spray dryer	SD-101 to SD-102	4 000	$[kg/h]$	2

3.1 Pre-processing of Starch

The process of breaking down starch into glucose can be divided into three steps: gelatinization, liquefaction, and saccharification. In the first step, gelatinization, a 90% starch slurry is diluted to 35%. The starch slurry is stored in storage tank TK-101, as shown in Table 1. In addition to water, Na_2CO_3, $CaCl_2$, and α-amylase are added. Na_2CO_3 lowers the pH to create an optimal environment for the amylase to function, while $CaCl_2$ stabilizes the enzyme. The slurry is mixed in mixer M-101 before passing through heat exchanger E-101 and then into jet boiler JC-101. The jet boiler injects steam into the slurry, raising the temperature to 105 °C. This steam is reused from the dryers in the downstream processing. The slurry is maintained at this temperature for five minutes while traveling through holding tubes, ensuring complete gelatinization.

During the liquefaction process, the slurry is cooled to 95 °C by reducing pressure and removing steam. It is held at this temperature for 60 minutes in stirring tank R-101. In this step, amylase hydrolyzes the α-1,4 linkages in both amylose and amylopectin, producing dextrins with a DE value of 8-12.

In the saccharification step, the slurry is cooled to 60 °C, and the pH is adjusted to 4 with HCl

before glucoamylase and pullulanase are added. The lower pH inactivates the α-amylase, while the temperature drop prevents retrogradation of the liquefied starch. The slurry is kept in stirring tank R-102 for 60 minutes while the enzymes break down the remaining linkages. Pullulanase breaks the α-1,6 linkages, and glucoamylase primarily breaks the α-1,4 linkages, with some activity toward α-1,6 linkages. The slurry is then heated to 85 °C in heat exchanger E-102 for a few minutes to stop the reaction. The heating medium used in E-102 is sourced from the dryers in the downstream processing. Finally, the slurry is cooled to 30 °C in E-103, as this is the optimal temperature for fermentation, and is diluted to the optimal concentration. The cooling medium in E-103 is sourced from reused wastewater in the centrifuge and filter in the downstream processing.

3.2 Reactor Design and Process Configuration

The reactor design was determined by evaluating the type of reactor that should be used for this process and whether the reactor should be operated as a continuous or batch process. Air-lift reactors offer several advantages compared to agitated reactors, such as lower costs associated with agitation and aeration, ease of scale-up, low shear characteristics, high oxygen transfer efficiency, and predictable flow patterns [34]. Air-lift reactors have also been widely used in filamentous fermentation and SCP production [34]. Due to these factors, air-lift reactors were chosen for this plant.

It is recommended that fermentation of *F. venenatum* should not run for longer than 1000 hours, as undesirable highly branched mutations will start to form [12]. Quorn has been reported to have an operating time of up to 6 weeks [11]. Given the potential for a 1000-hour operating time, a continuous operation process was selected. The reactor operating volume was set to 150,000 L or 150 m^3 [12]. Assuming that the operating volume should be 75% of the total reactor volume, a total reactor volume of 200 m^3 was chosen.

The specific growth rate for *F. venenatum* ranges between 0.17-0.2 h^{-1} [12]. For this process, an average specific growth rate of 0.185 h^{-1} was utilized. To prevent washout, a dilution rate of 0.15 h^{-1}, which is lower than the specific growth rate, was selected. The residence time of 6.67 hours was calculated using equation B.7 based on this dilution rate. Given the high operating time of 1000 hours, it was assumed that the reactors would operate at a steady state close to 100% of the time, with a growth rate near the specific growth rate (0.185 h^{-1}). The steady-state conditions outlined in B were used to determining a flow rate of 22.5 m^3/h for each reactor. The final biomass concentration exiting each reactor (x_f) was set to 20 [g/L], resulting in a biomass production of 450 [kg/h]. To achieve the annual goal of 20,000 metric tons, it was calculated that 6 reactors operating 7407 hours a year would be required. An operating time of 8000 hours was assumed to account for production stops, such as those caused by contamination, resulting in annual production of 21,645 metric tons of dry biomass. After 1000 hours, the content of reactors (R-107 to R-112) is pumped into the recovery tank (V-101), and the reactors are washed and sterilized at 120 °C for 20 minutes [35].

3.3 Inoculum Line and Reactor Operations

Directly initiating the growth of *F. venenatum* in the 200 m^3 reactors would be impractical, as the starting concentration required would be too high. Moreover, the growth rate might not be optimal at the beginning of the continuous flow, increasing the risk of an excessive dilution rate. To ensure *F. venenatum* achieves the highest possible growth rate, an inoculum line was designed for the process.

The inoculum line starts with a 2 L shake flask (LF-101), where the fungus is grown on LB media. It is assumed that LB media provides the fungus with all the necessary components for growth and does not limit its ability to reach the desired growth rate. Once the target biomass concentration is reached, the inoculum in the flask is transferred to a 20 L reactor (R-103). The reactors (R-103-R-106) are filled up with glucose from the PPW processing and minerals. However, these streams are not continuous and small compared to the other streams, and so they are neglected in the

process flow diagram (Figure 1). The batches operate at 75% of the total volume, resulting in an initial biomass concentration of 2.67 [g/L]. Using Monod kinetics and assuming that the specific growth rate is an average of values found in the literature, it was calculated using equation B.3 that a batch time of 10.89 hours would be needed for the final biomass concentration to reach 20 [g/L] (Appendix B).

After the batch is completed, the inoculum is transferred to the next reactor (R-104) with a total reactor volume of 200 L. Reactor R-103 is then sterilized with steam at 120 °C for 20 minutes before the next inoculum is added[35]. This pattern continues, with the reactor volume increasing 10x for every reactor moving up the inoculum line. The completed batch in R-104 is then transferred to reactor R-105 with a volume of 2000 L or 2 m^3, and then further transferred to reactor R-106 with a volume of 20 m^3. Finally, the completed batch is transferred to one of the reactors R-107 to R-112.

3.4 Design of Growth Conditions

LB media is only used for the 2 L shake flask. Utilizing LB media for the rest of the inoculum line and the 200 m^3 reactors would be cost-prohibitive. The use of different carbon sources was discussed in section 1.4. It was assumed that the black box model could be employed to find the stoichiometric coefficients for cell growth. The calculations are presented and described in detail in Appendix A.1, and the cell growth reaction was calculated to be:

$$C_6H_{12}O_6 + 1.85\,O_2 + 0.79\,NH_3 \longrightarrow 3.95\,CH_{1.8}O_{0.5}N_{0.2} + 2.05\,CO_2 + 3.63\,H_2O \tag{1}$$

The dry biomass composition of *F. venenatum* was assumed to be $CH_{1.8}O_{0.5}N_{0.2}$, often used as an average for dry biomass[36]. Since the black box model only provides four equations with five unknowns, an additional assumption was made to find the stoichiometric coefficient for biomass. Assuming that the biomass yield (Y_{xs}) is close to 95% of the maximized biomass yield of *F. venenatum*[37], this process achieves a biomass yield of 54%.

However, *F. venenatum* cannot grow on glucose alone. The fungus also needs a source of nitrogen and other elements, such as sulfur (S), phosphorous (P), potassium (K), magnesium (Mg), as well as trace minerals. Other components, such as $(NH_4)_2SO_4$, KH_2PO_4, Na_2HPO_4, and $MgSO_4 \cdot 7H_2O$, were added to accommodate these needs.

The black box model was expanded to include these compounds, and the calculations are provided in Appendix A.4. These calculations exclude trace minerals such as zinc (Zn), iron (Fe), and calcium (Ca), as it was assumed that sufficient amounts of these elements would be supplied through the water used to dilute the potato starch slurry and the water used to suspend the other media components.

Furthermore, it was assumed with the black box model that *F. venenatum* has an aerobic metabolism, where it consumes oxygen (O_2) and produces carbon dioxide (CO_2). To ensure that the growth of the fungi would not experience oxygen limitation, an additional 20% of air is supplied to the reactors. Both the amounts of O_2 consumed and CO_2 produced for the reactors are given in Appendix A.3. The airflow into the reactor passes through an air filter to ensure sterility.

3.5 Downstream Processing

Following fermentation, the biomass is transferred to holding tank V-101, designed to accommodate one week's production from a single fermenter. Next, the slurry undergoes dewatering through two disk stack centrifuges, S-101 and S-102, which efficiently remove water to achieve a dry weight of 20%[38;39].

The biomass concentration is further enhanced to 30% by extracting additional water using a vacuum drum filter, F-101. In the final stage, spray dryers SD-101 and SD-102 are employed to

evaporate 66% of the remaining water, yielding a final product with a 90% dry weight concentration. This downstream processing sequence ensures the production of a high-quality mycoprotein product with desirable moisture content for fish feed. The product is then packed and shipped to the customer.

4 Flowsheet Calculations

This chapter provides a summary of the calculations and modeling for the mass and energy balances of the process. Essential parameters, such as flow rates and temperatures for the main streams, are presented using tables for easy comprehension. It is crucial to perform a quality check on the calculated data, ensuring mass and energy conservation. Brief notes on the applied methods and fundamental equations are included here, with more detailed calculations available in Appendix A.

4.1 Mass Balance

Table 2 illustrates the calculated flow rates of the streams from the flowsheet in Figure 1. The components and stream temperatures are also described in the same table. A mass balance verification is performed in Appendix A.5, confirming that the mass is preserved.

Table 2: Flowrate, components, and temperature of all streams in SCP production plant.

Stream	**Flow rate** $[Kg/h]$	**Components**	**Temperature** [°C]
		Pre-processing	
S1	5 727.38	Slurry 90% Starch	20
S2	9 452.50	Water, $Na_2CO_3, CaCl_2$	20
S3	3.15	Alfa-Amylase	20
S4	15 183.03	Slurry 35% Starch	20
S5	15 183.03	Slurry 35% Starch	58
S6	-	Steam In	-
S7	15 183.03	Slurry Gelatinized	105
S8	-	Steam Out	-
S9	15 183.03	Slurry Liquefied	95
S10	15 183.03	Slurry Liquefied	60
S11	-	HCl + glucoamylase + pullulanase	20
S12	15 183.03	Slurry 8-12 DE	60
S13	15 183.03	Glucose slurry	60
S14	15 183.03	Glucose Slurry	85
S15	15 183.03	Glucose Slurry	30
S16	119 816.97	Water	30
S17	1 448.78	Ammonium Sulphate + other	30
		Inoculum and reactors	
S18	135 000.00	Glucose slurry	30
S19	41 911.02	Air in	30
S20	42 440.45	Air out	30
		Downstream	
S21	136 031.44	SCP and water	-
S22	122 428.29	Water Out	-
S23	13 603.14	SCP and water	-
S24	4 761.10	Water out filter	-
S25	8 842.04	SCP and water	-
S26	5 835.75	Water out from drier	-
S27	3 006.29	SCP out	-

4.2 Energy Balances

4.2.1 Heat exchangers

To calculate the heat exchange of the heat exchangers the general energy conservation principle were applied, and heat lost to the environment was assumed to be negligible.

$$Q = mc_p\Delta T \tag{2}$$

Q is the heat exchanged, ΔT is the temperature difference between the two streams, m is the mass and c_p is the average specific heat of the stream, based on the temperature range. Q was used to calculate the area of the heat exchangers:

$$Q = UA\Delta T \tag{3}$$

U is the heat transfer coefficient (assumed equal to $1.1\,\mathrm{kW\,m^{-2}\,K^{-1}}$), and A is the area if the heat exchanger. In this project, only the U-tube shell and tube heat exchangers were used due to their compatibility and price. The heat exchanged and the area was calculated from the equations above and summarized in Table 3.

Table 3: Heat transfer and area of all heat exchangers used in the system

Symbol	**Heat transfer** $[kW]$	**Area** $[m^2]$
E-101	684	16.17
E-102	171	12.61
E-103	171	10.78

4.2.2 Metabolism of *F. venenatum*

The reactors (R-107 to R-112) are all supplied with sufficient amounts of air, making it possible for the fungi to perform aerobic metabolism. This is an exothermic reaction, and so cooling of the reactors is needed to keep the temperature at an optimum, T = 30 °C. Assumptions made for the calculation are given in Appendix C. Based on these assumptions, the equation for aerobic metabolism becomes:

$$Q = \Delta H_{rxn} = -460\,\mathrm{kJ\,mol}\,O_2^{-1} \cdot n_{O_2} \tag{4}$$

Here n_{O_2} is the molar consumption of oxygen calculated, and Q is the heat that must be removed from the reactors.

4.2.3 Power consumption

Evaluating equipment power consumption is essential for understanding a plant's efficiency. Table 4 displays the energy usage of key components, allowing for an estimation of the plant's overall energy requirements and offering valuable insights for optimization.

Table 4: Power consumption of all major equipment. The footnotes indicate the sources of power consumption.

Equipment	**Symbol**	**Power consumption** $[kW]$
Pump	P-101 to P-116	600^a
CSTR	R-101	60^b
CSTR	R-102	60^b
Inoculum tank	R-104	0.3^b
Inoculum tank	R-105	3^b
Inoculum tank	R-106	30^b
Disc stack centrifuge	S-101 to S-102	190^c
Vacuum rotary filter	F-101	37^d
Spray dryer	D-101 to D-102	$7\ 846^e$
Total		**8 826**

[a] Alibaba[40], [b] Chemical Engineering Design, p. 625[41],
[c] Doran, p. 423[36], [d] Andritz[42],
[e] Energy Consumption of Industrial Spray Dryers[43].

5 Cost Estimation

This section presents the cost estimation for the production of single-cell protein (SCP) using *Fusarium venenatum* as a feed additive in the Norwegian aquaculture industry. The project aims to design a bioprocess plant for the annual production of 20,000 tons of *F. venenatum* mycoproteins and assess the technical and economic feasibility of the process using both conventional growth substrates and alternative waste materials. The cost estimation involves evaluating capital investments and operating costs based on the methodology provided by Ray Sinnott and Gavin Towler in their book *Chemical Engineering Design*[41].

5.1 Capital Investment

Capital investment refers to the initial funds required to establish and start operating an SCP production plant. The capital investment cost estimation includes costs for Inside Battery Limits (ISBL), Outside Battery Limits (OSBL), engineering and construction, and contingency.

5.1.1 Equipment Cost Estimation

To estimate the cost of individual equipment, the power-sizing relationship is used, which is represented by the equation:

$$C_e = a + b \cdot S^n \tag{5}$$

Where C_e is the cost of the equipment, a and b are constants, S is the equipment size, and n is the exponent. The constants (a, b, and n) can be found in Table 6.6 of Sinnott and Towler (2019)[41] for various types of equipment. The values provided in the table are based on 2007 prices.

To update the equipment costs to a more recent year, the Chemical Engineering Plant Cost Index (CEPCI) is employed. The equation for updating the costs is as follows:

$$C_{\text{updated}} = C_{2007} \cdot \frac{CEPCI_{\text{recent}}}{CEPCI_{2007}} \tag{6}$$

Where $C_{updated}$ is the updated cost of the equipment, C_{2007} is the cost calculated using the 2007 prices, $CEPCI_{recent}$ is the CEPCI value for the recent year, and $CEPCI_{2007}$ is the CEPCI value

for 2007. The $CEPCI_{recent}$ was assumed to be 720.2 in the year 2021 and 525.4 in 2007.

Table 5: Equipment list and prices for pre-processing, inoculum and reactors, and downstream stages for starch as carbon source. The footnotes indicate sources of equipment prices.

Equipment	**Name**	**Amount**	**Price** [USD]
Pre-processing			
Storage tank	TK-101	1	248 450
Pump	P-101	1	9 791
Pump	P-102	1	10 148
Mixer	M-101	1	3 143
Heat exchanger (U-tube shell and tube)	E-101	1	34 721
Jet cooker	JC-101	1	12 800[a]
CSTR tank (jacked agitator)	R-101	1	806 773
Pump	P-103	1	10 441
CSTR tank	R-102	1	806 773
Pump	P-104	1	10 441
Heat exchanger	E-102	1	34 267
Heat exchanger	E-103	1	34 018
Pump	P-105	1	16 384
Mixer	M-102	1	6 734
Inoculum and reactors			
Lab flask	LF-101	1	424[b]
0.02 m^3 tank	R-103	1	9 375[c]
0.2 m^3 tank	R-104	1	32 503[c]
2 m^3 tank	R-105	1	139 476
20 m^3 tank	R-106	1	494 293
reactor	R-107 to R-112	6	7 637 295[c]
Storage tank	TK-102	1	16 567
Pump	P-106 to P-111	6	56 962
Compressor	C-101 to C-106	6	152 268
Air filter	F-101 to F-106	6	544 566[c]
Compressor for airlift	C-107 to C-112	6	319 475
Air filter for airlift	F-107 to F-112	6	497 250[c]
Pump	P-112 to P-116	6	65 882
Downstream			
Recover tank (cone roof)	V-101	1	644 431
Centrifuge	S-101 to S-102	2	1 031 468
Filter	F-101	1	194 554
Spray dryer	SD-101 to SD-102	2	2 374 454
Total			**16.26 M**

[a] Alibaba[44], [b] Sigma Aldrich[45], [c] Matches[46].

5.1.2 ISBL (Inside Battery Limits)

ISBL costs are associated with the core process areas of the SCP production plant, including equipment, installation, piping, and instrumentation. The total ISBL cost involves equipment installation and can be calculated as the total updated cost of major equipment multiplied by a factor of 3.2 for fluid-solid processes[41].

5.1.3 OSBL (Outside Battery Limits)

OSBL costs include changes and additions to site infrastructure, such as utilities, waste treatment, storage, and other facilities outside the process area. The total OSBL cost was calculated as 40% of the ISBL cost.

5.1.4 Engineering and Construction Costs

Indirect costs include costs for engineering, construction, and contingency. These costs can be estimated by applying appropriate percentages to the sum of ISBL and OSBL costs. Engineering and construction costs are typically 20% of the direct costs (ISBL + OSBL), while contingency costs are estimated as a percentage of the total project cost (direct + indirect costs).

5.1.5 Contingency Costs

Contingency costs cover deviations from cost estimates and unexpected charges, estimated as 30% of the ISBL cost.

5.1.6 Total Investment

The total investment for the SCP production plant is a combination of total capital investment and working capital. Total capital investment includes direct costs (ISBL + OSBL) and indirect costs (engineering, construction, and contingency). The total capital investment sum provides an estimate of the initial capital investment needed to construct and commission the plant. Working capital typically represents 15% of the sum of ISBL and OSBL costs and covers production costs, product value, costs of spare parts, and other expenses.

Table 6: Total investment of the SCP product plant.

Category	**Price** $[USD]$
ISBL	52 019 620
OSBL	20 807 848
Engineering	14 565 494
Contingency	21 848 240
Working capital	10 924 120
Total	**136.42 M**

5.2 Operating Costs

Operating costs consist of fixed and variable costs, which contribute to the ongoing expenses required to maintain and operate the SCP production plant.

5.2.1 Variable Costs

Variable costs are expenses that vary with the production output, such as enzymes, raw materials, and electricity costs. As production levels change, these costs will fluctuate, impacting the overall operating costs of the plant.

5.2.2 Fixed Costs

Fixed costs include expenses such as rent, electricity, maintenance, laboratory fees, taxes, charges, and employment costs. These costs are incurred regardless of the production output and must be accounted for when assessing the economic feasibility of the SCP production plant. The number of workers was estimated using the following equation:

$$N = (6.29 + U)^{0.5} \tag{7}$$

and was estimated that the plant would need 6 workers on a shift-work basis, giving a four-shift rotation. The average salary for a control operator for a chemical process plant was estimated to be \$5,781 per month[47]. This gives a yearly operating labor cost of \$69,372 per worker. In addition, the plant requires supervision and laboratory expenses, which are estimated to be 25% and 10% of operating labor, respectively. Direct salary overhead was calculated as 40% of labor cost plus supervision. Property taxes and insurance were estimated to be 1.5% of ISBL, while rent of land was estimated to be 1.5% of (ISBL + OSBL). General plant overhead was 65% of total labor. Environmental charges were estimated to be 1% of (ISBL + OSBL).

Table 7: Variable, fixed and total operating costs of SCP production plant.

Component	**Price** $[USD/t]$	**Cost** $[USD/year]$
	Variable Cost	
Raw material	33	1 222 222
Enzyme	12 500	238 640
Minerals	1 485	1 959 044
Raw water	1.1	55 963
Wastewater	1.5	80 892
Packing	3	60 000
Electricity		3 530 520
Total		**7 147 351**
	Fixed Cost	
Operating labour		1 664 928
Supervision		416 232
Laboratory		166 492
Direct salary overhead		693 720
Maintenance		2 112 251
Property taxes and insurance		792 094
Rent of land		1 108 932
General plant overhead		3 266 819
Environmental charges		739 288
Total		**11 099 501**
Total operating cost		**18.25 M**

6 Investment Analysis

This chapter focuses on the investment analysis for the proposed bioprocess plant. This analysis is essential to assess the economic viability of the project and to make informed decisions regarding its feasibility. The analysis is conducted in three parts: Profit & Cash Flow, Economic Performance, and Sensitivity Analysis.

6.1 Profit & Cash Flow

To evaluate the profitability of the bioprocess plant, both the profit and cash flow must be examined. Profit represents the difference between total revenue and total operating costs, which include fixed and variable costs. Cash flow, on the other hand, signifies the net cash inflows and outflows over a specified period. Both profit and cash flow must be positive for the project to be considered economically viable.

The gross profit can be calculated using the following equation:

$$\text{Gross Profit} = \text{Revenue} - \text{Total Operating Costs} \tag{8}$$

where the revenue is found by multiplying the product price ($1.5 per kg[48], the same price as soy protein) with the sales volume per year.

A comprehensive breakdown of the plant's revenues, operating costs, depreciation, taxes, and net income should be provided. Additionally, a cash flow statement should be prepared to demonstrate the timing and magnitude of cash inflows and outflows throughout the project's lifespan.

The project's profitability was assessed by comparing the economic performance of producing SCP from potato starch and molasses. The real cash flow depends on taxes and depreciation, with a tax rate of 22% and a declining balance depreciation with an interest rate of 20%. The cumulative cash flow analysis revealed that the initial investment is higher for the PPW-based process due to increased capital costs for pre-processing equipment. However, the yearly profits are more substantial for the PPW-based process as a result of lower raw material costs. Consequently, the payback times are 8.7 years for the PPW-based process and 9.5 years for the molasses-based process.

6.2 Economic Performance

In this section, key financial metrics, such as Net Present Value (NPV), Return on Investment (ROI), and Internal Rate of Return (IRR), are used to assess the economic performance of the bioprocess plant.

The NPV, calculated with a 10% interest rate, gives the total value of the project based on the present value of future cash flows. ROI measures the ratio of profit over the investment, indicating the project's economic performance in offsetting the investment. IRR is the interest rate at which the project's NPV equals 0 at the end of its lifetime.

- **NPV**: The Net Present Value is the difference between the present value of cash inflows and the present value of cash outflows over the project's lifespan. It is calculated using the following equation:

$$NPV = \sum_{n=1}^{t} \frac{CF_n}{(1+i)^n} \tag{9}$$

  where CF_n represents the cash flow in year n, i is the interest rate, and t is the lifetime of the project given in years. The interest rate in this project is set to 10%. A positive NPV indicates that the project is expected to generate a profit and can be considered economically viable.

- **ROI**: Return on Investment measures the profitability of the investment as a percentage of the initial investment. It is calculated using the following equation:

$$\text{ROI} = \frac{\text{Net Profit}}{\text{Total Investment}} \times 100 \tag{10}$$

  A higher ROI signifies a more attractive investment opportunity.

- **IRR**: The Internal Rate of Return is the discount rate at which the NPV of a project is zero. In other words, it is the rate at which the project breaks even. A higher IRR indicates a more profitable investment and a shorter payback period.

A comparison of key economic performance measures for processes producing fungal proteins grown on starch from potatoes and fungal proteins grown on sugarcane molasses is shown in the table below.

Table 8 indicates that the PPW plant has a higher NPV, ROI, and IRR compared to the molasses plant, making it a more attractive investment opportunity.

Table 8: Economic performance comparison using starch and sugarcane molasses.

	PPW	**Molasses**
NPV [*$MM*]	-6.2	-14.9
ROI [%]	229	211
IRR [%]	9.3	8.1

6.3 Sensitivity Analysis

A sensitivity analysis examines the impact of changes in critical variables on the economic performance of the bioprocess plant. This analysis helps to identify the key factors affecting profitability and assess the risks associated with the project. The following variables were considered in the sensitivity analysis:

1. Revenue: The influence of changes in the market price of the final product and sales volume on the project's economic performance.
2. Total costs: The impact of fluctuations in the combined costs of raw materials, enzymes, minerals, electricity, and fixed costs on the project's profitability.
3. Investments: The effect of changes in equipment costs on the initial capital investment and overall project viability.
4. Raw materials cost: The impact of fluctuations in the cost of raw materials on the project's profitability.
5. Enzymes cost: The influence of fluctuations in the cost of enzymes on the project's profitability.
6. Minerals cost: The effect of changes in the cost of minerals on the project's economic performance.
7. Electricity cost: The impact of changing electricity consumption on the plant's operating expenses.

By conducting a sensitivity analysis, project stakeholders can better understand the potential risks and uncertainties associated with the bioprocess plant and develop strategies to mitigate these risks and optimize the project's economic performance.

Figure 2 illustrates the accumulated cash flow for starch and molasses, which is an essential component in the economic assessment of the two projects. The figure reveals that the project using starch reaches break-even after 8.7 years, while it takes 9.5 years when using molasses. It demonstrates that investors can recover their initial investments in almost 9 years using starch, with the remaining 11 years of the project generating a positive cumulative cash flow. Although the investment using starch might seem appealing, its internal rate of return (IRR) of 9.3% indicates that it falls slightly short of generating a positive net present value (NPV), which is calculated using a 10% interest rate.

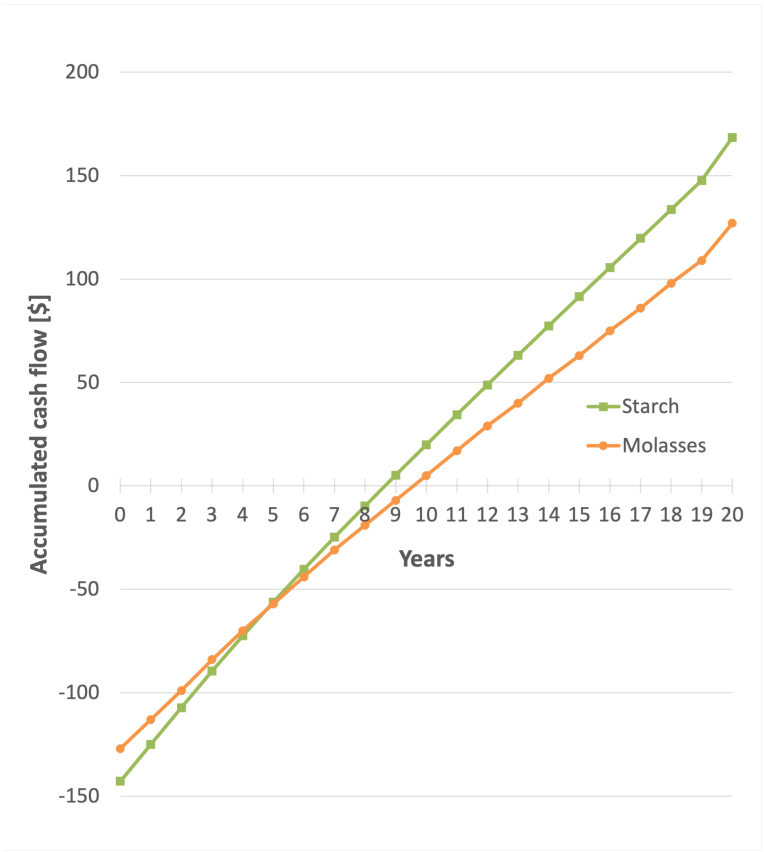

Figure 2: Accumulated cash flow using starch and molasses as carbon source.

The sensitivity analysis in the project evaluates how changes in key variables affect the Net Present Value (NPV) while keeping other variables constant. A 10% interest rate has been used to calculate the NPV. NPV gives the total value of the project based on the present value of future cash flows.

Figure 3 presents the sensitivity analysis of revenue, total costs, and investments affecting the NPV. This figure provides a big-picture view of the project's economic performance in response to changes in these critical variables. The analysis highlights the importance of managing revenues, controlling costs, and optimizing investments to ensure project profitability.

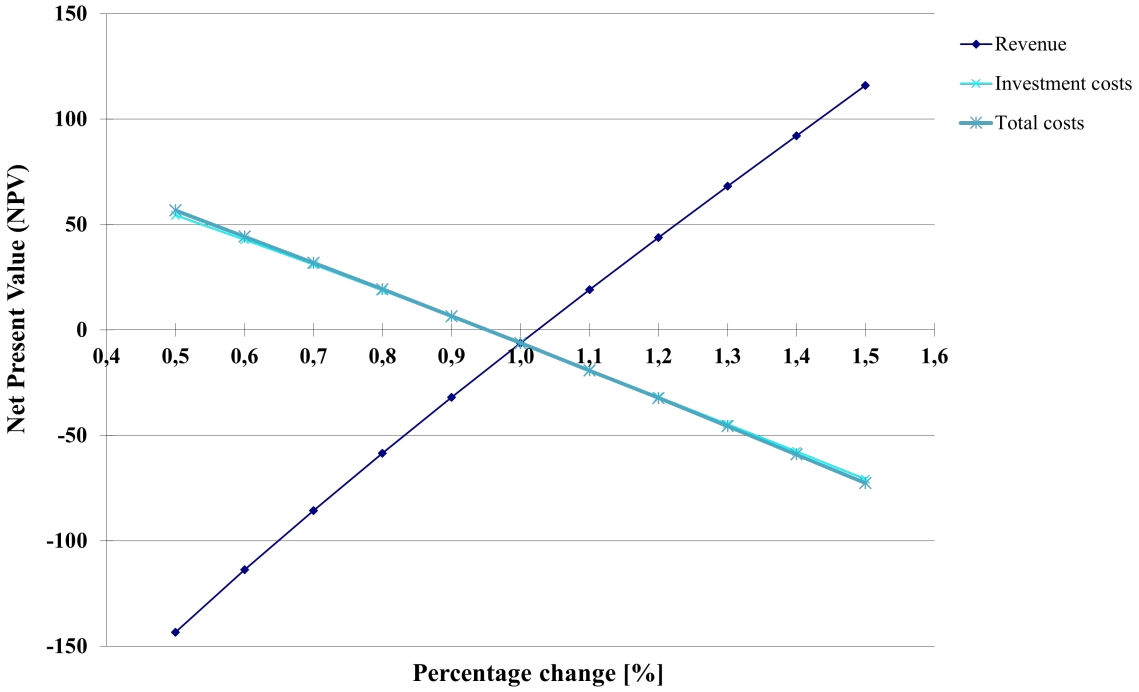

Figure 3: Sensitivity analysis of revenue, total costs, and investments affecting the NPV using starch.

Figure 4 focuses on the sensitivity analysis of fixed costs and components of variable costs affecting the NPV. This figure breaks down the variable costs into their components: raw materials, enzymes, minerals, and electricity. It shows that minerals and electricity costs have the most significant impact on the NPV, emphasizing the need to optimize these costs to maximize the project's economic performance.

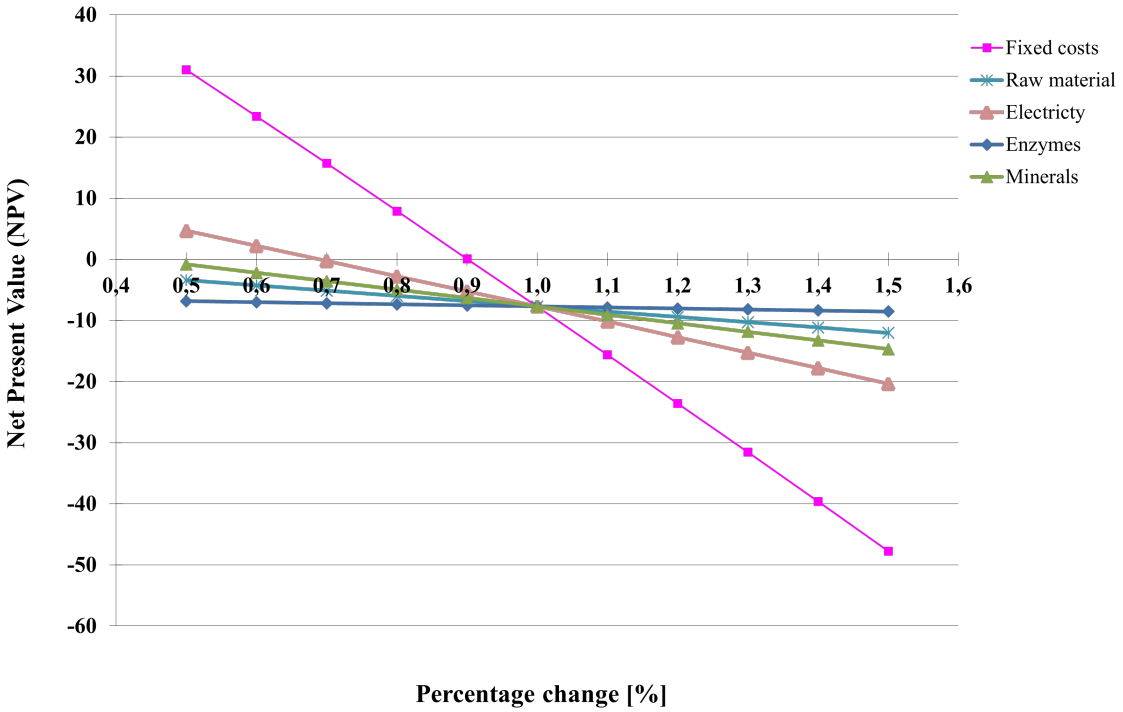

Figure 4: Sensitivity analysis of fixed and variable costs affecting the NPV using starch.

Figure 3 and 4 show how a change in economic components changes the NPV of the plant using

starch as a carbon source. Corresponding figures for the molasses plant is shown in Appendix D.3. The analysis for the PPW plant shows that electricity costs, fixed costs, investment costs, and revenue have the most significant impact on the NPV. Electricity is the largest variable cost, therefore it affects the NPV the most. A 10% change in electricity price changes the NPV with ± 2.48 M. A 10% change in fixed cost changes the NPV with ± 7.79 M. A 10% change in investment cost changes the NPV with ± 12.49 M. A 10% change in revenue results in an NPV change of ± 24.7 M.

The analysis shows that the mineral, enzyme, and raw material costs do not affect the NPV in any significant manner. Even a 50% decrease in cost would not make the plant profitable.

7 Discussion

7.1 Process Design

Several assumptions have been made during the design and modeling of this process plant. For the pre-processing and media, it was assumed that the raw material of 90% PPW only contains water and starch. However, it is likely that the PPW contains other components in addition to these two, such as other organic and inorganic materials. This assumption could impact the process in various ways. For instance, if the 90% PPW is a combination of starch and other compounds, the overall carbon source in the media would be lower than calculated. Consequently, there would not be enough carbon to sustain the growth of the 20,000 metric tons of *F. venenatum*. More PPW would be required to meet the carbon needs of the fungi, which would also influence the amount of water needed for different dilution steps. This would impact the variable costs of the plant. Additionally, it is assumed that the presence of other compounds would not affect the growth of *F. venenatum*. This assumption needs to be tested experimentally through an analysis of biomass growth to evaluate the feasibility of using PPW as an alternative carbon source.

Regarding the minerals found in the media, it was assumed that trace minerals, such as zinc (Zn), iron (Fe), calcium (Ca), and chloride (Cl), are present in sufficient amounts in the water used to dilute the PPW. To verify this assumption, the actual mineral content in the water source should be analyzed, and adjustments should be made if the levels are insufficient to support *F. venenatum* growth. If this assumption is not met by the water used for dilution, these trace minerals need to be added. This would lead to an increase in the variable costs of the process plant.

The major assumption for the black box model and the growth of *F.venenatum* is the biomass yield, $Y_{xs} = 0.54$. To bolster this assumption, the yield was set at 95% of the yield found in the literature, $Y_{xs} = 0.56$. Even when assuming a yield lower than the maximum, this assumption still could have major implications for the overall operation of the plant. If the biomass yield were to decrease, the present amount of glucose from potato starch and minerals found in the media would not be enough to meet the goal of 20,000 metric tons, as a lower yield would mean that more glucose is needed to achieve a similar dry weight of biomass. Thus, increasing the variable costs for both raw materials and minerals. However, if the plant would be able to operate at a biomass yield of $Y_{xs} = 0.56$, the present amount of glucose and minerals could sustain the production of dry biomass weight that would surpass the set goal of 20,000 metric tons. If the market demand is 20,000 metric tons, this would mean that the amounts of glucose and minerals needed could be lowered, which in turn lowers the variable costs. However, if the market demands are higher than the set goal of 20,000 metric tons, the excess dry biomass could also be sold. Which could increase the revenue for the plant.

In section 2, the required number of reactors and uptime were calculated. With six reactors, the uptime was found to be 8000 hours or 333 days. It was assumed that the remaining 32 days of the year would be sufficient to sterilize the reactors between uses. As the inoculum line only produces enough to supply one reactor at a time, there will be a slight delay between the startup of each reactor, allowing for the sterilization of one reactor while not completely stopping production of *F.venentaum*.

In section 4.1, the amount of steam used in the Jet Cooker (JC-101) and removed in the CSTR reactor (R-101) is nearly equal. The amount of steam added or subtracted would be negligible and not affect the overall mass balance. Furthermore, the impact on cost would be minimal, as the steam entering the jet cooker originates from the drying process and would require little to no extra energy to heat up. The steam from the drying process is also used as a heating medium in heat exchangers. It is assumed that 95% of the water used in the process can be reused, thus reducing wastewater costs. Some reused water is used in heat exchangers to cool down different streams.

The price assumptions made in this study were based on available literature and industry practices, but it is crucial to acknowledge that these assumptions may not accurately reflect reality. Therefore, additional experimental work and pilot-scale studies should be conducted to validate and refine these assumptions, ensuring the accuracy and reliability of the process calculations and design. It is assumed that the prices for enzymes, minerals, and equipment are realistic. Due to large variations in enzyme and mineral costs, the price was set at an average cost from different suppliers. The cost was taken from suppliers considered to be credible because of the significant differences in price. Most of the equipment cost was found using Sinnott and Towler[41]. However, some equipment was found from other sources, such as the reactors sourced from Matches, making it challenging to determine if the price is comparable to the rest of the plant.

For the operation of the PPW plant, the assumption was made that equipment functions perfectly all the time and that production would not be halted due to malfunction. Should the process be stopped because of faulty equipment it would lead to reduced production of SCP and a lower sales volume, reducing revenue. A particular weak point of the PPW plant is the downstream process. Because of the low number of units, the production would slow down or stop if any components malfunctioned, particularly the filter. Only one filter is needed for the flow rate of the process, however, if the filter malfunctions the plant could only run for an additional day before the recovery tank (V-101) would be full.

The one advantage the molasses plant has over the PPW plant is that the pre-processing is simpler. Not only does this reduce the initial investment cost, but the lower amount of components lower the risk of malfunctioning and stopping production. All the components are relatively simple and require little effort to replace should they break.

7.2 Investment Analysis

Compared to the traditional feedstock of molasses, the PPW plant has a higher investment cost, primarily due to the pre-processing of starch. The enzymatic hydrolysis requires specialized equipment, including two CSTR tanks—one for α-amylase and another for glucoamylase—and heat exchangers, which increase the initial investment cost. The initial investments are \$136 and \$120 million for PPW and molasses, respectively.

The higher initial investment is balanced by lower operational costs, primarily due to the reduced expense of raw materials. Although the PPW-based plant has a higher initial investment, its variable cost is significantly lower (\$3.37 M). Even when taking into account the additional expense of enzymes and minerals for hydrolysis, the variable cost for the PPW plant remains lower. This is attributed to the assumption that the raw material cost of PPW is only one-third the cost of molasses, as water generated from potato production is a waste product that companies must pay to dispose of and has limited or no practical applications.

Table 8 indicates that neither the PPW nor molasses plants are profitable over a 20-year period, given a 10% interest rate. The PPW plant has an NPV of -\$6.6 million, and the molasses plant has an NPV of -\$14.9 million; for a plant to be considered profitable, its NPV must be above 0. Although the project's NPV calculation is not viable with a 10% interest rate, the triple bottom line of utilizing starch from PPW—which offers environmental benefits and job creation at a national level—may attract investors who are willing to accept lower interest rates, such as 9% or even 8%. Under these conditions, the project could become financially viable and profitable, with anticipated NPVs of \$2.8 million and \$13.3 million, respectively. The PPW-based process

demonstrates superior Net Present Value (NPV), Return on Investment (ROI), Internal Rate of Return (IRR), and a shorter payback period, making it a more appealing investment option compared to the molasses-based process.

The sensitivity analysis of starch, as depicted in Figures 3 and 4, investigates how various economic factors impact the PPW plant's economy. The component with the greatest influence on the economy is revenue, which is divided into biomass and price. As the growth rate is consistently at its maximum and no biomass is lost during separation, it is not feasible to enhance biomass yield without making substantial modifications to the entire plant. Consequently, any revenue increase would stem from a price increase. Figure 3 illustrates that a 3% price increase would result in the plant breaking even. The investment cost also significantly affects the plant's economy; a 5% reduction in the initial investment would lead to a break-even point. Variable and fixed operational costs have a lesser impact on the plant's economy; a 35% reduction in electricity usage or a 10% reduction in fixed costs would be necessary to break even. Thus, increasing the price or reducing investment costs are more realistic approaches to achieving break-even, as these adjustments would be smaller.

Comparing the sensitivity analysis of molasses (Figures D.3 and D.2) to starch, some economic components have a similar effect on both analyses. For example, revenue and investment costs continue to have the largest impact on the economy. A 6% increase in revenue or a 13% decrease in investment cost would be required for the molasses plant to break even. Although the percentages are higher for the molasses plant compared to the PPW plant, the change in NPV per percent change remains comparable between the two plants. Consequently, both plants are approximately equally affected by alterations in revenue and investment cost. The most significant difference between the two plants is their sensitivity to operational cost changes, such as electricity, fixed costs, and primarily raw materials. While a 35% decrease in the electricity cost is necessary for the PPW plant to achieve an NPV of 0, even a 50% decrease in the electricity usage for the molasses plant would not make it break even (Figure D.2). Therefore, the molasses plant is less sensitive to changes in electricity usage. Moreover, a 20% reduction in fixed costs would be required for the plant to break even, which is unrealistic since fixed cost operations are assumed to be already optimized. The most significant difference arises from raw material costs. The PPW plant is not sensitive to changes in raw material costs, as even a 50% reduction in the material price will not result in the plant breaking even. In contrast, the molasses plant is significantly affected by changes in raw material costs, with a 26% decrease leading to a break-even point.

Not only is the molasses plant more sensitive to raw material costs, but several factors also render molasses an inferior choice for producing *F. venenatum*. The primary issue is that molasses is not produced locally, necessitating importation from another continent. As a result, the environmental impact would be considerably higher than that of PPW. Furthermore, the extended transportation could introduce logistical challenges. The holding tank for molasses is designed to contain only a week's worth of raw material required for production, necessitating a frequent supply. Any delays in the supply chain could lead to a production halt, potentially resulting in the annual target of 20,000 metric tons not being met. Additionally, the widespread use of molasses in multiple processes could drive up the price due to increased demand.

7.3 Recommendations

One direct approach to enhance the NPV of the PPW plant is to increase the product price. Initially, the price was assumed to be equivalent to that of soy protein; however, SCP feed presents several advantages. Firstly, since SCP is produced in Norway, its transportation costs would be lower compared to soy protein, which is produced abroad. Secondly, SCP production is more sustainable than soy protein production. Soy cultivation requires significant water and land resources. Soy protein yields approximately 3.17 tons per hectare, while the proposed plant produces 24,050 metric tons within a smaller area than is needed for soy production. The extensive use of soy protein has resulted in negative environmental impacts, such as deforestation, greenhouse gas emissions, water pollution, and widespread pesticide use[4]. Studies have shown a willingness to pay a premium price for sustainable products[49], making a price increase a realistic option. By leveraging the advantages of SCP and highlighting its sustainability attributes, the plant's financial

performance could be improved.

Further research into enhancing the downstream process is recommended, as it is the plant's most significant weak point. The downstream process makes the plant sensitive to both electricity usage and equipment malfunction. To reduce the plant's sensitivity to malfunction, more or larger storage tanks could be implemented after the reactors. This would allow the reactors to continue operating even if the downstream process was halted. Replacing the single filter with two smaller filters would also be beneficial. Investigating more energy-efficient methods of removing water from the product would enhance the plant's profitability. Electricity usage constitutes the largest variable cost (around 50%) for this plant. Therefore, efforts to minimize electricity usage could substantially impact the plant's profitability. The dryers have the highest electricity usage by a considerable margin, so exploring a more energy-efficient alternative to achieve the same dry weight percentage could be invaluable in optimizing the plant's profitability.

Additionally, further research into batch or fed-batch versus continuous operation of the reactors is recommended. A batch-operated process could maintain a higher biomass concentration than the continuous setup. The need for steady-state conditions with a low concentration of biomass out necessitates a highly diluted glucose slurry to achieve an inlet flow of 22.5 m^3. With the higher biomass concentration for batch/fed-batch operations, less water would be needed to dilute the glucose slurry. Consequently, a more efficient downstream process could be designed, as less water would need to be removed. This would lead to a reduction in electricity usage, which in turn would lower the operational cost of the plant.

8 Conclusions and Recommendations

This project aimed to design, model, and analyze the economics of a process for producing fungal single-cell protein (SCP) from PPW as a fish feed protein additive. The project compared the more environmentally friendly PPW plant to a traditional molasses-based plant in terms of profitability and processing design.

The economic analysis indicated that the PPW plant is more economically viable than the molasses plant, with a Net Present Value (NPV) of -$6.6 million as opposed to -$14.9 million. The PPW plant boasts a Return on Investment (ROI) of 229% and an Internal Rate of Return (IRR) of 9.3%. The sensitivity analysis highlighted that the NPV of the PPW plant is sensitive to changes in product price and investment cost, and that electricity was the most sensitive operational cost. Enzyme cost, raw material cost, and mineral cost had little to no impact on the sensitivity analysis. When comparing the sensitivity analysis of molasses to that of the PPW, the biggest difference was the sensitivity to raw material cost.

In addition to the economic benefits, the PPW plant exhibited a lower environmental impact than the molasses plant. Utilizing PPW as feedstock reduces the need for the transportation of molasses, which in turn reduces carbon emissions. Moreover, the PPW plant uses a local waste stream, making it unaffected by delays in carbon source shipments, as opposed to molasses.

Based on the findings of this study, the following recommendations are proposed:

- Investigate strategies to increase the product price by promoting the sustainability credentials and advantages of fungal SCP over traditional protein sources. Research has indicated a willingness to pay a premium for sustainable products, which could improve the financial performance of the PPW-based process.
- Conduct additional research into more energy-efficient methods for water removal from the biomass product. Reduction of energy usage would have the biggest impact on NPV out of all the variable costs.
- Examine the possibility of operating the reactor as a batch or fed-batch reactor in order to reduce the water consumption currently used for the dilution of glucose.

In conclusion, this study has demonstrated the potential of potato processing waste as a feedstock for producing fungal SCP for fish feed, with promising economic and environmental advantages compared to a traditional molasses-based process. The findings provide a solid foundation for further research and development in this area, and the recommendations outlined above offer a roadmap for bringing this sustainable protein additive to the aquaculture industry.

List of Symbols and Acronyms

Table 9: List of symbols

Symbol	Unit	Description
A	m^2	Area
a,b,c,d,e	mol/mol glucose	Stoichiometric coefficients of fermentation reaction
C_e	USD	Cost of equipment
cp	kJ kg^{-1} K^{-1}	Specific heat capacity
c_x	kg/kg	Mass fraction or concentration of compound x
D	h^{-1}	Dilution rate
F	kg/h	Flow rate
ΔH	kW	Change in enthalpy
i	%	Interest rate
k_d	-	Cell death number
MW_x	g/mol	Molar weight of compound x
$\dot{m}_i$	kg/s	Mass flow rate of compound i
N	-	Number of operators
$\dot{n}_i$	mol/s	Molar consumption rate of compound i
Q	kW	Power
S	-	Size parameter for equipment
T	°C	Temperature
ΔT	°C	Change in temperature
t	years	Lifetime of project
U	-	Numbers of processing units
U	kJ h^{-1} m^{-2} K^{-1}	Heat transfer coefficient
V	L	Volume
x_i	g/L	Initial biomass
x_f	g/L	Final biomass
$Y_{biomass}$	g/g	Biomass yield

Table 10: List of Greek symbols

Symbol	Unit	Description
μ	h^{-1}	Growth rate

Table 11: List of acronyms

Acronym	Description
PPW	Potato Processing Waste
CEPCI	Chemical Engineering Plant Cost Index
CF	Cash Flow
CSTR	Continuous stirred-tank reactor
DE	Dextrose equivalent
DW %	Dry weight
IRR %	Internal rate of return
ISBL	Inside battery limit
NPV	Net present value
OSBL	Outside battery limit
ROI %	Return on investment
SCP	Single cell protein
TEA	Techno-economic analysis
USD	United States dollars

References

[1] J.G. Da Silva. Feeding the world sustainably. https://www.un.org/en/chronicle/article/feeding-world-sustainably, 2012. Downloaded 06.02.23.

[2] S. Craig and L. Helfrich. Understanding fish nutrition, feeds, and feeding. https://fisheries.tamu.edu/files/2019/01/FST-269.pdf, 2017. Downloaded 06.03.23.

[3] Tim Searchinger Janet Ranganathan, Richard Waite and Craig Hanson. How to sustainably feed 10 billion people by 2050, in 21 charts. https://www.wri.org/insights/how-sustainably-feed-10-billion-people-2050-21-charts, 2018. Downloaded 06.03.23.

[4] EIT FOOD NORTH-WEST. Why alternative and sustainable fish feeds are needed. (https://www.eitfood.eu/blog/why-sustainable-fish-feeds-are-needed), 2021. Downloaded 07.03.23.

[5] Knut Henrik Rolland. The salmon farming industry in norway 2021 report. https://www.kontali.com/b/the-salmon-farming-industry-in-norway-2021-report. Downloaded 07.03.23.

[6] Reidun Lilleholt Kraugerud. Salmon feed is slowly changing. https://nofima.com/results/salmon-feed-is-slowly-changing/, 2022. Downloaded 07.03.23.

[7] M.H. Morowvat A.T. Nasseri, S. Rasoul-Amini and Y. Ghasemi. Single cell protein: Production and process. *American Journal of Food Technology*, 6:103–116, 2011. URL https://scialert.net/abstract/?doi=ajft.2011.103.116.

[8] Norma Julieta Salazar-López, Gabriel A. Barco-Mendoza, Shain Zuñiga-Martínez, J. Abraham Domínguez-Avila, R. Maribel Robles-Sánchez, Monica A. Villegas Ochoa, and Gustavo A. González-Aguilar. Single-cell protein production as a strategy to reincorporate food waste and agro by-products back into the processing chain. *Bioengineering*, 623, 2022. doi: https://doi.org/10.3390/bioengineering9110623.

[9] Yueming Dersjant-Li. The use of soy protein in aquafeeds. 01 2002.

[10] Yumi Kobayashi, Mohammad EL-Wali, Hörður Guðmundsson, Elísabet Eik Guðmundsdóttir, Ólafur H. Friðjónsson, Eva Nordberg Karlsson, Marja Roitto, and Hanna L. Tuomisto. Life-cycle assessment of yeast-based single-cell protein production with oat processing side-stream. *Science of The Total Environment*, 873:162–318, 2023. doi: https://doi.org/10.1016/j.scitotenv.2023.162318. URL https://www.sciencedirect.com/science/article/pii/S0048969723009348.

[11] Marilyn Wiebe. Myco-protein from fusarium venenatum: a well-established product for human consumption. *Applied Microbiology and Biotechnology*, 58:421–427, 2002. URL https://link.springer.com/article/10.1007/s00253-002-0931-x.

[12] Jack A. Whittaker, Robert I. Johnson, Tim J. A. Finnigan, Simon V. Avery, and Paul S. Dyer. The biotechnology of quorn mycoprotein: Past, present and future challenges. *Grand Challenges in Biology and Biotechnology*, 2020. doi: https://doi.org/10.1007/978-3-030-29541-7_3.

[13] Fataneh Hashempour-Baltork, Kianoush Khosravi-Darani, Hedayat Hosseini, Parastou Farshi, and S. Fatemeh S. Reihani. Mycoproteins as safe meat substitutes. *Journal of Cleaner Production*, 253:119958, 2020. ISSN 0959-6526. doi: https://doi.org/10.1016/j.jclepro.2020.119958. URL https://www.sciencedirect.com/science/article/pii/S0959652620300056.

[14] S. Fatemeh S. Reihani and Kianoush Khosravi-Darani. Influencing factors on single-cell protein production by submerged fermentation: A review. *Electronic Journal of Biotechnology*, 37:34–40, 2019. ISSN 0717-3458. doi: https://doi.org/10.1016/j.ejbt.2018.11.005. URL https://www.sciencedirect.com/science/article/pii/S0717345818300484.

[15] S. Taskila, M. Ahokas, V.H. Sotaniemi, M. Mäki, H.L. Malinen, M. Jaakkola, and J. Tanskanen. Conversion of potato peel waste to single cell protein by an acidophilic fungus. *Journal of Water Resource and Protection*, 10(5):522, 2018.

[16] B. Liu, Y. Li, J. Song, et al. Production of single-cell protein with two-step fermentation for treatment of potato starch processing waste. *Cellulose*, 21:3637–3645, 2014. doi: 10.1007/s10570-014-0400-6. URL https://doi.org/10.1007/s10570-014-0400-6.

[17] A. Tesfaw and F. Assefa. Co-culture: A great promising method in single cell protein production. *Biotechnology and Molecular Biology Reviews*, 9(2):12–20, 2014.

[18] Efstathia Tsakali, Konstantinos Petrotos, Angela D'Allessandro, and Panagiotis Goulas. A review on whey composition and the methods used for its utilization for food and pharmaceutical products. In *Proc. 6th Int. Conf. Simul. Modelling Food Bioind*, pages 195–201, 2010.

[19] I. Kiran Kumar and Saroj Mishra. Mass and energy balance in glucose and lactose metabolism in fusarium oxysporum. *Journal of Fermentation Technology*, 66(4):449–453, 1988. ISSN 0385-6380. doi: 10.1016/0385-6380(88)90013-1. URL https://www.sciencedirect.com/science/article/pii/0385638088900131.

[20] Pounikar Minakshi A Gomashe Ashok V and Gulhane Pranita. A liquid whey: A potential substrate for single cell protein production from bacillus subtilis ncim 2010. *International Journal of Life Sciences*, 2(2):119–123, 2014. URL https://oaji.net/articles/2014/736-1404211686.pdf.

[21] TINE. Email correspondence with tine. Private communication, 2023.

[22] P. Jelen. *Whey Processing*, pages 2739–2751. London Academic Press, 2003.

[23] Henning Jørgensen, Jan Bach Kristensen, and Claus Felby. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. *Biofuels, Bioproducts and Biorefining*, 1(2):119–134. doi: https://doi.org/10.1002/bbb.4. URL https://onlinelibrary.wiley.com/doi/abs/10.1002/bbb.4.

[24] S. F. S. Reihani and K. Khosravi-Darani. Influencing factors on single-cell protein production by submerged fermentation: A review. *Electronic Journal of Biotechnology*, 37:34–40, 2019.

[25] Anna Haug, Arne T Høstmark, and Odd M Harstad. Bovine milk in human nutrition–a review. *Lipids in health and disease*, 6:25, 2007. doi: 10.1186/1476-511X-6-25.

[26] Reeta Rani Singhania, Jitendra Kumar Saini, Reetu Saini, Mukund Adsul, Anshu Mathur, and Deepak Kumar Tuli. Waste derived bioeconomy in india: A perspective. *Renewable and Sustainable Energy Reviews*, 41:763–774, 2015.

[27] Arijit Das, Tathagata Paul, Suman Kumar Halder, Arijit Jana, Chandan Maity, Pradeep Kumar Das Mohapatra, Bikash R. Pati, and Keshab C. Mondal. Utilization of agro-industrial wastes for the simultaneous production of single cell protein and xylanase by thermomyces lanuginosus. *3 Biotech*, 4(1):41–47, 2014.

[28] ZY Zhang, B Jin, and JM Kelly. Production of lactic acid and byproducts from waste potato starch by rhizopus arrhizus: role of nitrogen sources. *World Journal of Microbiology and biotechnology*, 23:229–236, 2007.

[29] G. Suman, M. Nupur, S. Anuradha, and B. Pradeep. Single cell protein production: A review. *International Journal of Current Microbiology and Applied Sciences*, 4:251–262, 2015.

[30] Marc J. E. C. van der Maarel. *Starch-Processing Enzymes*, chapter 14, pages 320–331. John Wiley & Sons, Ltd, 2009. ISBN 9781444309935. doi: https://doi.org/10.1002/9781444309935.ch14. URL https://onlinelibrary.wiley.com/doi/abs/10.1002/9781444309935.ch14.

[31] O.A. Adetunji E.F. Aransiola, E. Betiku and B.O. Solomon. Myproduction of baker's yeast (saccharomyces cerevisiae) from raw cassava starch hydrolyzates in a bioreactor under batch process. 5(1):98–103, 2006. doi: 10.3923/biotech.2006.98.103.

[32] Vera Meyer, E. Basenko, J. Philipp Benz, Gerhard Braus, Mark Caddick, Michael Csukai, R.P. Vries, Drew Endy, Jens Frisvad, Nina Gunde-cimerman, Thomas Haarmann, Yitzhak Hadar, Kim Hansen, Robert Johnson, Nancy Keller, Nada Krasevec, Uffe Mortensen, Rolando Perez, Arthur Ram, and Han Wosten. Growing a circular economy with fungal biotechnology: A white paper. *Fungal Biology and Biotechnology*, 7, 04 2020. doi: 10.1186/s40694-020-00095-z.

[33] Quorn™: a story about single cell protein. controlledmold.com, 2022. URL https://controlledmold.com/quorn-a-story-about-single-cell-protein/.

[34] Si-Jing Wang and Jian-Jiang Zhong. Bioreactor engineering. In *Bioprocessing for value-added products from renewable resources*, pages 131–161. Elsevier, 2007.

[35] Beth Junker, Michael Lester, James Leporati, John Schmitt, Michael Kovatch, Stan Borysewicz, Waldemar Maciejak, Anna Seeley, Michelle Hesse, Neal Connors, et al. Sustainable reduction of bioreactor contamination in an industrial fermentation pilot plant. *Journal of bioscience and bioengineering*, 102(4):251–268, 2006. URL https://www.sciencedirect.com/science/article/pii/S1389172306706624.

[36] P. M. Doran. *Bioprocess Engineering Principles*. Elsevier, 2013.

[37] Asha Byju Thomas, Trupti Dattatray Shetane, Ranu Goutam Singha, Rabindra K Nanda, Sushilkumar S Poddar, and Ajinath Shirsat. Employing central composite design for evaluation of biomass production by fusarium venenatum: In vivo antioxidant and antihyperlipidemic properties. *Applied biochemistry and biotechnology*, 183:91–109, 2017. URL https://link.springer.com/article/10.1007/s12010-017-2432-5.

[38] AK Athnasios and M Quantz. Yeasts. *Ullmann's Encyclopedia of Industrial Chemistry*, 2000.

[39] Shona L Pahl, Alison K Lee, Theodoros Kalaitzidis, Peter J Ashman, Sanjeev Sathe, and David M Lewis. Harvesting, thickening and dewatering microalgae biomass. In *Algae for Biofuels and Energy*, pages 165–185. Springer, 2013.

[40] Top quality centrifugal pump water pump clean water pump factory. alibaba.com, 2023. URL https://www.alibaba.com/product-detail/Top-Quality-Centrifugal-Pump-Water-Pump_1600082716073.html?spm=a2700.galleryofferlist.normal_offer.d_title.7b16e4e4q7LH4H.

[41] Gavin Towler and Ray Sinnott. *Chemical engineering design: principles, practice and economics of plant and process design*. Butterworth-Heinemann, 2021.

[42] Vacuum and pressure drum filters, yu and pyu. https://www.andritz.com/group-en, 2023. URL https://www.andritz.com/products-en/group/separation/disc-drum-filters/vacuum-drum-filter-yu.

[43] C. Baker and K. McKenzie. Energy consumption of industrial spray dryers. *Drying Technology - DRY TECHNOL*, 23:365–386, 02 2005. doi: 10.1081/DRT-200047665.

[44] Price of a jet cooker. Alibaba.com, 2023. URL https://www.alibaba.com/product-detail/Liquefication-Jet-Cooker-For-Starch-Syrup_1600106409829.html.

[45] Lab flask for start of inoculum line. sigmaaldrich, 2023. URL https://www.sigmaaldrich.com/NO/en/product/sigma/cls431255.

[46] Matches. matche.com, 2014. URL https://matche.com/equipcost/Filter.html.

[47] Gjennomsnittlig månedslønn i ulike yrker, fordelt på sektor. SSB, 2023. URL https://www.ssb.no/arbeid-og-lonn/lonn-og-arbeidskraftkostnader/statistikk/lonn.

[48] Soy protein concentrate spc 65%/ concentrate soy protein. alibaba.com, 2023. URL https://www.alibaba.com/product-detail/soy-protein-concentrate-spc-65-concentrate_1600311963098.html?spm=a2700.galleryofferlist.normal_offer.d_title.22b16d9c07xym7.

[49] Guzhen Zhou, Wuyang Hu, and Wenchao Huang. Are consumers willing to pay more for sustainable products? a study of eco-labeled tuna steak. *Sustainability*, 8(5):494, 2016. ISSN 2071-1050. URL https://www.mdpi.com/2071-1050/8/5/494.

[50] Lab course Biochemical Engineering TBT4140. NTNU, 2022.

[51] Benkun Qi, Jianquan Luo, and Yinhua Wan. Chemical conversion of molasses for production of levulinic acid and hydroxymethylfurfural. *Research and Advances: Environmental Sciences*, 2018.

[52] Annelies E. van Diepen, Jacob A. Moulijn, and Michiel Makkee. *Chemical Process Technology*. Wiley, 2013.

Appendix

A Mass Balance Calculations

A.1 Black Box Model

Assuming that there is no production of secondary products as the main focus here is the biomass production, the black box model gives the following stoichiometric reaction with glucose as a carbon source:

$$C_6H_{12}O_6 + a\,O_2 + b\,NH_3 \longrightarrow c\,CH_{1.8}O_{0.5}N_{0.2} + d\,CO_2 + e\,H_2O \tag{A.1}$$

Where a,b,c,d and e are the stoichiometric coefficients for oxygen, nitrogen source, biomass, carbon dioxide and water respectively. Based on this reaction it is possible to set up elemental balances for the different elements:

Carbon:

$$6 = c + d \tag{A.2}$$

Hydrogen:

$$12 + 3b = 1.8c + 2e \tag{A.3}$$

Oxygen:

$$6 + 2a = 0.5c + 2d + e \tag{A.4}$$

Nitrogen:

$$b = 0.2c \tag{A.5}$$

Which gives us five unknowns and four equations. It is not possible to solve for five unknowns with only four equations, and so we need to introduce another equation:

$$Y_{xs} = \frac{c \cdot Mw_{biomass}}{Mw_{glucose}} \tag{A.6}$$

Where Y_{xs} is the biomass yield based compared to the substrate, c is the stoichiometric coefficient for biomass, and Mw_x is the molecular weight where x is either biomass or glucose. The calculations for the molecular weights are shown in equations A.7 and A.8, and the values are given in Table A.1. The biomass yield found in the literature is stated to be at 56% [37]. For these calculations the yield was assumed to be around 95% of the value found in the literature, and it is also given in Table A.1.

Glucose:

$$Mw_{glucose} = 6 \cdot 12[g/mol] + 12 \cdot 1[g/mol] + 6 \cdot 16[g/mol] \tag{A.7}$$

Biomass:

$$Mw_{biomass} = 12[g/mol] + 1.8 \cdot 1[g/mol] + 0.5 \cdot 16[g/mol] + 0.2 \cdot 14[g/mol] \tag{A.8}$$

Table A.1: Values used to calculate the stoichiometric coefficient c. The calculation for the molecular weight of glucose and biomass are shown in equations A.7 and A.8. The biomass yield is around 95% of the value found in the literature.

	Value	**Unit**
$Mw_{glucose}$	180	[g/mol]
$Mw_{biomass}$	24.6	[g/mol]
Y_{xs}	0.54	-

By inserting the values in Table A.1 into a modified version of equation A.6, we can calculate the stoichiometric coefficient c:

$$c = \frac{Y_{xs} \cdot Mw_{glucose}}{Mw_{biomass}} = \frac{0.54 \cdot 180\ [g/mol]}{24.6\ [g/mol]} \tag{A.9}$$

Which gives us that c = 3.95. By substituing c into equations A.2, A.3, A.4 and A.5 we can calculate the stoichiometric coefficients for oxygen, N-source, CO_2 and water, and they are all given in Table A.2.

Table A.2: All the stoichiometric coefficients a,b,c,d and e were calculated using modified versions of equations A.4, A.5, A.9, A.2 and A.3 respectively.

Stoichiometric coeff	Value
a	1.85
b	0.79
c	3.95
d	2.05
e	3.63

The full black box model with all of the stoichiometric coefficients are given in equation 1 in section 3.

The stoichiometric coefficients can then be used to determine the amount of glucose, N-source and oxygen needed to sustain the growth of *F. venenatum* in the reactors.

A.2 Pre-processing of Starch

In the pre-processing stage, the raw materials undergo initial treatment to prepare them for the subsequent stages of the bioprocess. The main purpose of this step is to ensure that the feedstock is suitable for the cultivation of the fungal biomass. This section will outline the mass balance calculations for the pre-processing phase, considering the primary input and output streams.

During the pre-processing stage, the starch feedstock is hydrolyzed into glucose by the action of amylases. This enzymatic hydrolysis can be represented by the following equation:

$$n \cdot C_6H_{10}O_5 + n \cdot H_2O \xrightarrow{Amylases} n \cdot C_6H_{12}O_6 \tag{A.10}$$

In equation A.10, n represents the molar amount of the starch, and the conversion of the starch ($C_6H_{10}O_5$) into glucose ($C_6H_{12}O_6$) is facilitated by the presence of amylases and water (H_2O). The mass balance calculations involve determining the required amount of amylases and water for the hydrolysis process, as well as calculating the expected glucose output based on the initial starch input. The convertion rate of starch to glucose is 97%.

Table A.3: The conversion rate of starch to glucose

	Inn		**Out**	
	Starch	Water	Glucose	Rest
Moles	1	1	1	0
Theoretical weight	162 g	18 g	180 g	0 g
Actual weight	162 g	18 g	174.6 g	5.4 g

To perform these calculations, it is necessary to know the molecular weights of the compounds involved and the initial mass of the starch feedstock. Additionally, the efficiency of the amylases and the extent of hydrolysis must be considered. Based on this information, the mass balance can

be calculated for each input and output stream, allowing for a better understanding of the material flows and requirements in the pre-processing stage of the bioprocess plant.

A.3 Air

The hourly air consumption was calculated using the stoichiometric relationship between glucose and oxygen, 1:1.85. The number of glucose moles per reactor per hour is 4629.6 [moles/h], which corresponds to 8570.5 [moles/h] of oxygen needed. Using the molar mass of oxygen, we find that 274.5 [kg/h] of pure oxygen is required. Assuming air composition with 21% oxygen:

$$\frac{274.5\ [kg/h]}{0.21} = 1306\ [kg/h] \tag{A.11}$$

1306 [kg/h] of air is needed to sustain aerobic growth. Additionally, a 20% excess is supplied to the reactor to avoid oxygen limitation, resulting in a total air intake of 1567.2 [kg/h] per reactor.

The black box calculations were also used to estimate the amount of CO_2 produced, with a glucose to CO_2 ratio of 1:2.05. Using the same number of moles of glucose per reactor yields:

$$4629.6\ [moles/h] \cdot 2.05 = 9485.1\ [moles/h] \tag{A.12}$$

Multiplying 9485.1 [moles/h] by the molecular weight of CO_2 (44 [g/moles]) results in 417.3 [kg/h] of CO_2 produced. Additionally, 79% of the incoming air consists of inert N_2 gas, making the total mass of the outlet air 1655.4 [kg/h].

A.4 Minerals

The media calculations were conducted by considering only the components needed in larger quantities, such as nitrogen (N), phosphorus (P), sulfur (S), potassium (K), and sodium (Na). It is assumed that the trace ions, such as calcium, iron, chloride, zinc, etc., found in the water added to the glucose slurry and used to dilute the media components are sufficient to promote growth. The composition of *F. venenatum* is assumed to be similar to the biomass composition of *E. coli* [50]. Moreover, it is assumed that the amounts of P, S, K, and Na needed are so small that they do not affect the black box calculations. Thus, the same stoichiometric coefficient c = 3.95 is used for these calculations.

Table A.4: Composition of *F. venenatum*. The left column shows the elements, and extra, used for the media. The right most column shows the elements on a C-mol basis, used to calculate the stoichiometry as with the black box model.

Element	Percent[%]	C-mol basis
Carbon (C)	53	1
Nitrogen (N)	12	0.22
Sulphur (S)	1	0.019
Phosphorous (P)	3	0.057
Magnesium (Mg)	0.5	0.009
Calcium (Ca)	0.5	0.009
Potassium (K)	1	0.019
Sodium (Na)	1	0.019
Chloride (Cl)	0.5	0.009
Iron (Fe)	0.2	0.004

By adding the c-mol basis numbers from Table A.4 to the black box model we get the following biomass:

$$CH_{1.8}O_{0,5}N_{0,2}S_{0,019}P_{0,057}K_{0,019}Na_{0,019}Mg_{0,009} \tag{A.13}$$

The media components are specified in section 3.4, which gives the following elemental balances:

KH_2PO_4:

$$g = 0.019 \cdot c \tag{A.14}$$

Na_2HPO_4:

$$2h = 0.019 \cdot c \tag{A.15}$$

$MgSO_4 \cdot 7H_2O$:

$$i = 0.009 \cdot c \tag{A.16}$$

These calculations does not include nitrogen (N) og sulphur (S), as this stocihiometric coefficient is found in the simplified version of the black box model (coefficient b). However:

$$1\ mole\ (NH_4)_2SO_4\ = 2\ moles\ NH_4^+ \tag{A.17}$$

Which was accounted for in the amount of ammonium needed.

Table A.5: Stoichiometric coefficients for KH_2PO_4, Na_2HPO_4 and $MgSO_4 \cdot 7H_2O$ calculated from equations A.14,A.15,A.16

Stoichiometric coefficient	**Value**
g	0.075
h	0.038
i	0.037

Finally, it was possible to relate the components with the glucose through stoichiometry, and the mass for needed per hour for all of the components were calculated.

A.5 Check of Mass Conservation

Table A.6 illustrates the mass conservation for the whole process. The relative difference between the total mass flow in and out is 0.06% and the mass flow can thus be considered balanced.

Table A.6: Conservation of mass flows in the process.

Component	**Mass in** $[kg/h]$	**Mass out** $[kg/h]$
90% Starch	5 727	-
Water, Na_2CO_3, $CaCl_2$	9 455	-
α-amylase	0.5	-
Water (for dilution)	119 817	-
Ammonium sulfate (and other media components)	1 449	-
Air	41 911	-
CO_2, N_2	-	42 440
Wastewater centrifuge	-	122 428
Wastewater filter	-	4 761
Water out of dryer	-	5 836
SCP (90% solids)	-	3 006
Total	**178 360**	**178 471**

The concervaton of mass was calculated using equation:

$$\dot{m}_{out} = \dot{m}_{in} + \dot{m}_{generated} - \dot{m}_{consumed} \tag{A.18}$$

B Monod Kinetics

B.1 Batch Inoculum Line

The four reactors (R-103 to R-106) are operated in batches. The growth of biomass is assumed to follow Monod kinetics for cell culture in a batch process:

$$\frac{dx}{dt} = (\mu - kd) \cdot x \tag{B.1}$$

where x is:

$$x = x_0 e^{(\mu_{max} - kd) \cdot t} \tag{B.2}$$

For the batches it is assumed that there is no or negligible cell death, simplifying equation B.1:

$$t_b = \frac{1}{\mu_{max}} \cdot ln \frac{x_f}{x_0} \tag{B.3}$$

By substituting the values discussed in section 3.3 into equation B.3, we get a batch time of 10.89 hours.

B.2 Continuous for Reactors

Reactors (R-107 to R-112) are operated as continuous reactors. Since the operating time is 1000 hours, lag phase was assumed to be negligible. the mass balance on cell basis gives:

$$F \cdot x_i - F \cdot x + \mu x \cdot V - k_d x V = 0 \tag{B.4}$$

Where F is the flow rate, x_i and x are initial biomass yield and x is the final biomass, V is the volume, μ is the growth rate while k_d is the cell death number. The initial biomass $x_i = 0$, the final biomass concentration was assumed to be $x_f = 20$ [g/L,] and cell death is assumed to be less than growth rate, simplifying equation B.4:

$$\mu \cdot x \cdot V = Fx \tag{B.5}$$

Further, it is assumed that the reactors operate at steady state close to 100% of the time, which means that μ (growth rate)= D (dilution rate). This simplifies equation B.5:

$$D = F/V \tag{B.6}$$

Residence time:

$$\tau = \frac{1}{D} \tag{B.7}$$

C Energy Balance Calculations

C.1 Aerobic Metabolism

The energy balance equation for a cell culture is:

$$-\Delta H_{rxn} - M_v \Delta h_v - Q + w_s = 0 \tag{C.1}$$

Where ΔH_{rxn} is the heat of reaction, $M_v h_v$ is evaportaion, Q is heat to be removed (power) and W_s is shaft work. Since the reactors are operated at T= 30 °C we assume that evaporation is close to zero, and is negligible. Further, the reactors (R-107 to R-112) are all airlift, and so there is no shaft work. This simplifies the equation:

$$Q = -\Delta H_{rxn} \tag{C.2}$$

Where heat production is directly proportional to O_2 consumption, and the energy released per mole O_2 consumed is 460 KJ.

D SCP Production with Molasses

Molasses is the conventional carbon source used for SCP production by Quorn [12]. Both sugar cane and sugar beet molasses can be used. Table D.1 illustrates the average composition of sugar cane molasses [51]. This composition is used as the basis for the calculations ahead and sugar cane molasses is further referred to as molasses.

Table D.1: Composition of sugar cane molasses

Component	Percent [%]
Water	18.8
Sugars	52.4
Organics	17.7
Inorganics	11.1

The only difference between the process design for SCP production from molasses as compared to PPW (see section 3) is the pre-processing. It is therefore only this part that is described in this appendix.

D.1 Pre-processing of Molasses

Figure D.1 shows a process flow diagram of the pre-processing of molasses [52]. Table D.2 illustrates the major equipment used in the process, with respective sizes and prices. The raw molasses slurry is initially stored in storage tank TK-101. Water is added to dilute the raw molasses. This simplifies the pumping and fermentation. HCl is added to reach a pH of 4, where some organic materials precipitate [52]. The slurry is mixed in mixer M-101 before getting centrifuged in disk stack centrifuge S-101. Insolubles are removed during the centrifugation. The slurry is further heated rapidly in jet cooker JC-101 at a temperature of 137 °C, before staying in holding tubes for a couple of minutes to ensure proper sterilization [52]. The steam used here originates from the dryers in the downstream processing, as described for the PPW plant in section 3. The sterilization is performed to reduce the risk of contamination in the process, especially during fermentation. Later, the sterilized molasses slurry is cooled to 30 °C in cooler CO-101, a temperature considered ideal for the fermentation. Finally, water is added to dilute the molasses slurry to the necessary concentration before it is sent to the reactors for fermentation.

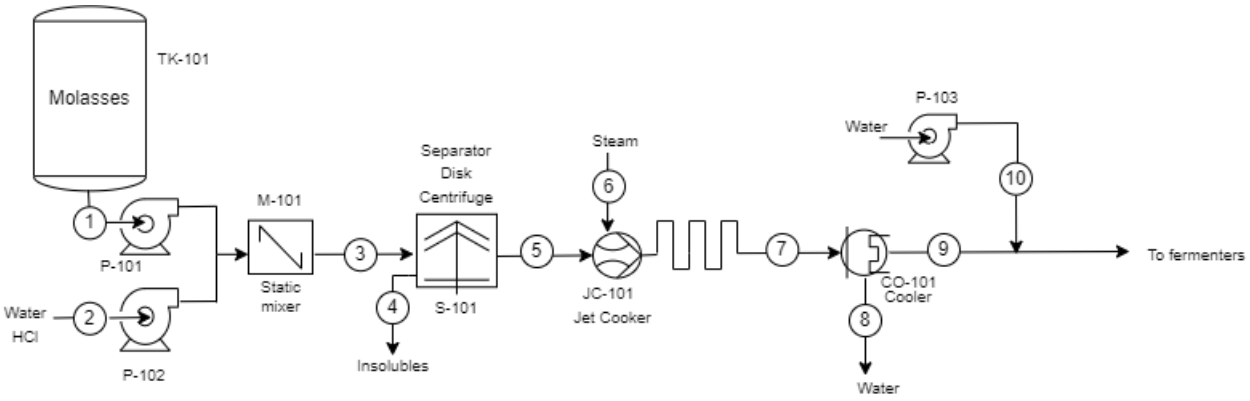

Figure D.1: Process flow diagram of molasses pre-processing.

Table D.2: Equipment list and prices for pre-processing stage for molasses. The footnote indicate source of equipment price.

Equipment	**Name**	**Amount**	**Size**	**Price** [USD]
Pre-processing				
Storage tank	TK-101	1	1 300 m^3	295 140
Pump	P-101	1	2.1 L/s	10 003
Pump	P-102	1	7.1 L/s	11 532
Mixer	M-101	1	9.2 L/s	4 112
Centrifuge	C-101	1	41 cm	378 962
Jet cooker	JC-101	1	30 m^3	12 800[a]
Cooler	CO-101	1	31 L/s	244 665
Pump	P-103	1	15 659 L/s	15 659
Total				**972 873**

[a] Alibaba[44]

D.2 Mass Balance of Molasses

Figure D.3 illustrates the calculated mass flows for the streams in the pre-processing of molasses from Figure D.1. These were calculated in the same way as for the PPW-based process described in Appendix A and section 2.

Table D.3: Flowrate of all streams in pre-processing of molasses

Stream	**Flow rate** [kg/h]	**Components**
S1	9 542	Cane Molasses Slurry
S2	15 873	Water, HCl
S3	25 415	Cane Molasses Slurry
S4	1 689	Insolubles
S5	23 726	Cane Molasses Slurry
S6	-	Steam In
S7	23 726	Cane Molasses Slurry
S8	23 726	Cane Molasses Slurry
S9	-	Steam Out
S10	11 274	Water
S11	135 000	Cane Molasses Slurry

D.3 Cost Estimation and Sensitivity Analysis of Molasses

Table D.4 shows the total investments of the molasses plant.

Figure D.2 presents the sensitivity analysis of revenue, total costs, and investments affecting the NPV of the molasses plant. This figure provides a big-picture view of the project's economic performance in response to changes in these critical variables. The analysis highlights the importance of managing revenues, controlling costs, and optimizing investments to ensure project profitability.

Figure D.3 focuses on the sensitivity analysis of fixed costs and components of variable costs affecting the NPV. This figure breaks down the variable costs into their components: fixed costs, raw materials and electricity. It shows that raw materials and fixed costs have the most significant impact on the NPV, emphasizing the need to optimize these costs to maximize the project's economic performance.

Table D.4: Total investments for SCP production using molasses.

Category	**Price** $[USD]$
Total Investment	**120 571 119**
ISBL	45 975 641
OSBL	18 390 257
Engineering	12 873 180
Contingency	19 309 769
Working capital	9 654 885
Operating cost	**21 587 122**
Variable cost	11 279 975
Fixed cost	10 307 147

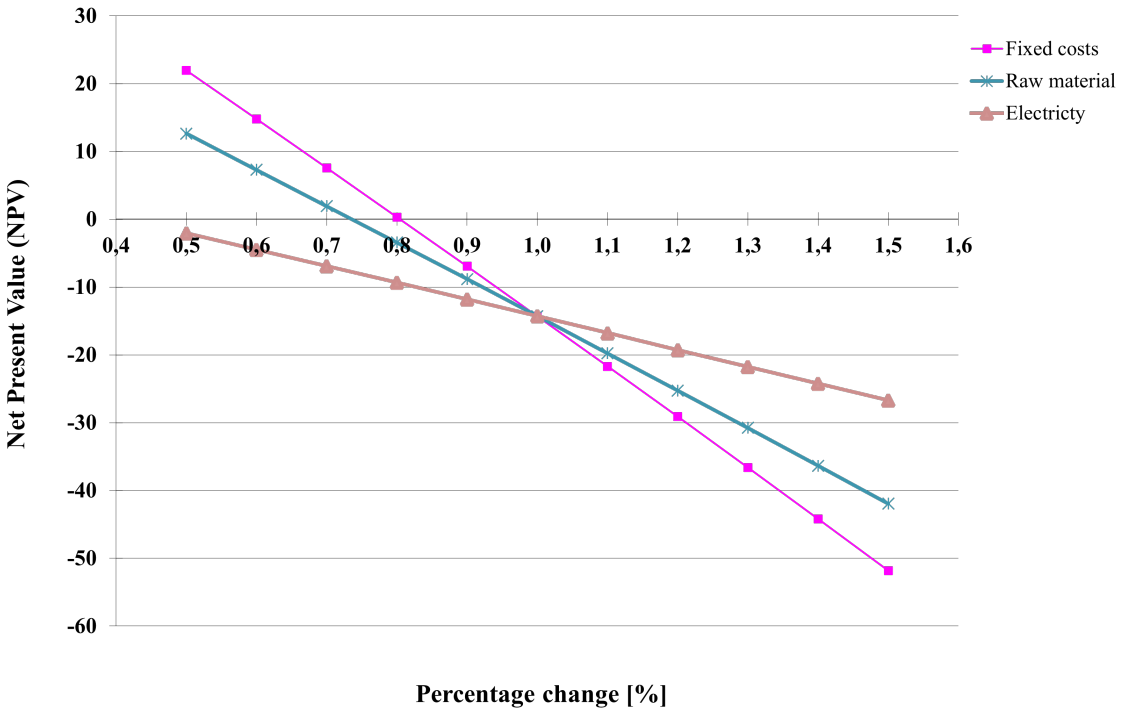

Figure D.2: Sensitivity analysis of fixed and variable costs affecting the NPV using molasses.

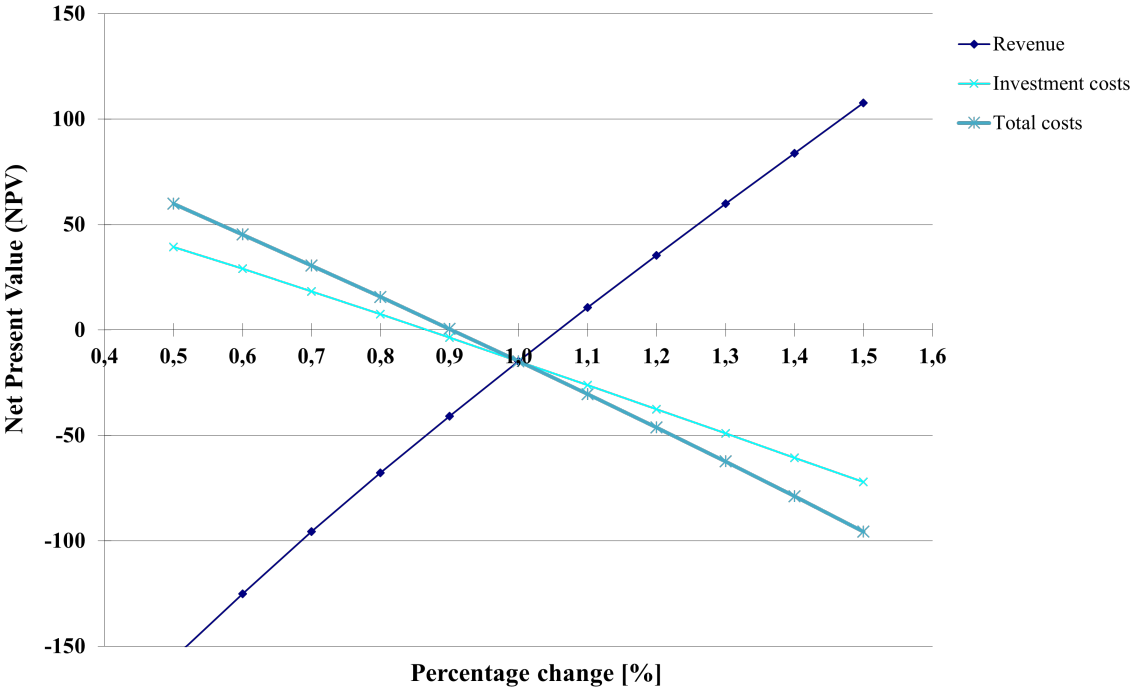

Figure D.3: Sensitivity analysis of revenue, total cost and investment costs affecting the NPV using molasses.

E Email Correspondence with Tine

Gmail **Lars Føleide <zyronbackup@gmail.com>**

Myse til NTNU

Mathilde Ulrikke Søderholm Thu, 23 Mar at 12:56
<Mathilde.Ulrikke.Soderholm@tine.no>
To: Aleksander Mittet <aleksandermittet@gmail.com>, lars.foleide@gmail.com <lars.foleide@gmail.com>, jonama@stud.ntnu.no <jonama@stud.ntnu.no>, andreeas.wold@ntnu.no <andreeas.wold@ntnu.no>

Hei igjen,

Her kommer litt mer info!

- Tilvirkningskosten er på ca. 50 øre/liter
- Transportkostnaden er ca. 50 øre/liter (her bruker vi tankbiler)
- Prisen ligger på ca. 1-2 kr/liter. Men varierer veldig ut ifra tørrstoffinnholdet.
- Sur myse utgjør en liten andel av mysene i TINE. Vi får kun surmyse fra Cottage Cheese, og produksjonen av Cottage Cheese var på ca. 7,6 millioner liter i fjor.
- Sur myse har en litt høyere verdi enn søt myse grunnet kvaliteten/holdbarheten. Vi har f.eks en kunde som er villig til å betale 5-10 øre mer for sur myse.
- Anlegget som produserer mest myse er TINE Jæren med ca. 28 millioner liter i 2021.

Mvh Mathilde

TINE SA

[Quoted text hidden]