Conceptual Design and Operation of an Integrated Mycoprotein Fermentation Process Focusing on Side Stream Resource Recovery

Master Thesis
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Cover image: Made using Gencraft with the prompt "A food industry factory in a field. The field is covered in multicolored magic mushrooms"

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Populärvetenskaplig sammanfattning

Svampens guldgruva: Från avlopp till fenomenal hållbarhet!

Mycoprotein är ett köttalternativ som de flesta svenskar ätit någon gång i sitt liv. Men trots att det är betydligt mer miljövänligt än vanligt kött har det fortfarande stora brister speciellt när det kommer till vattenutsläpp. I en värld där rent vatten är en bristvara finns det mycket att vinna på att kunna återvinna vattnet men kanske går det också att skapa nya värdefulla produkter samtidigt.

Marknaden för köttalternativ har växt enormt mycket de senaste åren och kommer bara att bli större. En av de stora spelarna är den svampbaserade produkten mycoprotein som är en fantastisk källa för protein och omättade fetter. Kommersiellt går den under många namn men är kanske mest känd under märket “Quorn”. Produkten har funnits sedan 1985 och många framsteg har gjorts när det kommer till produktionen av svampen men än så länge finns det knappt någon information om dess avloppsvatten. Då över 50% av jorden befolkning lever i områden med hög vattenstress samt många av dem även i områden där vattenstress skapat proteinsvält, hade det varit gynnsamt om vattnet och kanske andra avloppsrester från mycoprotein-produktionen kunde återanvändas eller säljas.

Följaktligen gjordes det en studie på potentiella alternativ för återvinning och återanvändning av avloppsvatten från en mycoprotein-produktion. Arbetet gjordes i samarbete med Tetra Pak, som har påbörjat nya projekt inom biomassa-fermentering. Via ett simuleringsprogram på datorn byggdes det upp en fermenteringsprocess för mycoprotein. Detta användes sedan för att utvärdera vattenavloppet då ingen tidigare gjort några tester på dess innehåll. Därefter skapades det några olika alternativ för att behandla vattnet bland annat med hjälp av evaporatorer och membran (sil med extremt små hål), som även dessa simulerades. Ur detta hittades fyra produkter: vatten till återanvändning, gödsel, näring till återanvändning, och fiskmat.

Tyvärr gav inget av alternativen någon vinst men då det endast var simuleringar samt första arbetet utfört på detta området finns det mycket potential för förbättring. Alternativen hade definitivt kunnat öka mycoproteinets hållbarhet, speciellt produktionen av gödsel. Dessutom hade vattenåteranvändning kunnat möjliggöra att processen används på ställen där det råder protein- och vattenbrist. Skapandet av fiskmat hade kunnat minska behovet av småfisk som foder, vilket hade lett till mer hållbara fiskodlingar. Dessutom hade fiskmaten kunnat fungera väl som även hund- och kattmat. Arbetet är en start på förhoppningsvis många framtida arbeten för att förbättra hanteringen av mycoproteinets avloppsvatten.

Abstract

Mycoprotein from the fungi Fusarium Venenatum A3/5 has been used as a meat alternative for a long time but little attention has been paid to the waste it creates. When the fungi is fermenting it uses a lot of water which is sent to the drain after the process is finished. This water contains large quantities of both nutrients and biomass. As circular economy is becoming more important for environmental sustainability, these wastewaters offer great potential for resource recovery. The aim of this report was therefore to evaluate the feasibility of resource recovery from a semi-batch production of mycoprotein. A conceptual design of the process was made using SuperPro Designer to get an idea of the wastewater characteristics. A framework for deciding which resource recovery option to choose, based on techno-economic analysis and sustainability, was also created. This was then used to simulate and evaluate four different resource recovery scenarios in SuperPro Designer. Two of them used membrane filtration. One was only recovering water and the other one was recovering water and creating fertilizer at the same time. Another option was to reuse the water and nutrition using a treatment of membranes, ion exchange, and activated carbon. The last tested resource recovery method was to create fish feed powder using evaporation and spray drying. A modified conceptual design of the downstream production line was also tested where pasteurization of the fermentation broth was done directly after the fermenter, instead of after several dewatering stages.

The conceptual design predicted three wastewater streams, the first two with low concentrations of biomass but high amounts of water and nutrients and one stream with low amounts of water but with a lot of biomass. Water (76-82 wt% recovery) and fertilizer with a nutritional value of 20.5:5.5:3.5 ($N:P_2O_5:K_2O$) were extracted using the first two streams. The operating cost of only water recovery was too high to be compensated by the lowered fresh water need. By creating fertilizer as well, the total emissions were greatly lowered and the cost of operation was almost met. To decrease the cost of these treatments a sterilizing method that does not use heat could be used as pasteurization was by far the largest expense. Reuse of nutrition could not be profitable using the treatment method in this study. The ion exchange was too expensive both in capital cost and operating costs due to the large amount of salt in the wastewater. Fish feed creation from the third stream was the most economically feasible option even though it also did not compensate for its operating costs. The biomass recovered in the fish feed was about 30 wt%. The modified conceptual design showed that heat treatment before would lower the required amount of heat treaters needed but make the wastewater streams more similar which would make separation for resource recovery more difficult. None of the resource recovery options had a positive net present value. This could potentially be changed by assuming other selling prices, especially for the fish feed, or changing some of the cost parameters of the simulation which might not have been applicable such as the cost for yard improvements.

List of Abbreviations

BOD	Biological oxygen demand
CIP	Clean in place
COD	Chemical oxygen demand
DFC	Direct fixed capital
DW	Dry weight
FDC	Facility-dependent costs
GAC	Granular-activated carbon adsorption
MF	Microfiltration
MT	Metric tone
NF	Nanofiltration
NPV	Net present value
RO	Reverse Osmosis
PFD	Process flow diagram
TDS	Total dissolved solids
TEA	Techno-economic analysis
thOD	Theoretical oxygen demand
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TP	Total phosphor
TRL	Technology readiness level
TS	Total solids
TSS	Total suspended solids
UF	Ultrafiltration
WW	Wet weight

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1 Introduction

1.1 Problem Formulation

The process of creating mycoprotein from Fusarium Venenatum varies between companies but all include some type of inoculation of the fungi, growth of the fungi, heat treatment for RNA-reduction and sterilization, and removal of excess water. A lot of research has been conducted on the growth of the fungi as well as the final product but the wastewater has been largely ignored. As the population and standard of living are growing the demand for more efficient resource handling is increasing, which makes it imperative for companies to limit, recycle, or repurpose their waste. For mycoprotein, this might not only be environmentally but also economically beneficial, as the waste contains vast amounts of protein, nutrients, and water. (McGeorge, 2019)

1.2 Aim

The report aims to create a conceptual design of a semi-batch mycoprotein fermentation in SuperPro Designer to postulate potential methods for resource recovery from its wastewater streams. A framework of how to evaluate the techno-economic feasibility and sustainability of resource recovery will also be created and used to assess the options for mycoprotein wastewater. The selected options as well as a potential heat treatment modification on the existing process will be simulated and evaluated using SuperPro Designer.

2 Background

Mycoprotein created from the filamentous fungi Fusarium Venenatum A3/5 can be found in most European grocery stores as a meat alternative. The process of creating mycoprotein has been around for a long time and has been commercially sold since 1985. (Finnigan et al., 2017) The largest manufacturer of mycoprotein is Marlow Foods Ltd producing mycoprotein by the name "Quorn" but there are several other mycoprotein businesses such as Mycorena, MycoTechnology, Inc., Kernel Mycofoods, and others. (Choudhury, 2022) The fungi has been used as a meat substitute due to its hyphae having similar width and length as animal muscle fibers and the lack of mycotoxins. (Wiebe, 2002) The biomass produced by Fusarium Venenatum is usually referred to as mycoprotein which is the RNA-reduced fungi. (Moszczyński & Tabarowski, 2018) The mycoprotein contains a high percentage of protein (40-60 wt% dry weight) with a bioavailability matching that of animal protein (Dunlop et al., 2017; Wikandari et al., 2022). As the European demand for protein is said to double by 2050 and mycoprotein provides a more sustainable protein alternative, it has gained a lot of interest. (Eitfood, 2023) The market for mycoprotein has been increasing steadily over the years and has a projected market size for 2023 of 976 Million USD and a compound annual growth rate between 2022-2023 of 12.6%, far outreaching the average for the food industry in that period (-1.4%) and the expected food industry growth for 2023-2028 (3.4%). (Choudhury, 2022; *Statista*, n.d.) The waste produced by mycoprotein will increase proportionally with this growth and as such more investigation on the treatment of the waste is needed.

2.1 Processes

2.1.1 Quorn Process

There are different ways of producing mycoprotein from Fusarium Venenatum, however, the by far most referenced in the literature is the Quorn process. The Quorn process is a continuous fermentation where the fungi are grown in an airlift reactor, to which nutrients and oxygen are constantly fed and biomass is removed. The concentration of biomass in the reactor is about 10-15 g/L wet basis. The biomass is then heat treated at 68℃ for 20-45 minutes to reduce the RNA content from ～8 wt% to under the allowed limit of 2 wt%. (Lonchamp et al., 2022; Risner et al., 2023) This heat treatment step also causes the cells to be leaky, which causes a loss of up to 30% in biomass in the following centrifuge. In the centrifugation step, the water is reduced to about 75 wt%. (Finnigan et al., 2017) The mycoprotein is then sent to a vacuum chiller which freezes the biomass at 0℃. Depending on the intended usage, the biomass will then undergo other procedures to make the final product. (Risner et al., 2023) The process is usually run for 6 weeks, at which the highly branched mycoprotein becomes more dominant compared to the sparsely branched. (Finnigan et al., 2017)

2.1.2 Semi-batch Process

The process that will be used as a base for the simulation is done semi-batch-wise over 22 hours The process begins with the inoculation of the Fusarium Venenatum. It then goes through three stages of seed fermenters before entering the final large fermenters. After it has grown, the tank is emptied and the excess water is mostly removed from the broth containing the biomass in two consecutive drainer washers before it enters a heat pasteurization module where the fungi are simultaneously killed and the RNA is lowered to the allowed limit of 2 wt%. The final excess of water in the mycoprotein is thereafter removed in a parallel set of decanters and the product is frozen to create the meat-like texture. A simplified block diagram of the process can be seen in Figure 1.

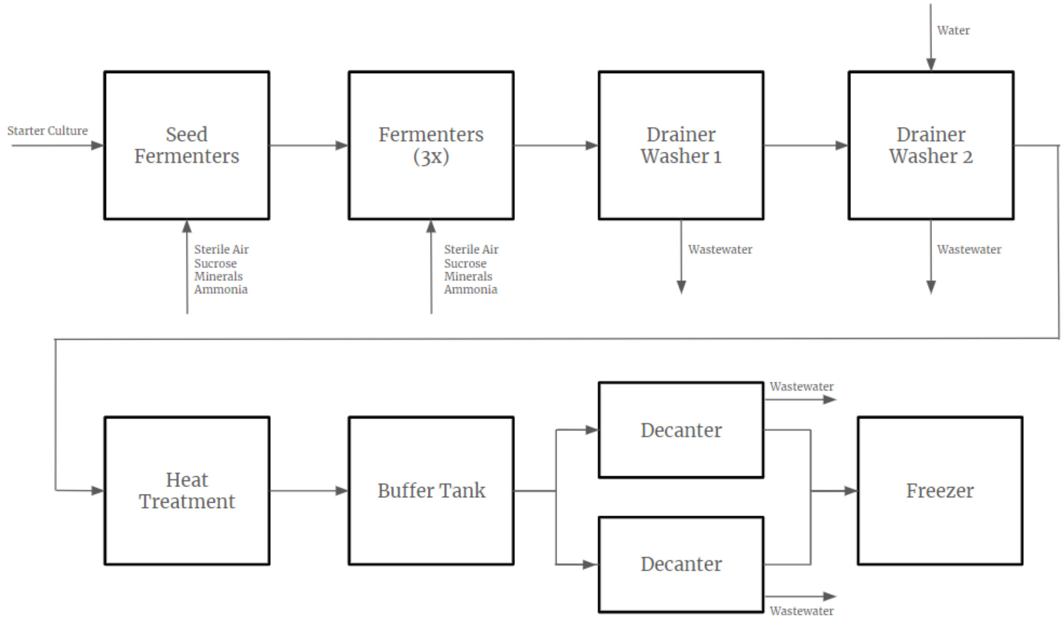


Figure 1. Block flow diagram of the simulated process.

2.2 Circular Economy

In March 2020 the European Commission agreed on the circular economic action plan (CEAP), which is a large step towards sustainable growth. The plan includes monitoring product consumption within the EU but also restricting industrial waste. (*European Commission*, n.d.) Unlike linear economy, circular economy focuses on a cradle-to-cradle approach where the waste created should be able to be partly or fully reused in a new product's “life cycle”. This approach applies to users of the product, where a circular economy would entail recycling the product or reusing it for another purpose, as well as industries that should have efficient resource handling and processing of waste. (Baratsas et al., 2022) For example, the food industry stands for a total of 5 million tonnes or 11 kg per person of food waste annually in the EU. (*European Commission*, 2023) Globally, food production wastes have reached about 320 million tonnes per year and is predicted to reach 16 tonnes per second in 2030. (Sridhar et al., 2021) A lot of the industry's food waste is present in their wastewater. For example, the amount of protein present in food processing wastewater is enough to supply the entire global aquaculture, not to mention the amount of potential fuel lost in starchy waters. (Durkin et al., 2022) As such, resource recovery from wastewater is a vital part of the incorporation of a circular economy in food processing.

2.2.1 Water Scarcity

As of 2023 at least half of the world's population live in high water-stressed conditions. (Kuzma et al., 2023) High water stress in the case of countries such as Singapore, which have laws and regulations governing technological developments in such a way that water is highly reused and recycled, does not lead to a water crisis. Nonetheless, 60% of irrigated crops are grown in regions with extremely high water stress. Food scarcity due to water shortages is already a large issue in many developing countries where millions face starvation. (Kuzma et al., 2023)

With only about 777 L/kg, mycoprotein uses considerably less water than other high-protein alternatives such as beef (15415 L/kg), chicken (4325 L/kg), and tofu (2523 L/kg). Thus, mycoprotein is a lot more suitable for water-stressed regions. (Finnigan et al., 2017) However, the current mycoprotein fermentation processes still require high amounts of water compared to vegetables (322 L/kg), and might therefore not be viable for regions with extremely high water stress. Should there be a way to reuse the water, the freshwater requirement could potentially become lower than that of vegetables and provide a more water-efficient food source. (McGeorge, 2019)

Furthermore, the most common type of malnutrition in the world is kwashiorkor and marasmus, which are directly related to a lack of protein/energy. (Muller, 2005) As mycoprotein offers a high protein content, does not depend on the weather, and is suitable with most religions' dietary restrictions, it could be of great use for countries suffering from drought-based starvation. However, drought-based regions usually must restrict water usage, which may still cause issues regarding the process.

2.3 Techno-Economic Analysis

Techno-economic analysis (TEA) is done to evaluate the economics of a process to compare financial gain or costs. It can for example be used to compare new technology to existing ones or to support decisions regarding the purchase of certain equipment. (*US Department of Energy*, n.d.) In this section, a framework for techno-economical and sustainability analysis of resource recovery is presented.

2.3.1 Decision Support Framework

For wastewater, which might contain a lot of different components, the most economically viable resource recovery option could be difficult to determine by only assessing the components. As such, for this study and future studies of resource recovery, a decision diagram was created to aid in the assessment of sustainable and techno-economically good resource recovery alternatives. The diagram is based on a model done by Silk et al. (2020) but has been modified to fit the circumstances of this thesis. Some of these changes include adding an assessment of resource recovery where multiple components can be sold as a whole instead of separating them each individually, removing the economic calculations for which the company has its own programs, and adding technology readiness level (TRL) to the wastewater stream separation methods. Figure 2 below shows the decision support framework, with each pane being described below.

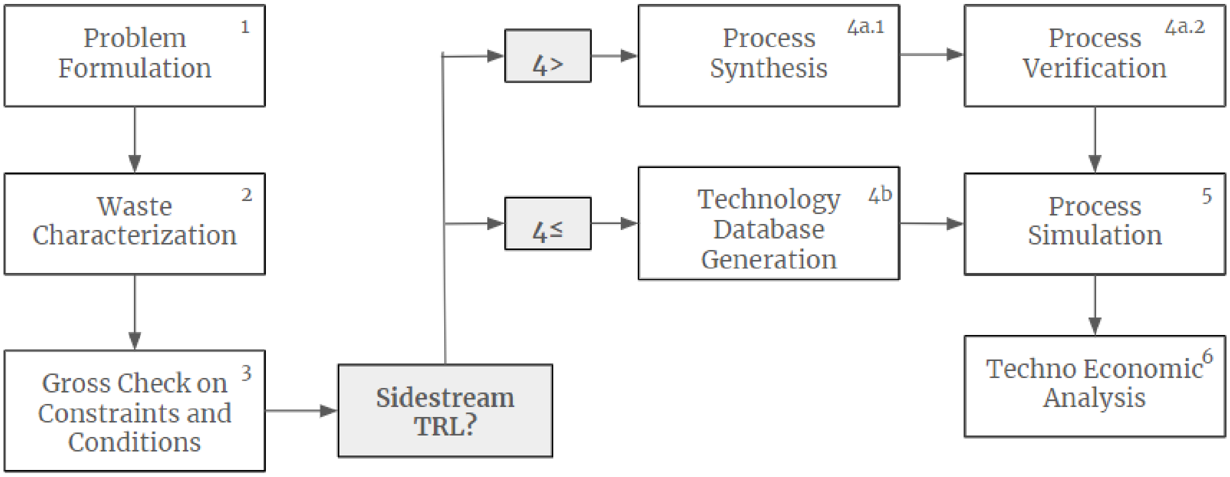


Figure 2. Decision support framework for TEA and sustainability of resource recovery.

Step one is the problem formulation. In this phase, the reason for wanting to do a resource recovery is defined, such as environmental concerns, legal concerns, and potential profit. The end objective is also defined here. For example, if the end objective is to create a fertilizer, it might not be necessary to specify all molecules present in the stream or to separate them. However, this should be done if the objective is general profitability. Furthermore, mathematical conditions that should be maximized such as profit or environmental benefits are defined alongside constraints such as minimal profitability or maximum water reuse.

In the second step, all information available about the streams applicable for resource recovery is gathered either from experiments or literature reviews. In this stage, any potential reactants, inhibitors, and dangerous components should be identified. The degree to which this is done is based on the objective defined in the last step. For example, if it is to be used for fish food, components that might be harmful to the fish should be tracked.

In the third step, the environmental impact of components in the stream like chemical oxygen demand (COD), total organic carbon (TOC), presence of heavy metal, sulfate content, etc, should be evaluated. These values should be monitored to see if they exceed the limit for the environmental objective defined in Step 1. The cost of sending this to communal wastewater should also be calculated. Likewise, the potential profit of each component or group of components should be defined in this step. The components/group of components that maximize the conditions defined in step 1 and that do not defy any constraints, should be considered first for resource recovery. If it does not yield good results further on in the diagram, the second best option should be chosen, then the third, and so on.

In the first decision gate of the diagram, the TRL of the sidestream separation is determined by studying the literature on the intended separation. A TRL of 4 means that the technology has been tested and validated on a lab scale. (*European Commission*, 2020) If the TRL is less than 4 then the next step is process synthesis. If the TRL is 4 or above then the next step is technology database generation.

To synthesize a separation process from the beginning requires knowing what thermodynamic properties distinguish the different components. From this, a potential separation based on the largest thermodynamic differences can be made, such as a distillation sequence for differences in volatility. Jaksland et al. (1995) describe this method in detail. In general, the separation with the largest differences in properties will be the least expensive as well. (Silk et al., 2020) The second step to process synthesis is process verification, in which a literature review is performed to determine the feasibility of such a separation. This could include determining if azeotropes exist, if there are heat-sensitive components, or if any of the equipment can be destroyed by a certain component.

For a technology database generation, on the other hand, a literature review should be conducted to find all separation possibilities that have been tested. Thereafter, they should be selected based on how well they satisfy the conditions and constraints from step 1.

When a separation method has been chosen, it should be simulated in a suitable program to get an idea of stream sizes and effectivity. This simulation should be used as a basis for techno-economic analysis. If it does not adhere to the required constraints or if there might be a solution that fulfills the conditions better, another separation process should be chosen or if this does not yield better results another component/group of components in step 3 should be chosen.

2.4 Resource Recovery

Resource recovery is a vital part of having a circular economy. As the production of mycoprotein leads to a lot of biomass and nutrient-rich wastewater, multiple resources could be recovered. (Finnigan et al., 2017) There are multiple components in the wastewater such as salts, sucrose, biomass, in the shape of cells, leaked intracellular compounds, and ammonia. These also come in varying concentrations in the different wastewater streams. Consequently, an evaluation of the most economical and sustainable treatment for each stream is needed.

2.4.1 Nutritional Value

All added chemicals to the process are well-defined except for the biomass and its intracellular components. In this section, an overview of the biomass composition is therefore given in order to get a better understanding of the potential uses except for human consumption. The usual mycoprotein nutritional constitution can be seen in Table 1, where wet weight (WW) assumes 75 wt% water content and 0 wt% water content in dry weight (DW).

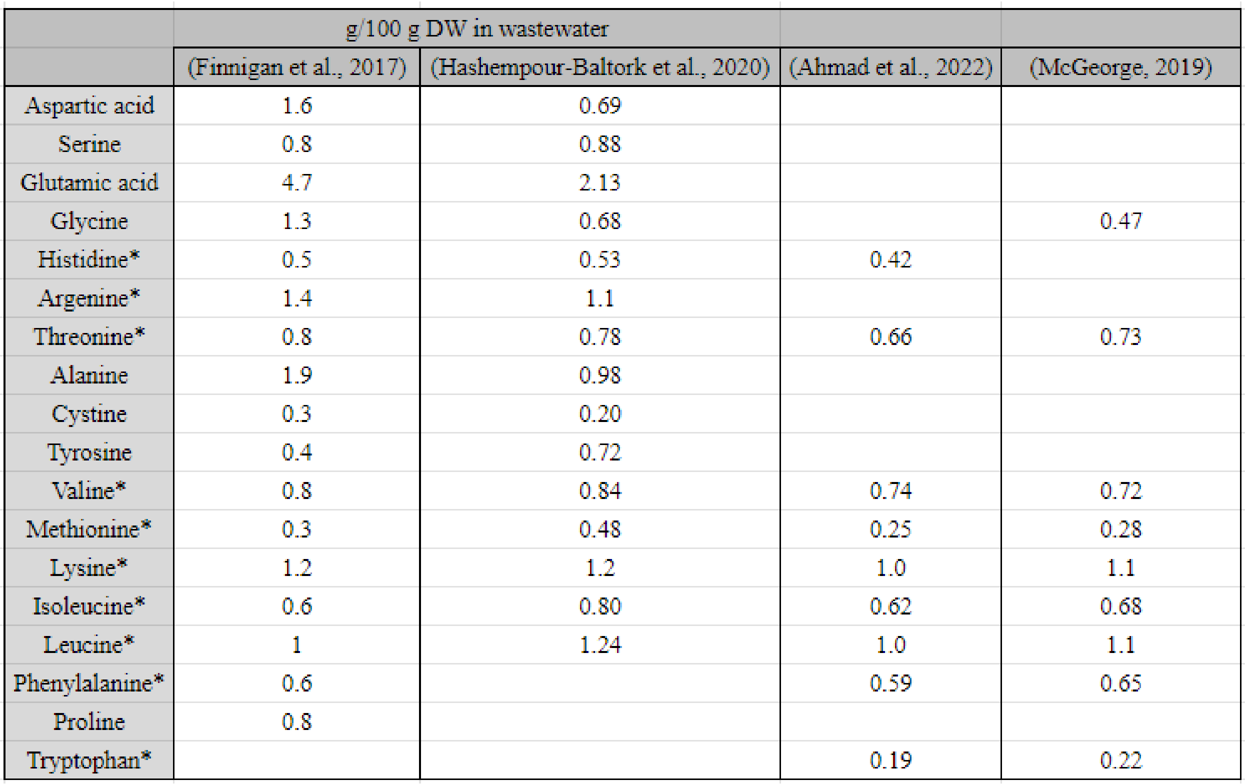
Table 1. Standard mycoprotein nutritional content.(Ahmad et al., 2022)

	g/100 g DW	g/100 g WW

Moisture	0	75
Protein	45	11.25
Saturated fatty acids	2.4	0.6
Total fat	11.6	2.9
Total fiber	24	6
Sugar	2	0.5
Total carbohydrate	12	3
Kcal	340	85

It can be seen from the table that mycoprotein is both rich in protein and unsaturated fats. Furthermore, several studies have been made on the specific amino acid distribution of mycoprotein which can be seen in Table 2. The asterisk after some amino acids shows the amino acids that are required in high amounts in fish food. (Craig and Helfrich, 2002)

Table 2. The amino acid constitution of mycoprotein

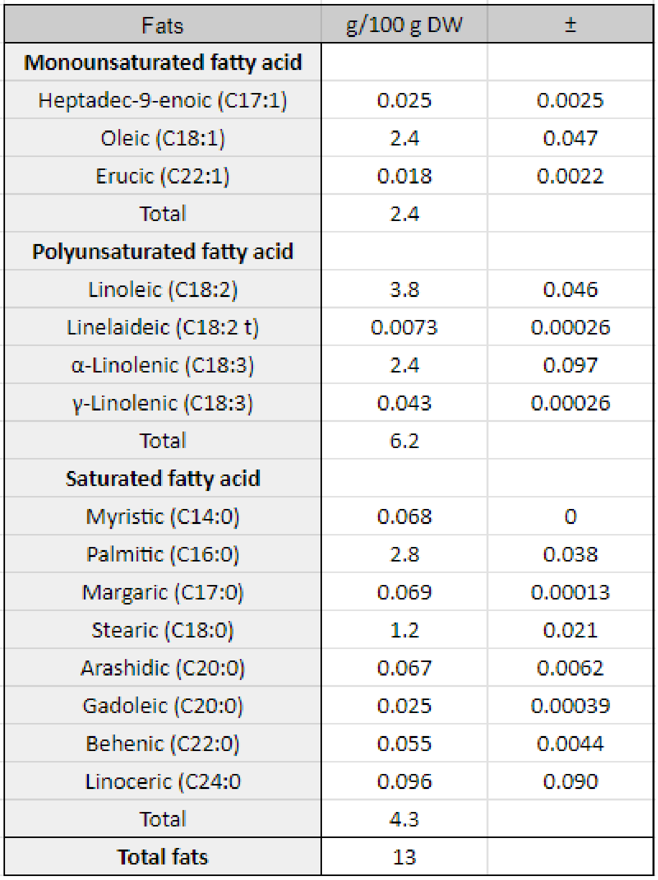


	g/100 g DW in wastewater			
	(Finnigan et al., 2017)	(Hashempour-Baltork et al., 2020)	(Ahmad et al., 2022)	(McGeorge, 2019)
Aspartic acid	1.6	0.69		
Serine	0.8	0.88		
Glutamic acid	4.7	2.13		
Glycine	1.3	0.68		0.47
Histidine*	0.5	0.53	0.42	
Argenine*	1.4	1.1		
Threonine*	0.8	0.78	0.66	0.73
Alanine	1.9	0.98		
Cystine	0.3	0.20		
Tyrosine	0.4	0.72		
Valine*	0.8	0.84	0.74	0.72
Methionine*	0.3	0.48	0.25	0.28
Lysine*	1.2	1.2	1.0	1.1
Isoleucine*	0.6	0.80	0.62	0.68
Leucine*	1	1.24	1.0	1.1
Phenylalanine*	0.6		0.59	0.65
Proline	0.8			
Tryptophan*			0.19	0.22

**Important in fish feeds*

Similarly, studies have been made on the fat contents of mycoprotein, which can be seen in Table 3.

Table 3. Fat composition in mycoprotein. (Hashempour-Baltork et al., 2020)



Fats	g/100 g DW	±
Monounsaturated fatty acid		
Heptadec-9-enoic (C17:1)	0.025	0.0025
Oleic (C18:1)	2.4	0.047
Erucic (C22:1)	0.018	0.0022
Total	2.4	
Polyunsaturated fatty acid		
Linoleic (C18:2)	3.8	0.046
Linelaideic (C18:2 t)	0.0073	0.00026
α-Linolenic (C18:3)	2.4	0.097
γ-Linolenic (C18:3)	0.043	0.00026
Total	6.2	
Saturated fatty acid		
Myristic (C14:0)	0.068	0
Palmitic (C16:0)	2.8	0.038
Margaric (C17:0)	0.069	0.00013
Stearic (C18:0)	1.2	0.021
Arashidic (C20:0)	0.067	0.0062
Gadoleic (C20:0)	0.025	0.00039
Behenic (C22:0)	0.055	0.0044
Linoceric (C24:0	0.096	0.090
Total	4.3	
Total fats	13	

The mineral content of mycoprotein can be seen in Table 4.

Table 4. The mineral content of dry-weight mycoprotein.(Finnigan et al., 2017)

Minerals	g/kg DW biomass
Ca	1700
Mg	1850
K	4000
Na	200
Fe	30
Zn	400
Cu	1050
Mn	20

2.4.2 Fertilizer

Before evaluating separation methods the end goal needs to be defined, which requires identifying what the components in the wastewater could be used for. As the wastewater stream will contain only dissolved solids, except for biomass and ammonia, separating each component will most likely be very costly. The biomass as a large suspended solid could be separated using filters and gravimetric methods, as previously showcased in the process by the drainer washer and decanter. Ammonia has a low boiling point and can be removed by distillation. The dissolved nutrients on the other hand have very similar thermodynamic abilities and would therefore be difficult to separate. (Patnaik, 2003) As such the resource recovery will focus on options where the content of the stream can be used directly after dewatering and with minimal removal of a specific component.

One such option is using the wastewater to produce fertilizer. The most important nutrient in plant fertilizer is ammonia followed by phosphorus and potassium. The content of these is given as weight ratios N:P_2O_5:K_2O. Ammonia is usually supplied as either ammonium nitrate, calcium ammonium nitrate, urea, ammonium sulfate, or nitrate salts. As these would all require extra processing to create from aqueous ammonia, they are likely not very economically viable although it would have to be tested. (Levy, 2023) Aqueous ammonia can be used directly as a fertilizer in a 25 to 29 wt % NH_3 solution but is less common due to transportation costs. (*Oklahoma State University*, n.d) Only 10%-15% of nitrogen is supplied in liquid form in the UK. In this report, it will be assumed that the mycoprotein plant is close enough to agriculture so that aqueous ammonia is a viable fertilizer. (Levy, 2023) Monopotassium phosphate can be used directly as a phosphor and potassium source and has a nutrient content of 0:53:34. (*North Dakota State University*, 2010; Schrödter et al., 2000) Other nutrients required in a larger amount are sulfur, calcium, and magnesium. Both sulfur and magnesium can be supplied via magnesium sulfate and extra nitrogen and sulfur can be supplied via ammonium sulfate (20:0:0). (Levy, 2023; *North Dakota State University*, 2010) Calcium is usually supplied as lime but can be given as calcium chloride, which has a quick solubility. (*Anorel*, 2024) Trace minerals such as copper, iron, chlorine, molybdenum, manganese, boron, and zinc, which are usually supplied as salts, are also required. (Levy, 2023) However, to be able to use the wastewater for fertilization, the sugar content needs to be kept low. While a certain amount of sucrose can be beneficial to the growth of crops, it can also reduce the biodiversity of the soil and attract insects. (Gao et al., 2022; Morrow et al., 2019) Gao et al. (2022) found that a 3% sucrose solution gave the highest yield for soapberry growth.

2.4.3 Fish Feed

Another viable option if there is a lot of biomass in the stream, is creating fish feed. Aquaculture is the fastest-growing food industry and is also highly dependent on protein availability. Furthermore, the demand for sustainable options for fish feed is very high as commercial fishing has already caused overfishing of several smaller fishes that are used in the feed. (Eitfood, 2023)

The most expensive part of fish food is the protein, with lysine and methionine, often being the most limiting amino acids. Fish have 10 essential amino acids, all of which can be found in mycoprotein (see Table 2). Usually, the content of the fish feed is composed of 18-50% protein, 10-25% fats, 15-20% carbohydrates, less than 10% trace nutrients, less than 7% fibers, and less than 1.5% water. (Craig and Helfrich, 2002) Although depending on the fish, these values can vary tremendously, for example, grouper fish feed usually needs 40-45% protein, 1-10% fat, 0.1-8% fiber, and 0.8-95.5% water, which is closer to the nutritional values of mycoprotein (see Table 1). Unsaturated fats are also highly beneficial for fish, which there is a lot of in mycoprotein. (Soong, 2016) To be able to use the wastewater streams for fish feed, the ammonia concentration would, however, need to be minimized as it is toxic to fish at concentrations of 0.05 mg/L and above. Additionally, ammonia contributes to algae growth. (*FDACS*, n.d.) Commercial fish feed is also usually supplied as either granules pellets or powder, powder usually being the cheapest option as it does not require extra processing to get its shape. (*Yushunxin*, 2023) Fish feed can not only be used for fish but is also regularly mixed into livestock feed as an extra protein source. (*ACAF*, 2001)

2.4.4 Reuse of Nutrition

McGeorge (2019) did a study on the possibility of reusing the centrate (wastewater from centrifugation) from the Quorn process, where the centrate was sterilized by either filtering or autoclavation, and harmful metabolites were removed by activated carbon. The glucose and ammonia content was then replenished in the recycled centrate to the concentration found in the original broth, while the minerals were added in the same amount as in the original broth. It is important to note that no RNA reduction step was performed in the study, which could affect the composition of the centrate. The activated carbon could successfully remove 30% of the total amino acid content and 100% of the nucleotides. Both the filtered and autoclaved centrate had an increase in biomass yield with a 77% and 90% increase respectively, compared to the non-recycled broth. The reason for the increase in biomass could be because not all of the amino acids were removed and could be used as a nutrient but it could also be because of the higher amount of minerals present in the recycled broth. However, branching was also affected by using the recycled media, which in turn affected the texture of the mycoprotein. (McGeorge, 2019) McGeorge also found that calcium and iron affect the branching of the fungi and if the concentration of these were increased in the recycled centrate, it could have caused the morphology change. Users of the process studied in this thesis have had similar results using water that has been recycled, indicating that there is a large risk with recycling unless all components are measured and replenished each time so that the broth stays constant. (Ghanbari, 2024)

2.4.5 Other Resource Recovery Options

The most crucial recovery is the water which would decrease mycoproteins' water footprint, allowing it to be produced in high water-stressed areas but also the carbon footprint of the associated water treatment in communal wastewater facilities. Different potential uses of the wastewater which will not be explored in this report is using it as an emulsifier, foaming agent, gelling agent, and as an umami flavoring. (Finnigan et al., 2017)

2.5 Wastewater Treatment Methods

Food industry wastewater tends to be very complex and can vary a lot between different branches. Subsequently, there are a lot of wastewater treatment methods used such as gravimetric separation, coagulation, adsorption, oxidation, evaporation, microbiological degradation etcetera. However, recently more and more industries are using membranes as they offer a more sustainable and cost-effective treatment method. They have the advantage of not needing any extra chemical additives while being relatively fast and compact compared to options such as bioreactors and granular filtration. They are also non-destructive to the components in the wastewater, which makes them especially useful for resource recovery. (Pervez et al., 2021)

When treating wastewater with membranes, several filtration steps are generally used in decreasing size to reduce fouling. For example, the largest component in the stream would be the whole fungi cells, which could be removed by microfiltration (MF). Some intracellular components such as proteins and DNA would be stopped by ultrafiltration (UF), while the dissolved solids except monovalent ions would be stopped by nanofiltration (NF). Lastly, reverse osmosis (RO) would remove everything except for water. (Lipnizki, 2019)

2.5.1 Demineralization

Due to the risk of salts present in reused water affecting the hyphae structure, extra care needs to be taken to make sure that it is not present in any substantial concentrations. Common methods for demineralization include membrane filtration, distillation, solvent extraction, and freezing. However, these methods are very costly for diluted streams such as the one in question. Instead, methods such as precipitation, electrosorption, and ion exchange are more suitable for dilute streams, although they usually are more compound-specific. (Alkhadra et al., 2022) Precipitation is specific and also requires the addition of another chemical. Electrosorption, on the other hand, is adsorption aided by an electrical field and has a broader range of ions it can adsorb. It is also specifically used to treat reverse osmosis permeate. (Alkhadra et al., 2022; *Water Innovations*, n.d.)

Ion exchange binds ions while releasing a substitute ion back into the feed. While ion exchange typically binds either cations or anions, mixed bed ion exchangers that can remove both also exist and are typically used as demineralization after reverse osmosis. Such a system can achieve a resistivity of 18.2 MΩ·cm. A downside is that alkalis and acids are needed for regeneration. (Alkhadra et al., 2022; *Purolite*, n.d.)

2.5.2 Sterilization

Since the wastewater stream from the drainer washer will not have undergone pasteurization, the fungi will still be alive. Therefore it needs to be sterilized before being used as a product. This can be done by heat treatment as in the main process part. Other methods include ozone, chlorination, and ultraviolet radiation. (Wen et al., 2020)

2.6 Legal Specifications

Apart from the environmental and potential economic benefits of reusing water, water treatment might be legally required. For example, sulfates and ammonia which might exist in high concentrations, are both corroding to concrete, and consequently, the amount allowed in industry wastewater in Sweden is 60 mg/L ammonia and 400 mg/L sulfates. The conductivity also has a maximum limit of 500 mS/m to avoid corrosion of steel. As there might be a lot of biomass in the wastewater the limit for suspended solids also needs to be considered as it can cause blockages. In Sweden, this limit is 40 mg/L. (*Svenskt vatten*, 2019) Both the total phosphorus and total nitrogen content are limited as well to 0.3 mg/L and 10 mg/l respectively. (*Mittskanevatten*, 2021) There are limits on the biological oxygen demand (BOD) to COD ratio as well, to ensure that the material is easily degradable. As the organic components of the stream are either sugar or fungi, it will be assumed that they can be degraded easily.

3 Method

3.1 Conceptual Design

Before any simulation of resource recovery could be tested, a general framework for the existing process had to be made. As there were no studies on the wastewater streams from this process, the framework would be the only guidance to what might exist in them. Furthermore, unit operations that would not directly affect the wastewater streams such as seed fermenters, freezers, and water sterilization, were omitted from the simulation. As such, the final framework only consists of the main fermenters, drainer washers, heat pasteurization, decanter, and some pretreatment to simplify future changes to the recipe. The simulation is done as a batch process over 22 hours with 340 batches a year. The actual process is semi-batch but due to the lack of knowledge on the fermentation reaction kinetics, batch was chosen instead and the fermentation time was shortened to limit the time to 22 hours. Unless otherwise specified, the presets in SuperPro Designer were used.

3.1.1 Streams

Determining the streams that would enter the fermenter was the initial step in the simulation process. In the real process, different streams of water, substrate, and minerals are mixed before entering the fermenter. For this purpose, blending tanks were put in but the economy and process time were not included in the results of the simulation as only the downstream process was of interest. The mineral stream was created by mixing water with minerals in powder form. These minerals were then added to a substrate mixture which was also created by blending powder substrate and water. The components found in the mineral and substrate stream can be seen in Table 5. Sodium molybdate was omitted from the simulation due to very low concentrations.

Table 5. Components of the mineral and substrate powders.

Minerals	Substrate
EDTA	Ammonium sulfate
Calcium chloride	Monopotassium phosphate
Zinc sulfate	Magnesium sulfate
Iron sulfate	Calcium chloride
Boric acid	
Manganese sulfate	
Copper Chloride	
Potassium iodine	
Sodium molybdate	

A blending tank was also used to create the sucrose stream of 66.5 wt% and aqueous ammonia of 24.5 wt%. The substrate/mineral stream, ammonia stream, and sucrose stream were then connected to the fermenter together with a stream of process water and air. The air was preset to 0.5 VVM by SuperPro Designer.

3.1.2 Fermentors

The fermentors consist of 3 tanks, which were simulated as parallel units (pink icon next to the fermenter in Figure 3). The tank operates at 35°C. (Ghanbari, 2024) The reactor starts with an initial amount of Fusarium Venenatum seed in it. The same amount is left after the fermentation for the next batch. This was not simulated as it does not change the outcome and adds a lot of complexity in terms of removing the broth and only partly the biomass.

3.1.3 Reactions

To get an approximation of what might be left of the nutrients in the broth after fermentation, the uptake of nutrients and conversion to biomass needed to be estimated. This was done by calculating a stoichiometric reaction for the fermentor based on values found in the literature. Dry mycoprotein has the empirical formula $CH_{1.98}O_{0.647}N_{0.173}P_{0.0151}$ (MW=27.221 g/mol). (Upcraft et al., 2021) It also had a experimental maximum yield on sucrose of 0.342 g biomass/ g sucrose or 4.35 mol/ empirical biomass/ mol sucrose for an initial seed size of 10% v/v, which is the same amount used in the real process. (Thomas et al., 2017) The only carbon source is sucrose, the nitrogen source is both ammonium sulfate and ammonia, and the phosphorus is from monopotassium phosphate. From this, an elemental balance could be set up as follows using the non-ionic versions of the components and assuming optimal growth:

$$1\text{ sucrose} + 0.0657\ H_3PO_4 + 0.753\ NH_3 + 22.69\ O_2 \rightarrow 4.295\text{ Biomass} + 7.65\ CO_2 + 15.84\ H_2O$$

The reaction enthalpy for this reaction was set to -3700 kcal/kg based on the oxygen conversion. (Ferreira et al., 2023) To get the phosphoric acid and ammonia used in the above reaction, the following dissociation reactions were also added:

$$(NH_4)_2SO_4+2H_2O\rightarrow 2NH_3+SO_4^{2-}+2H_3O^+$$
$$KH_2PO_4 + H_2O \rightarrow H_3PO_4 + KOH$$

To estimate the mineral consumption, the theoretical maximum produced dry weight of biomass was calculated, based on the empirical balance and the assumption that sucrose is the limiting reactant and will be fully consumed. This value was then multiplied by the amount of trace mineral present in the mycoprotein according to Table 4 to get an estimation of how much mineral will be used to create the mycoprotein. To get a stoichiometric value relating to the sucrose input, the mineral needed in mol was divided by the sucrose needed in mol for the same amount of biomass. The resulting stoichiometry can be seen in Table 6.

Table 6. The stoichiometry for the minerals is based on sucrose.

Minerals	Amount (mol/kmol Sucrose)
Ca	4.96
Mg	8.90
K	12.0
Fe	0.0628
Zn	0.715
Cu	0.0368
Mn	0.532

Only zinc, copper, and manganese were not supplied in excess and were therefore assumed to be fully consumed without limiting the reaction, as there are no studies on the effect on the yield the lack of these minerals would have. Usually, mycoprotein fermenters are limited by the amount of carbon source and not micronutrients. (Finnigan et al., 2017) Metals can only be absorbed by the fungi after dissolution, so reactions relating to the dissolution had to be taken into consideration. (Robinson et al., 2021) All the minerals will be fully dissolved at 35℃. (*Sigma-Aldrich*, n.d.) The solubility at 20℃ can be seen in Table 7.

Table 7. The solubility of the stream components.

Minerals	**Solubility (g/100 g H_2O (20°C))**	**Source**
EDTA	5.0	(*Acros Organics*, n.d.)
Calcium chloride	74.5	(Patnaik, 2003)
Zinc sulfate	53.8	(*Sigma-Aldrich*, n.d.)
Iron sulfate	26.6	(*Sigma-Aldrich*, n.d.)
Boric acid	5.0	(*Sigma-Aldrich*, n.d.)
Manganese sulfate	62.9	(Patnaik, 2003)
Copper Chloride	77.0	(*Sigma-Aldrich*, n.d.)
Potassium iodine	144.5	(*Sigma-Aldrich*, n.d.)
Sodium molybdate	65.3	(Patnaik, 2003)
Ammonium sulfate	75.4	(*Sigma-Aldrich*, n.d.)
Monopotassium phosphate	22.7	(*Sigma-Aldrich*, n.d.)
Magnesium sulfate	35.6	(*Sigma-Aldrich*, n.d.)
Sucrose	201.9	(Patnaik, 2003)

It was assumed that no reactions and precipitation would occur. The dissolution reactions were as follows:

$$MgSO_4 \rightarrow Mg^{+2} + SO_4^{-2}$$
$$CaCl_2 \rightarrow Ca^{+2} + 2Cl^{-}$$
$$ZnSO_4 \rightarrow Zn^{+2} + SO_4^{-2}$$
$$FeSO_4 \rightarrow Fe^{+2} + SO_4^{-2}$$
$$MnSO_4 \rightarrow Mn^{+2} + SO_4^{-2}$$

$$CuCl_2 \rightarrow Cu^{+2} + 2Cl^-$$

$$KI \rightarrow K^+ + I^-$$

$$KOH \rightarrow K^+ + OH^-$$

The dissolution reactions were not directly added, as SuperPro Designer does not have a database to simulate cations. They were instead indirectly added to other reactions. The mineral stoichiometry was added to the biomass empiric reaction and the added mass due to minerals was added to the empiric biomass to preserve the mass balance (27.685 g/mol). Due to the stoichiometric size difference, the reaction was split into two as follows:

$$4.96CaCl_2 + 8.90MgSO_4 + 0.0492ZnSO_4 + 0.0628FeSO_4 + 0.1353MnSO_4 + 0.00896CuCl_2 + 12.0KOH \rightarrow \text{Biomass minerals} + 9.94Cl^- + 12.0OH^- + 9.07SO_4^{2-}$$

and

$$1\ \text{sucrose} + 0.0657\ H3PO4 + 0.753\ NH3 + 22.69\ O2 + 0.001\text{Biomass minerals} \rightarrow 4.295\ \text{Biomass} + 7.65\ CO2 + 15.84\ H2O$$

Manganese sulfate, copper chloride, and zinc sulfate stoichiometry were changed to fit the amount added. Potassium is added both through the monopotassium phosphate and potassium iodide. As the added amount of monopotassium phosphate is 80,000 times larger than the added amount of potassium iodide, the potassium requirement will be assumed to be satisfied from the monopotassium phosphate alone. The concentration of biomass out of the reactor was known and was equivalent to a 54.7% sucrose conversion.

SuperPro Designer can keep track of intracellular and extracellular components. In the simulation, the reactions and separations involving biomass are recorded on a dry mass basis. The water content of the mycoprotein is however specified as 75 wt%, which is the final water content in the mycoprotein product. In the simulation, water will therefore always exist in at least a ¾ ratio together with mycoprotein in a stream to account for the intracellular water (unless fully dried).

3.1.4 Drainer Washers

The fermentation broth goes through two sets of drainer washers using Tetra Pak Drainer Washer 2. (*Tetra Pak*, n.d.-a) The broth is washed by water while it travels through the drainer washer by a conveyor belt that simultaneously acts as a filter allowing liquid to be drained out. In this process, only the second drainer washer will be washing and draining while the first only acts as a drainer. No specification on the amount of dissolved components or biomass that follows the liquid exists. As such, an assumption was made that the drainer washer only removes components by diluting the fermentation broth so that the water that is drained out has the same concentration of dissolved components as the broth left behind. This assumption has several disadvantages such as disregarding that the washing water might be pressurized, that it might have another temperature, and how long the contact time between the broth and washing water is. The first drainer washer reduces the broth by 90% while the second drainer washer reduces the broth together with washing water by 70%. There is no knowledge of how much biomass is removed in the drainers except from that there are losses. The losses of biomass were therefore assumed to be 0.9% and 0.7% respectively for the first and second drainer washers, based on a similar cheese curd process. (Ghanbari, 2024)

The percentage of removed dissolved components ($x_{removed}$) for the drainer washers could be calculated based on the following equations:

$$x_{removed} = \frac{M_{water}}{M_{water\ tot}} \qquad \text{eq. 1}$$

and

$$M_{removed} = x_{removed} \cdot M_{nutrition} + M_{water} + x_{removed\ biomass} \cdot M_{biomass} \qquad \text{eq. 2}$$

Where M_{water} is the amount of water removed from the drainer, both coming from the water added and the water which has been removed from the original stream, $M_{water\ tot}$ is the sum of the water in the fermentation broth and the added washing water, $M_{removed}$ is the mass of the liquid drained out, $M_{nutrition}$ is the mass of dissolved components (everything that is not biomass or water), $x_{removed\ biomass}$ is the percentage of biomass removed and $M_{biomass}$ is the ingoing amount of biomass (WW).

3.1.5 Pasteurization

Pasteurization was done at 85°C for 30 min. No changes in the stream happened in the simulation before and after pasteurization. However, it was assumed that the heat would cause a 30% leakage from the biomass, similar to that of the Quorn process. (Finnigan et al., 2017)

3.1.6 Decanters

A decanter centrifuge separates solids from a liquid by having a rotating axis that increases the g forces within the decanter, therefore allowing for faster sedimentation. (*Alfa Laval*, n.d.-a) The process includes two parallel decanters. As with the drainer washers, the only available information on the efficiency of the separation is the drained amount of liquid. A decanter separates particles based on the density difference between the solid and liquid, based on Stokes' law. (Bridges & Robinson, 2020) This model has several difficulties when it comes to the simulation, as the size and total non-empirical weight of the biomass are unknown. Hence, an estimation of the removal efficiency of the biomass can not be calculated using standard centrifugation equations. By assuming that the pasteurization has caused a 30% leakage and that the constitution of the leakage acts as a dissolved component in water, it is possible to get an idea of the removal efficiency. (Finnigan et al., 2017) Dissolved components such as sucrose, substrate, and minerals will not be affected by centrifugation. (Prabhu, 2022) The removal percentage (x) can followingly be calculated as

$$\square = \frac{\square_{\square\square\square\square\square\ \square\square\square\square\square\square\square}}{\square_{\square\square\square\square\square\ \square\square\square\square\square\square\square}} \qquad \text{eq. 3}$$

and

$$\square_{\square\square\square\square\square\square\square\square} = \square \cdot \square_{\square\square\square\square\square\ \square\square\square\square\square\square\square} + \square \cdot \square_{\square\square\square\square\square\square\square\square\square\square} + \square \cdot 0.3 \cdot \square_{\square\square\square\square\square\square\square\square} \qquad \text{eq. 4}$$

Where $M_{water\ removed}$ is the mass of water drained and $M_{water\ initial}$ is the extracellular water in the decanter feed.

It should be noted that the assumption that the material from the biomass leakage acts as a dissolved solid is a rather crude approximation since it constitutes both protein, fats, RNA, and fibers, which are not dissolvable and can be separated using centrifugation. Another assumption made in the simulation is that the leakage will have the same constitution as the original biomass. The leakage will mostly contain intracellular components and not components from the cell walls but as there is no study on the exact component release, it will be disregarded. (Finnigan et al., 2017) The final process flow diagram (PFD) of the conceptual design can be seen in Figure 3.

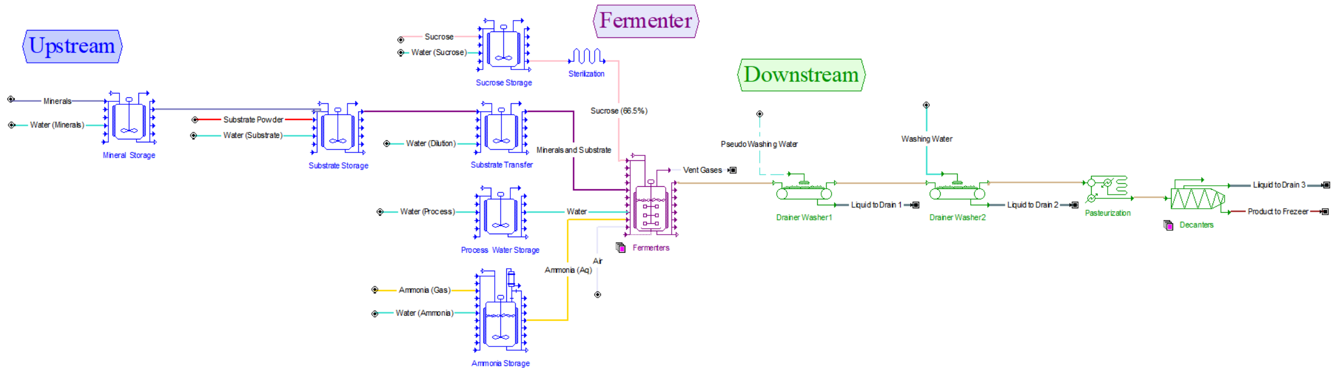


Figure 3. The PFD of the conceptual design.

3.2 Separation Method

There are three streams that go to the drain, one from drainer washer 1, one from drainer washer 2, and one from the decanters. The centrate from the decanters will, based on the assumption that 30% of biomass is lost, contain the largest amount of biomass and the least amount of water, while drainer washer 1 will have the highest amount of everything else. Due to the addition of water, drainer washer 2 will have the most dilute stream. As the streams have quite different concentrations, there is a potential for separate treatment methods for each stream and also different techno-economic objectives. For example, the stream from the decanters is suitable for biomass recovery for fish feed but less so for water recovery, while the stream from drainer washer 1 is more suitable for water recovery and use as fertilizer. However, the cost of separate systems might outweigh the product income. The sizing of all equipment was based on SuperPro Designer's inbuilt size calculations and the condition that only one unit be used (compared to several parallel units).

3.2.1 Water Reuse

The first option which was tested, was only reusing the water from the drainer washers by membrane filtration. As the wastewater from the decanters contained little water and high amounts of biomass, it was deemed unfavorable to add it to the water reuse system. Firstly, the two drainer wastewater streams had to be mixed using a mixer. Secondly, heat treatment (sterilizer (130℃)) taking a total of two hours was added to kill the microorganisms so that they could not grow on the membranes. Longer process time in this case lowers the capital cost due to lower throughput. This was followed by microfiltration to remove the whole cell, then nanofiltration to remove the majority of the ions, the rest of the biomass, and the ammonia and sucrose partially, and then reverse osmosis to remove the rest.

The microfiltration flux was set to 220 $L/m^2/h$ based on Tomasula et al. (2011) who removed Bacillus anthracis from milk. It was assumed that all whole cells would be retained but that the heating step had caused 30% of the biomass to become intracellular leakage, which would not be stopped by the membrane, and that the water recovery percentage was 95%, resulting in a rejection coefficient of 88%. (Tomasula et al., 2011)

The nanofiltration was based on Sjölin et al. (2019) study on the purification of sucrose from molasses. For 8 bar the flux was set to be 100 $L/m^2/h$ and the sucrose retention 60%. The $MgSO_4$ rejection was not specified for the membrane used in the study. Instead, nanofilters from Alfa Laval were used as a reference for salt rejection, which was ≥99%. (*Alfa Laval*, n.d.-b) As the individual rejections are hard to determine, it was assumed that all ions had a rejection of 99%. It was also assumed that the rejection of the biomass components was total. Ammonia was set to have a removal of 66% based on a study by Awadalla et al. (1994), which gave a rejection of 82%. The water recovery percentage was set to 90%.

The same condition for water recovery was also set for RO. However, according to PCI Membranes (2019), it is possible to concentrate sucrose to 30% using RO, which would mean a higher water recovery. The salt and sucrose rejection was set to 99.98%. (*PCI Membranes*, 2019) Awadalla et al. (1994) also found that ammonia removal for reverse osmosis was >99%. The flux was set to 20 $L/m^2/h$ based on standard RO fluxes. (Dhawan, n.d.) All the filtration steps were set to take two hours. To avoid scaling the concentration of each compound in the retentate was made sure to be far under the solubility limits seen in Table 7 as the concentration would be higher closer to the membrane boundary.

Because previous attempts have shown changes in morphology in the product after reusing membrane-treated water, an ion exchange mixed bed was added as a last step, reducing the ions to 18.2 MΩ·cm or 0.055 µS/cm, which is the conductivity of pure water. (*Water Innovations*, n.d.) Sulfuric acid of 6% and NaOH of 4% were used for regeneration with SuperPro Designer calculating the amount needed. (*Veolia*, n.d.) Sterilization after the ion exchange was not added, as the existing process already contains a water sterilization part and it was thus assumed that it can be used for the reused water as well. The PFD for the membrane process can be seen in Figure 4 below.

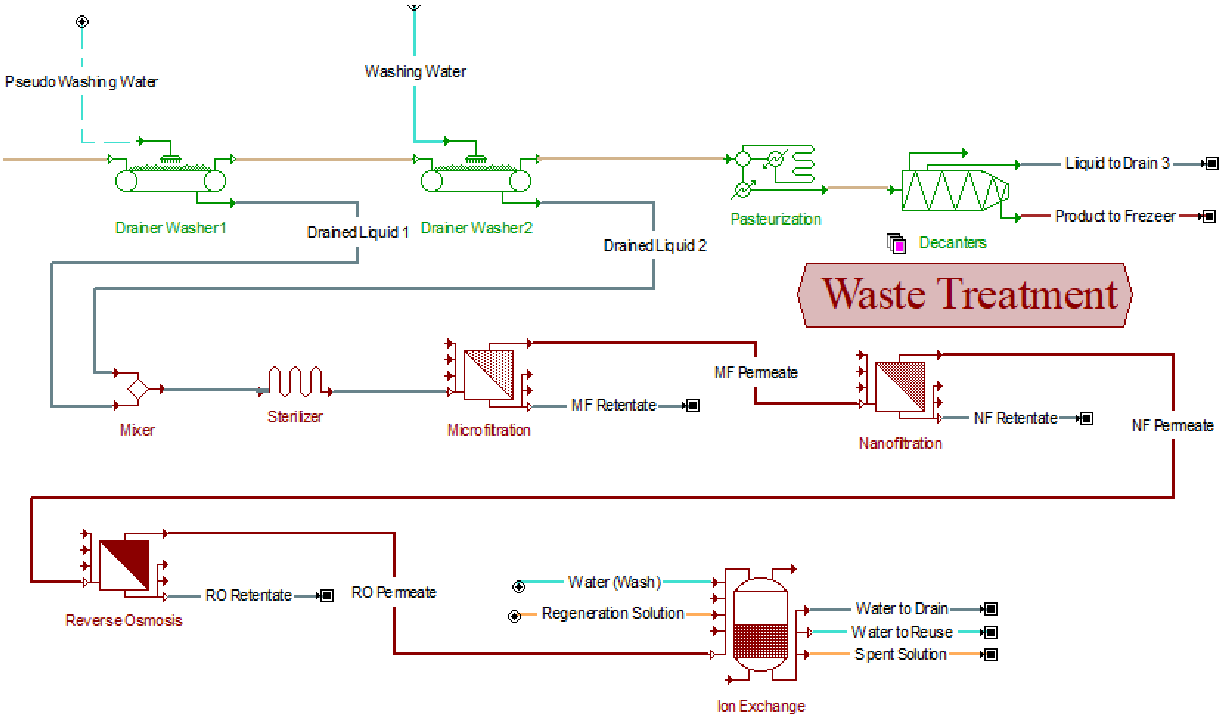


Figure 4. The PFD for membrane treatment for water reuse.

3.2.2 Fertilizer

To be able to use the wastewater streams from the drainer washers as fertilizer, the sucrose content needs to be lowered. This is not an easy task as sucrose is similar in size to the other components, is in the middle range of boiling points, and has high solubility. (*NCBI*, n.d.) Bacterial degradation would also not be viable as the nutrients would be used up as well. Instead, it was decided that the sucrose conversion would be increased and the added amount of sugar decreased to keep the biomass content the same. The process is not yet at the point where this would be plausible, but in the future, the conversion is something that would be improved. The sucrose conversion was set to 96%, which is a conversion that can typically be achieved in fermentation. (Huang & Reardon, 2021) To keep the biomass content the same as in the conceptual design, the added amount of sucrose was lowered by 42%. As the biomass needed to be removed and the ammonia concentrated to about 25 wt%, a membrane sequence was used again. The same procedure used for water reuse was used to create both fertilizer from the NF retentate and water for reuse. However, the water recovery was increased to 97.5% in the nanofilter, which is possible to achieve by recycling and backwashing water by cross flow. (Bi et al., 2014) As the largest part of the dissolved solids comes from the sucrose, the decrease of sucrose should make way for a higher water recovery.

3.2.3 Reuse of Nutrition

The wastewater from the drainer washers will also contain a lot of sucrose, ammonia, and water, and is, therefore, suitable for reuse of nutrition. Before it could be reused, biomass, minerals, and harmful metabolites needed to be removed. This was done by following the water reuse procedure to the microfiltration. To remove the leaked biomass, ultrafiltration was added with the assumption that 98% of the remaining biomass would be removed and that the water recovery was 95% and the flux was 150 $L/m^2/h$. (Dhawan, n.d.) After the ultrafiltration, the mixed ion exchange bed described in the water reuse part was used to lower the ions to 18.2 MΩ·cm or 0.055 µS/cm. The reduction percentage of each ion species was assumed to be identical. After the ions had been removed, granular activated carbon adsorption (GAC) was used to remove harmful metabolites and nucleotides. It was assumed that this would remove all the leftover biomass but not have a pronounced change on the rest of the components. The GAC was set to take two hours with water as the washing liquid. Compared to the study by McGeorge (2019) this process has both heat treatment, ultrafiltration, and ion exchange. The PFD can be seen in Figure 5 below. The re-entry of the nutrients for reuse to the reactor was not simulated. Instead, the decrease in nutrient use was calculated manually.

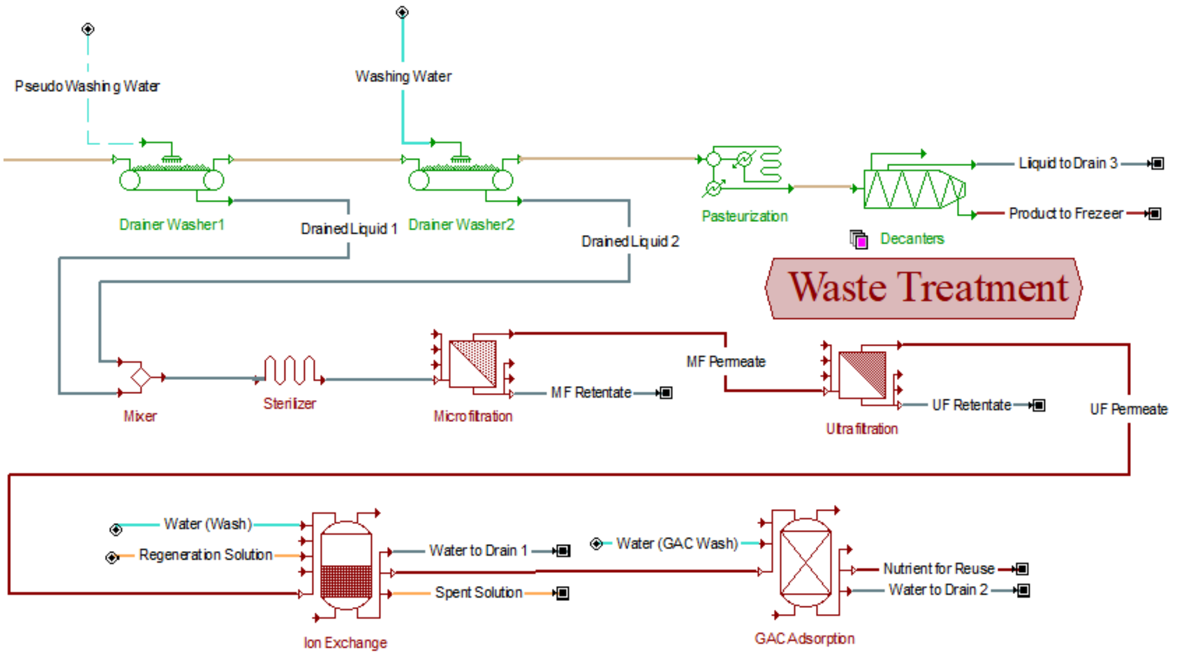


Figure 5. The PFD for nutrient reuse.

Reuse of nutrition requires testing of the water to determine the amount of nutrition left, as well as more stringent control over the feed streams so that the nutritional content at the beginning of each batch is the same.

3.2.4 Biomass Recovery

The wastewater stream from the decanters will have a high biomass content, which makes it suitable for creating fish feed. As the stream contains the same ingredients found in the product but more diluted, it will be assumed that everything except ammonia is fish-safe. Consequently, the stream only needs to be dried and purged from ammonia. For this, an evaporator followed by a spray dryer was used. The spray dryer was modeled after Tetra Pak's spray dryers, which have a recommended liquid input of a maximum of 52%. (Ghanbari, 2024) As such, it was assumed that the evaporator would lower the water content to this level and remove the ammonia as well. The dryer was used to create fish feed powder. The moisture content for powder created in a spray dryer is usually 1.5% to 5%. (*Tetra Pak*, n.d.-b) Using SuperPro Designer's inbuilt Spray dryer, with its presets for temperature and automatic calculation of air and steam usage, the decanter wastewater stream was dried for two hours to 5% total water content (including intracellular water). The PFD for the biomass recovery can be seen in Figure 6.

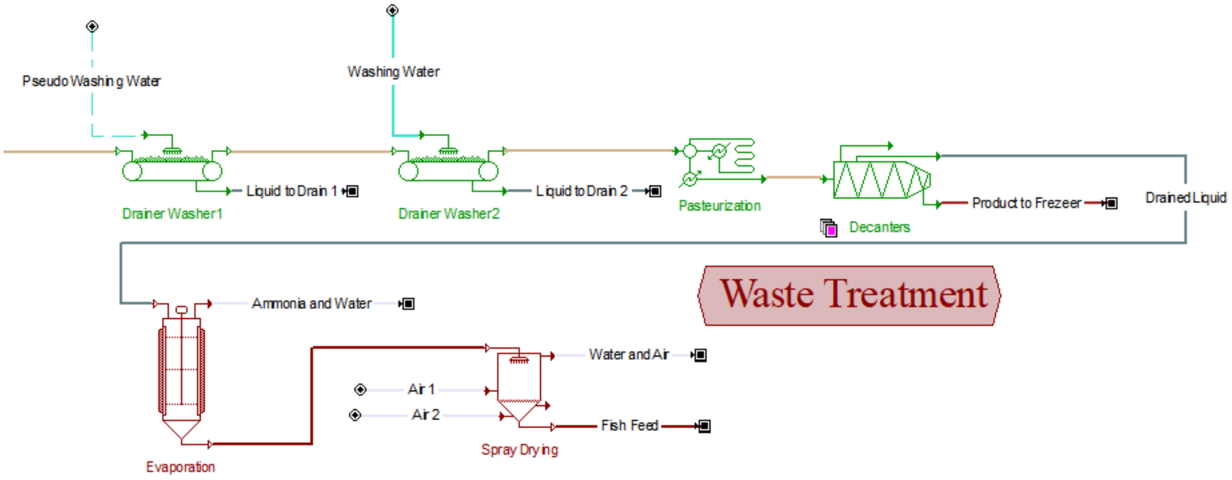


Figure 6. The PFD for the biomass recovery.

3.3 Modified Conceptual Design

The pasteurization module is placed after the drainer washers, but for the reuse of water or the creation of fertilizer, the drained liquid from the drainer washers also needs to be heat treated. An analysis of what would happen if the pasteurization module came before the drainer washer was therefore performed. The heater was assumed to be the same size as the original one and the process time the same which would therefore require multiple units. If the time for the heating process was increased, the throughput would be lowered and consequently also the needed amount of units. Further investigation of what happens if the parameters of the pasteurization are changed was not made. The heater was again assumed to cause a 30% leakage of biomass. The leaked biomass was assumed to be so small that it would not be stopped by the drainer and be removed in the same way as the dissolved solids. It was also assumed that the total mass removal in each unit would be the same as in the original one. As such, the loss of biomass in the first drainer was 28.5%, and in the second one 2.62%. The PFD for the modified conceptual design can be seen in Figure 7 below.

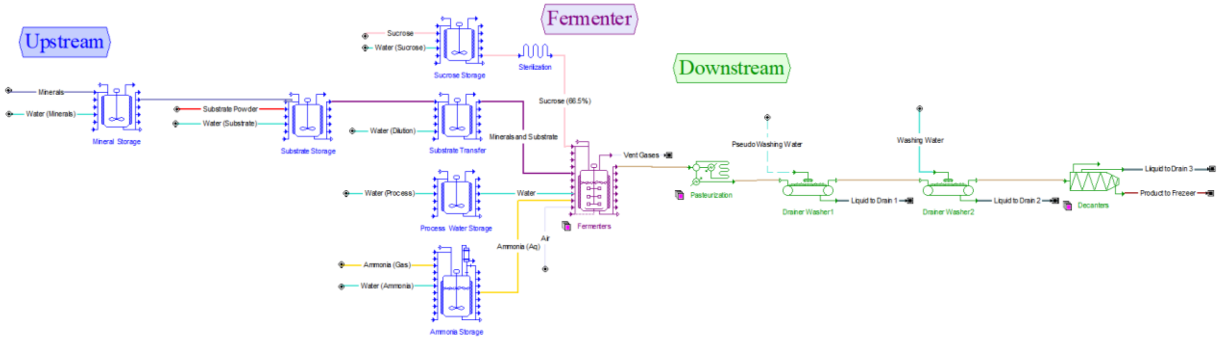


Figure 7. The pasteurization modified PFD.

3.4 Environmental Analysis

Apart from the potential economic benefits, there is a push towards more sustainable waste treatment, which includes reducing the total amount of waste created. All waste streams were aqueous streams, with all components being treated as dissolved solids except for the mycoprotein which is a suspended solid. (*UNM Health Science Center*, n.d.) The simulation did not include clean-in-place (CIP), which would also contribute to the wastewater.

Two organic components existed in the stream, the sucrose and the mycoprotein. The COD and theoretical oxygen demand (thOD) were calculated based on the assumption that everything can be fully oxidized. The results can be seen in Table 8. (*Aropha*, 2023) It was assumed that all carbon in the mycoprotein is organic and contributes to TOC. Likewise, it was assumed that all nitrogen in the mycoprotein would contribute to the total Kjeldahl nitrogen (TKN).

Table 8. Environmental properties of sucrose and mycoprotein.

	Sucrose	Biomass (DW)
COD (g O_2/g)	1.122	1.225
ThOD (g O_2/g)	1.122	1.245
TOC (g C/g)	0.4207	0.4408
Phosphorus ratio (g P/g)	0	0.01719
TKN (g N/g)	0	0.08897

3.5 Techno-Economic Analysis

The TEA calculations in SuperPro Designer use the process model to estimate the purchase costs of the equipment, utility use, cost of feed, and income from the product. These are then multiplied with different cost factors to get the operating cost, revenue, and capital cost which together are used to calculate the profitability of the plant. For the conceptual design, the cost of the blenders was not added to the TEA as they were not part of the actual process but were put in to simplify future changes of recipes. The TEA did also not include CIP costs or costs before the fermenter or after the decanter, such as packaging, freezing, and transportation. The TEA of the conceptual design was compared to an online questionnaire for TEA specifically made for bioeconomy (*Scaler.bio*). For the resource recovery, TEA was only done on the new parts added to the conceptual design, and the cost for the conceptual design parts was set to zero.

3.5.1 Capital Costs

The capital cost for the existing process was based on industrial values and the size comparison of different purchasing costs can be seen in Figure 8.

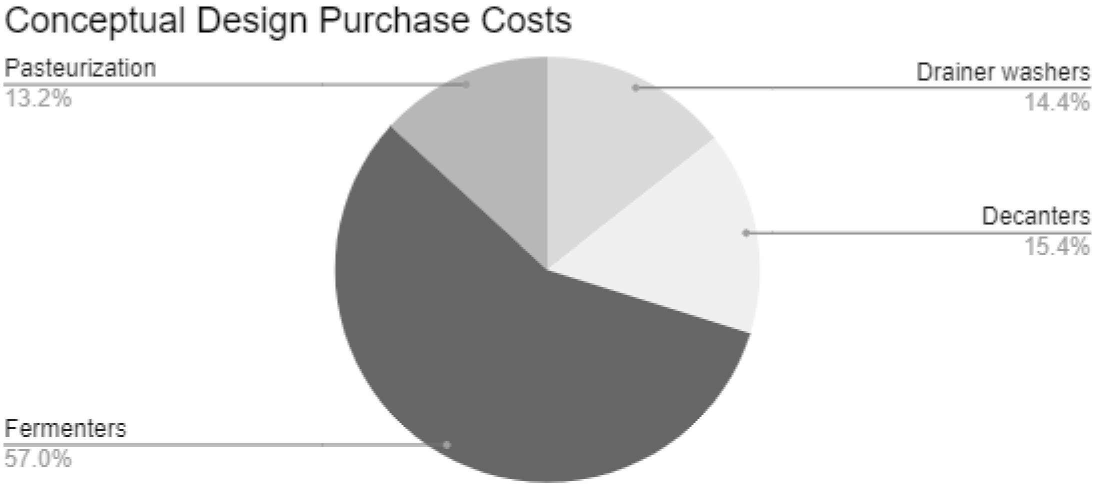


Figure 8. Pie chart of the purchase costs

The installation factor specific to each unit can be seen in Table 9. These factors were preset in the unit operations. The installation factors were multiplied by the purchase cost to get an estimate of the installation cost.

Table 9. Installation cost factors

Unit operation	Installation factor
Fermenter (50 m^3)	0.3
Drainer washer (one unit)	0.5
Sterilizer/Pasteurization	0.5
Decanter (one unit)	0.5
MF/UF/NF	0.5
RO	0.4.
Ion Exchange	0.3
Evaporator	0.2
Spray Drier	0.5
GAC	0.3

Based on the total purchasing cost of the units, several direct and indirect costs were added based on cost factors preset in SuperPro Designer to get the direct fixed capital (DFC), which is defined as DFC=Direct costs + Indirect costs + Other costs. The factors used to calculate direct costs can be seen in Table 10.

Table 10. Direct cost factors. The factors are multiplied by the purchase cost to get an estimation of the cost center.

Direct Costs	Factor (factor x purchase cost)
Piping	0.35
Instrumentation	0.4
Insulation	0.03
Electrical Facilities	0.1
Buildings	0.45
Yard Improvements	0.15
Auxiliary Facilities	0.4
Unlisted Equipment Purchase Cost	0.2

To the direct costs, the installation cost and purchase cost of the main equipment were added, as well as the installation cost of the unlisted equipment (Unlisted Equipment Purchase Cost x 0.5). The indirect costs are for the engineering and construction and are the direct cost times the factor 0.25 and 0.35 respectively. Other costs which include contractor's fee and contingency, were factors of 0.05 and 0.1 times the sum of the indirect and direct costs. Startup and validation costs were preset to 5% of the DFC and the company's working capital was preset to cover 30 days of labor, waste treatment, utilities, and raw materials.

3.5.2 Operating Costs

The operating costs include facility costs, labor costs, lab costs, cost of utilities, and costs of materials. The cost of the nutrition and water can be seen in Table 11 below. The price of biomass was set to 5.04 $/kg which Upcraft et al. (2021) calculated as the price of production using the Quorn process.

Table 11. The cost of different components in the feed.

Component	$/MT	Date	Source
Sucrose	881	Nov 2023	(European Commission, 2024)

Ammonium sulfate	179.5	Jan 2024	(*ECHEMI*, 2024-a)
Monopotassium phosphate	418	Jan 2024	(*ECHEMI*, 2024-b)
Magnesium sulfate	333	Dec 2023	(*Chemanalyst*, 2024-a)
Calcium chloride	278	Jun 2023	(*Chemanalyst*, 2024-b)
EDTA	1945	Jul 2023	(*Chemanalyst*, 2024-c)
Zinc sulfate	480	Jan 2024	(*Global Sources*, 2023-a)
Iron sulfate	200	Jan 2024	(*Global Sources*, 2023-b)
Boric-acid	600	Jan 2024	(*Global Sources*, 2023-c)
Manganese sulfate	600	Jan 2024	(*Global Sources*, 2023-d)
Copper Chloride	900	Jan 2024	(*Global Sources*, 2023-e)
Potassium iodide	818	Jan 2024	(*Global Sources*, 2023-f)
Water (Sweden)	5.7	Average 2023	(Svenskt vatten, 2023)
Ammonia	600.26	Dec 2023	(*S&P Global*, 2024)

The facility-dependent cost (FDC) is the sum of the maintenance cost, depreciation, and miscellaneous costs. The maintenance cost was preset to 0.1 times the purchase cost. No depreciation was assumed and the miscellaneous costs which include insurance, local taxes, and factory expenses were preset to 1%, 2%, and 5% of DFC. The labor cost was 69 $/h. Laboratory costs, quality costs, and quality assurance costs were preset to 15% of the labor cost. For utilities, presets for the different costs were used. The costs can be seen in Table 12.

Table 12. The cost of utilities.

Item	Cost
Electricity	0.1 ($/kWh)
Chilled water	0.5 ($/MT)
Cooled water	0.1 ($/MT)
Steam	32 ($/MT)

The economic evaluation used to calculate for instance the net present value (NPV) was done using solely assumptions provided by SuperPro Designer. These can be seen in Table 13.

Table 13. Assumptions for economic evaluation of the process.

Item	Assumption
Construction Period	30 months
Startup Period	4 months
Project Lifetime	15 years
Inflation	4%
NPV Interest Low	7%
NPV Interest Medium	9%
NPV Interest High	11%
Income Taxes	25%
Loan Interest (DFC 10 years)	9%
Loan Interest (R&D, working capital, front royalties 6 years)	12%
DFC Outlay First Year	30 % DFC
DFC Outlay Second Year	40 % DFC
DFC Outlay Third Year	30 % DFC
Depreciation Period	10 years
Salvage Value	5 % DFC

3.5.3 Resource Recovery Cost

Some costs specific to the resource recovery can be seen below in Table 14. The membranes were expected to be changed every 1000 operating hours, the GAC every 40000 h, and the ion exchange resin every 20000 h.

Table 14. Various costs specific to resource recovery.

Cost Center	Cost	Source
H_2SO_4	59 $/MT	(*Chemanalyst*, 2024-d)

NaOH	570 $/MT	(*ECHEMI*, 2024-c)
MF Membrane	400 $/m^2	SuperPro Designer Preset
UF Membrane	400 $/m^2	SuperPro Designer Preset
NF Membrane	400 $/m^2	SuperPro Designer Preset
RO Membrane	15 $/m^2	SuperPro Designer Preset
Ion Exchange Resin	2 $/L	SuperPro Designer Preset
GAC	4 $/kg	SuperPro Designer Preset

3.5.4 Byproduct Price

For the water and nutrient reuse, the price was chosen to be the same as the purchase price of each component, as the need to buy “fresh” components would be lowered. However, for the fish feed and fertilizer, this could not be done as the mixture of components might be less or more valuable than the individual components. Instead, market prices for these specific uses were found. The price of the fish feed was not easily determined, as the prices can vary heavily. For example, nutritional yeast-based powder feed with ≥40% protein can be sold for 1.6 $/kg, while another high protein powder is sold for 5 $/kg. (*Alibaba*, n.d.; *Fiskfoder*, 2023) As mycoprotein has high protein digestibility and contains large amounts of unsaturated fats, which are preferred for fish feed, it might have a higher price than nutritional yeast. (Craig and Helfrich, 2002) The price of the fertilizer was determined using a method described by Levy (2023) which uses the $N:P_2O_5:K_2O$ ratio. The price of calcium chloride was also added to the fertilizer in the same way. (*Custom Hydronutrients*, 2023) The other constituents were present in considerably smaller quantities and were therefore not added to the final price.

4 Results

4.1 Conceptual Design

Three wastewater streams could be identified from the conceptual design. Of the total wastewater, the drainer washer 1 is responsible for 85%, drainer washer 2 for 12%, and the decanters for 3%. In Figure 9 the distribution of components in the drainer washer 1 stream can be seen. These components make up a total of 3.94 wt% of the stream, with the rest being water.

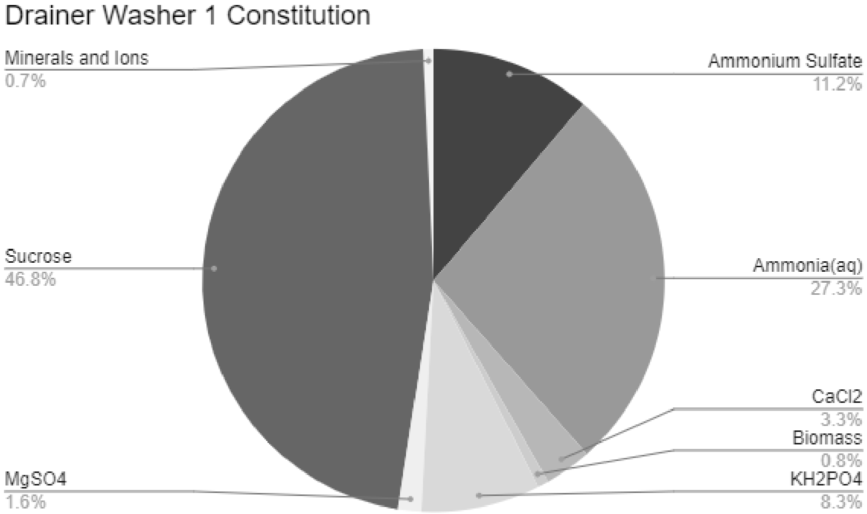


Figure 9. Percentage distribution of components in the liquid drain from drainer washer 1.

It can be seen that sucrose makes up almost half of the component, closely followed by ammonia. There is very little biomass in the stream (measured in wet weight). In Figure 10 the distribution of components in the drainer washer 2 stream can be seen. The components make up 2.06 wt% of the stream.

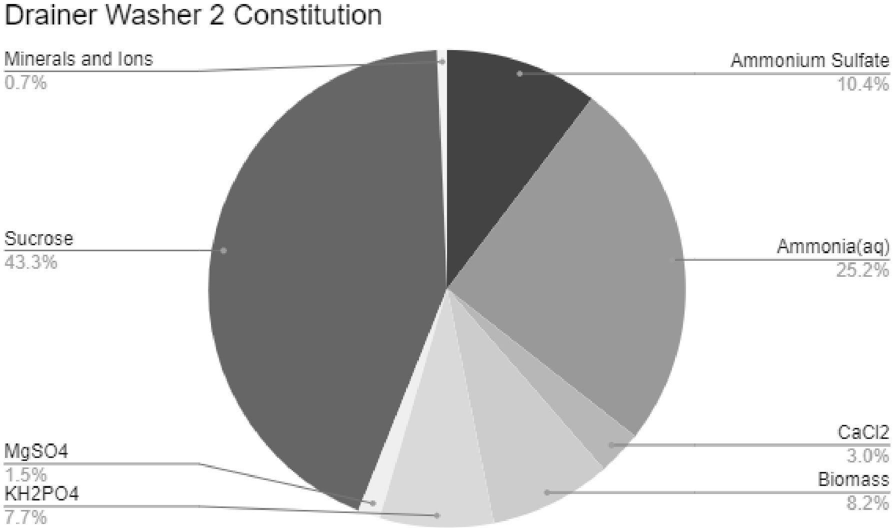


Figure 10. Percentage distribution of components in the liquid drain from drainer washer 2.

It can be seen that the percentage of biomass has increased with the others decreasing slightly compared to drainer washer 1. In Figure 11 the distribution of components in the decanters stream can be seen. These components make up a total of 29.2 wt% of the stream with the rest being water.

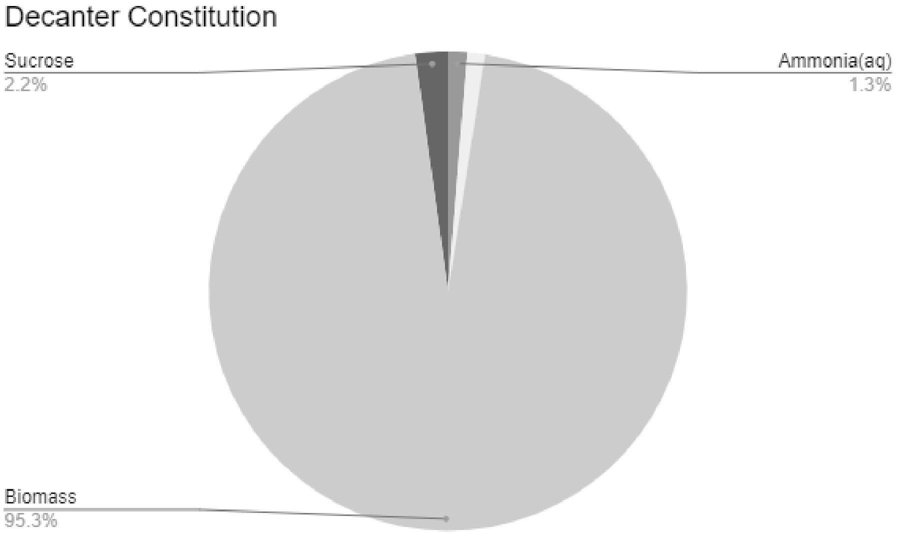


Figure 11. Percentage distribution of components in the liquid drain from the decanters. The segment not marked represents the rest of the components which make up less than 1.2 wt% of the total components (except water).

As suspected this stream consists mostly of biomass with the rest of the constituents making up less than 5 wt%. Figure 12 shows how much of the initial added component will go into the drain and the amount of biomass out of the fermenter that will be lost.

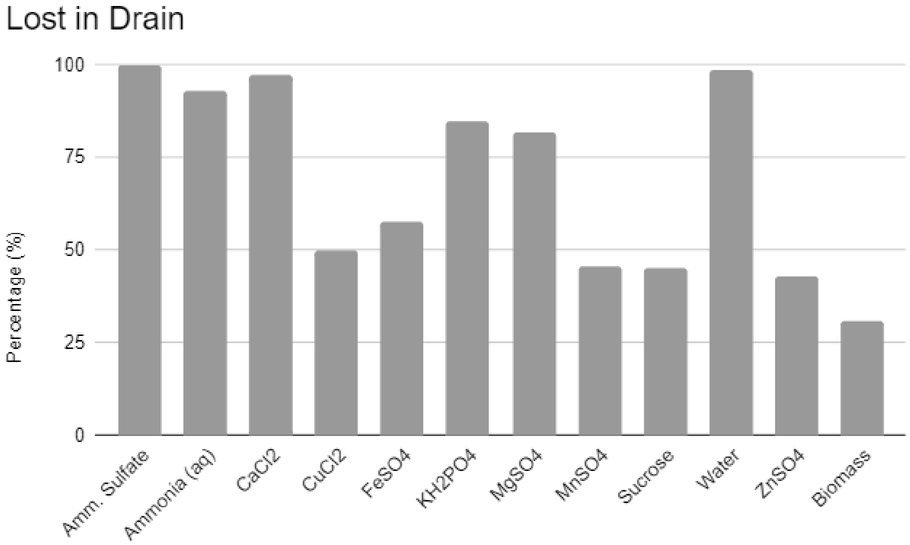


Figure 12. The percentage of added components which is lost in the wastewater as well as the percentage of biomass out of the fermenters lost in the wastewater.

As can be seen in the figure, most of the ammonium sulfate, ammonia, and calcium chloride will not be depleted. Magnesium sulfate and monopotassium phosphate are also not used in large quantities. There is also a large amount of sucrose left.

4.1.1 Economics of Conceptual Design

The capital costs were split into three parts, with the direct costs contributing 54% to the capital costs, the indirect costs 33%, and the contractor's fee & contingency the rest. The percentage of which each different subpart contributes to the total capital costs can be seen in Figure 13.

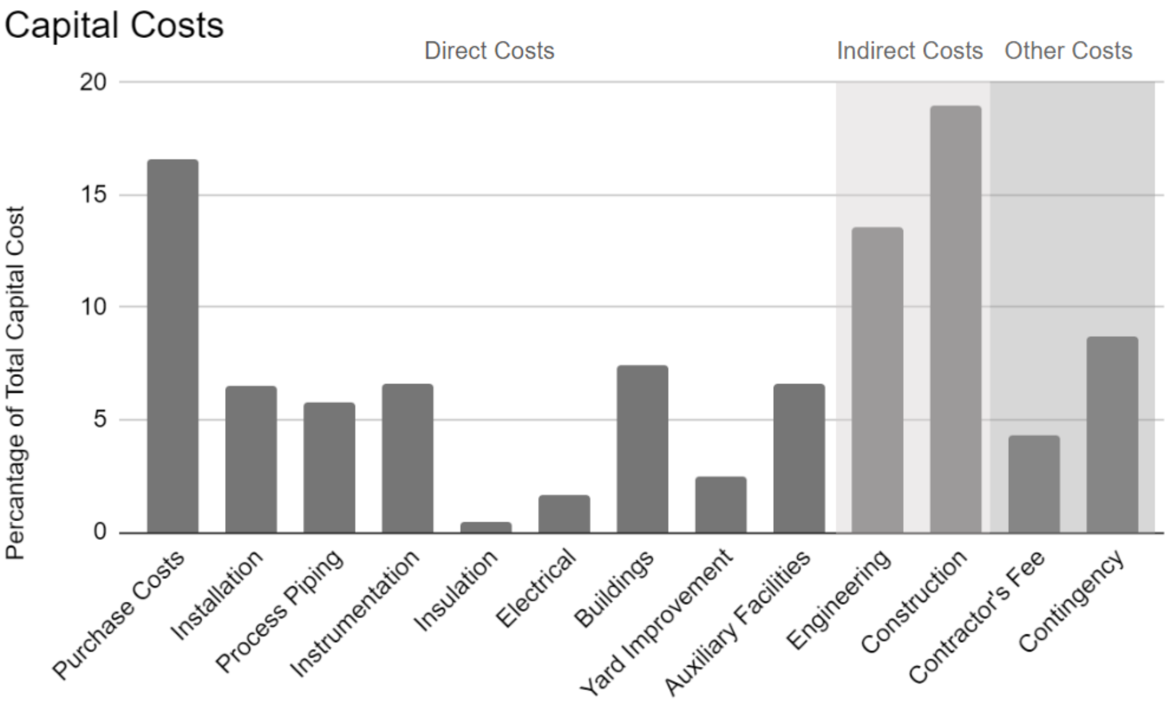


Figure 13. The percentage each part contributes to the total capital costs.

It can be seen that construction costs are the largest cost followed by the purchasing cost and engineering. Similarly, Figure 14 shows the distribution of operating costs.

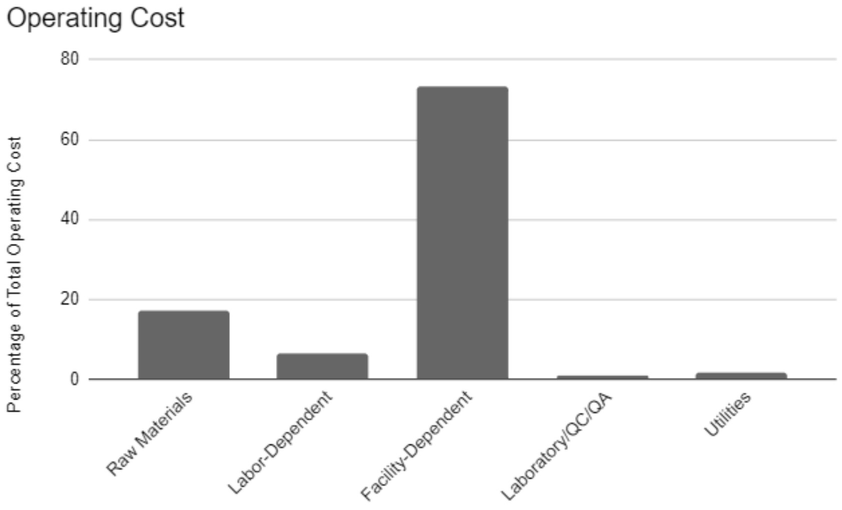


Figure 14. The percentage each part contributes to the total operating costs.

FDC are by far the largest contributor to the operating costs. The comparison of the potential profit of each component lost in the wastewater streams can be seen in Figure 15.

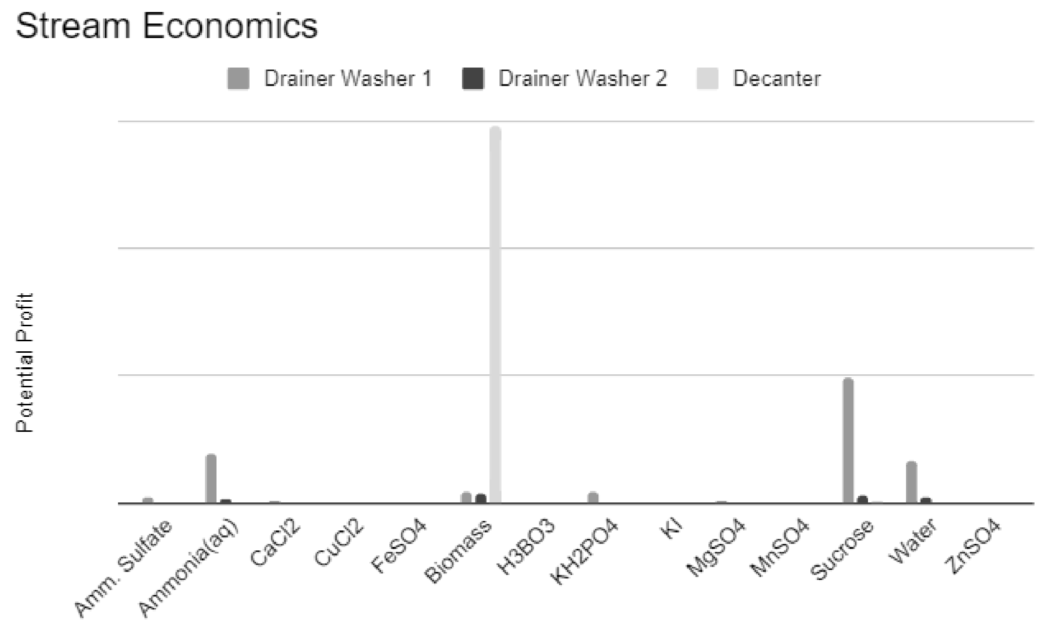


Figure 15. Comparison of the cost of each component lost in the three wastewater streams.

The biomass in the decanters wastewater makes up the largest potential profit. It should however be noted that the potential profit of biomass is calculated based on the production price and not on the price of fish feed, which is lower. Sucrose is the second most valuable and is mainly lost in drainer washer 1, where water and ammonia also contribute to profit loss. The rest of the constituents of the different streams do not constitute a remarkable amount of potential profit.

The questionnaire (Scaler.bio) used to assess the TEA gave similar results as the SuperPro Designer, with about 27% higher capital cost and operating costs. The largest difference could be seen for the purchase cost, where the questionnaire predicted a 75% higher cost. The questionnaire does, however, include the whole process economy, not only that of the fermenters and downstream treatment.

4.2 Resource Recovery

4.2.1 Water Reuse

The amount of water that could be saved compared to the initial amount of water added was 76%. Three retentate waste streams spent ion exchange regeneration streams, and a wash stream were created. The waste from the ion exchange was fairly low due to the rather small amount of ions left after the RO. The relative size of the purchase cost of each piece of equipment can be seen in Figure 16.

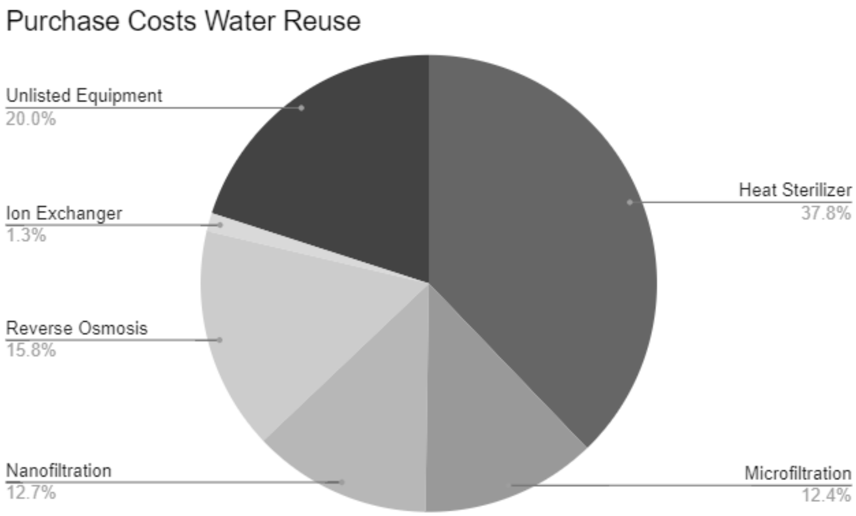


Figure 16. The relative purchase cost of each piece of equipment for water reuse.

It can be seen that the heat sterilizer makes up the largest part of the purchasing cost.

4.2.2 Fertilizer

The water recovery for the fertilizer was 82%. The nutritional value of the fertilizer became 20.5:5.5:3.5 (N:P_2O_5:K_2O) and the price for the fertilizer was 678 $/MT. The sucrose content was only 0.9 wt%. The process has one less waste stream than only water reuse, which becomes the fertilizer instead. The relative size of the purchase cost of each piece of equipment can be seen in Figure 17.

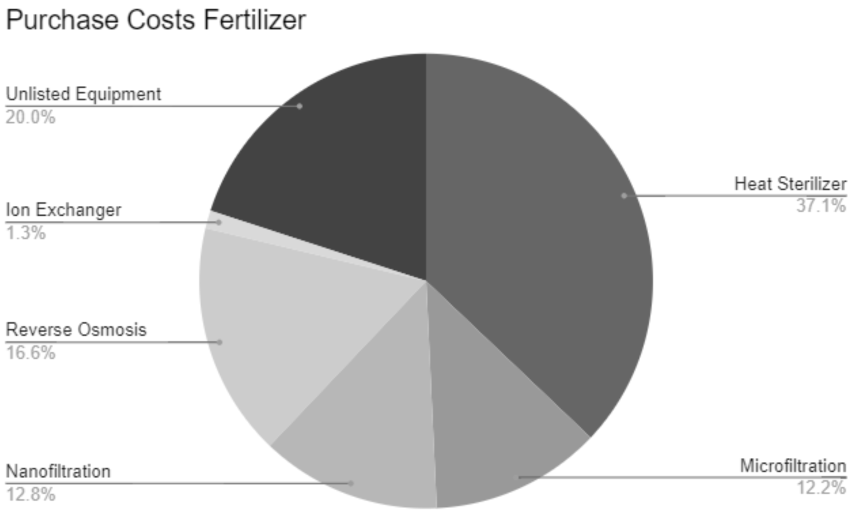


Figure 17. The relative purchase cost of each piece of equipment for water reuse and creation of fertilizer.

Yet again, it is the heat sterilizer that is the most expensive part of the purchasing costs.

4.2.3 Nutritional Reuse

While it was possible to recover a lot of ammonia, sucrose, and water, the water consumption in the washing stage of the ion exchange was several times higher than the water regained, and the cost of the NaOH and H_2SO_4 far surpasses the potential gains from the nutrient reuse. As no profit could possibly be made and more waste was amassed using this system compared to the original one without treatment, further analysis was disregarded.

4.2.4 Biomass Recovery

About 30% of the biomass per dry weight is recovered and can be used as fish feed. The nutritional value of the fish feed is difficult to predict as the biomass will primarily be leaked intracellular mass but the biomass makes up 83 wt% of the fish feed and sucrose 7.5 wt%. The ammonium sulfate concentration was 1.8 wt%. The process creates two waste streams, one with ammonia and water and one with only water. The distribution of the purchase costs can be seen in Figure 18.

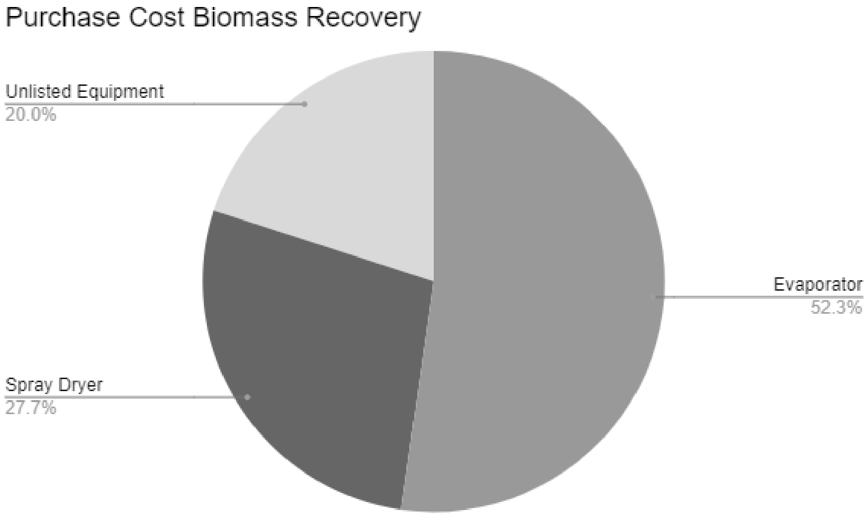


Figure 18. The relative purchase cost of each piece of equipment for biomass recovery.

As can be seen in the figure the evaporator makes up over half of the total purchase costs.

4.2.5 Economic Comparison Resource Recovery

The size comparison of the operating costs for the different resource recovery options can be seen in Figure 19. The largest contributing operating cost to all three options was the FDC, making up over 90% of the total costs. The gross margin was -1666.39% for the water recovery, -236.88% for the water and fertilizer, and -258.69% for the biomass recovery.

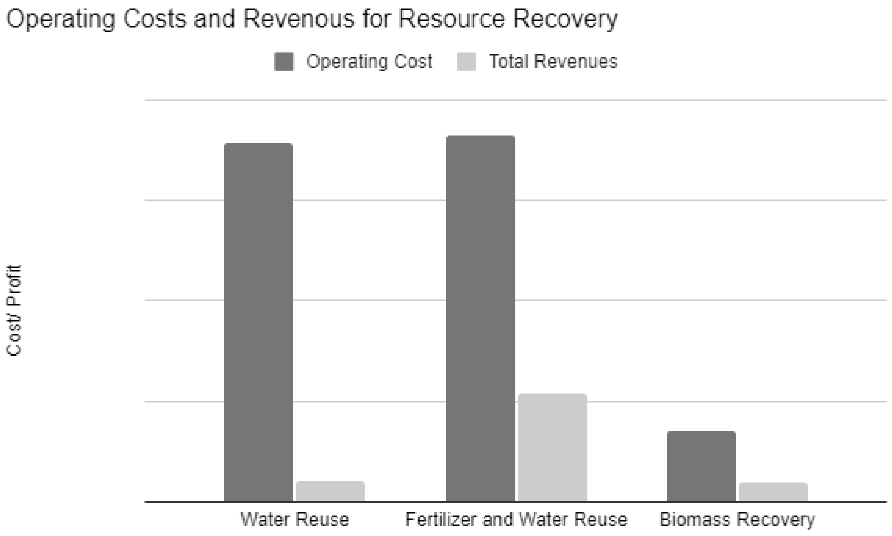


Figure 19. Size comparison of operating costs and revenue for the resource recovery.

The operating cost for the fertilizer and water reuse is marginally larger than only water reuse but has a lot higher revenue. Both biomass recovery and water reuse resulted in similar revenue but biomass has less operating costs.

The comparison in size of required capital investments can be seen in Figure 20. Apart from the purchase cost of the equipment, the largest contributors to the capital costs are construction costs followed by engineering costs and installation of buildings, which make up 38% of the total capital costs.

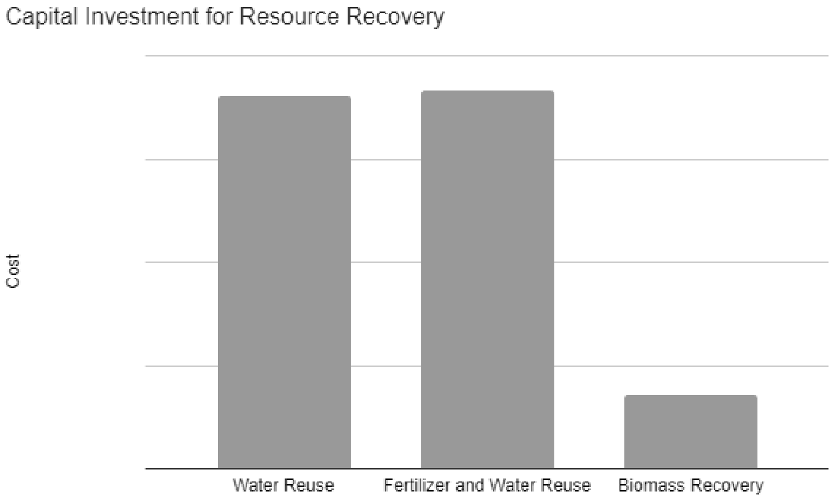


Figure 20. The size comparison of capital cost for the resource recovery.

The capital costs for water reuse and fertilizer creation are similar, while biomass recovery is a lot cheaper.

For the water reuse revenue to match the operating cost, the price has to be 101 $/MT, and for the NPV to be positive the price has to be above 131.6 $/MT. For water reuse and fertilizer creation the fertilizer price, which provides the largest revenue, would need to be 2690 $/MT, an almost 300% increase from the calculated price. For the NPV to be positive the price of the fertilizer needs to be above 3562 $/MT. For the fish feed these values are 5.74 $/kg (260% increase) and 7.52 $/kg.

4.2.6 Environmental Comparison Resource Recovery

The comparison of different wastewater values for the waste streams can be seen in Figure 21, where TP stands for total phosphor, TS for total solids, TSS for total suspended solids, and TDS for total dissolved solids. For the fertilizer creation and water reuse the COD, ThOD, TS, and TDS are way lower mainly due to the reduced amount of sugar and increased conversion of it.

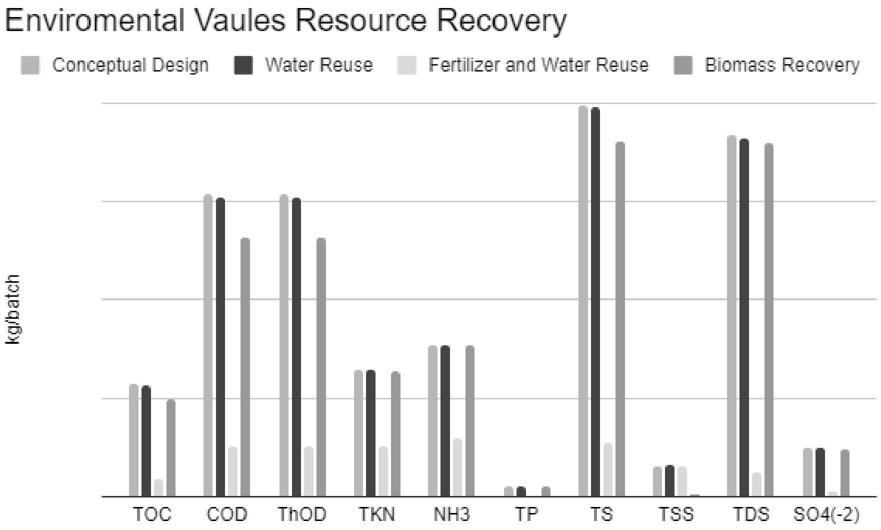


Figure 21. Comparison of different wastewater values for the aqueous waste.

It can be seen that the creation of fertilizer has the highest impact on all water quality values except TSS and would therefore also have the lowest burden on communal wastewater facilities. The water reuse does not lower any values as its wastewater would have the same constituents only with a lower amount of water. The biomass recovery lowers the COD and ThOD by a bit but its largest impact is on the TSS, which is almost completely removed.

4.3 Modified Conceptual Design

The modified design required 18 pasteurization modules to pasteurize the broth out of the fermenter in the same amount of time as the original pasteurizer. In the separation process above, sterilizers were used instead of pasteurization to ensure safety for reuse and prevention of biological growth on the membranes. If pasteurization modules with the same specification as the original one were used, the entire process would use 20 pasteurization modules due to the addition of wash water in drainer washer 2. The wastewater composition from the different units was very similar, which can be seen in Figures 22, 23, and 24.

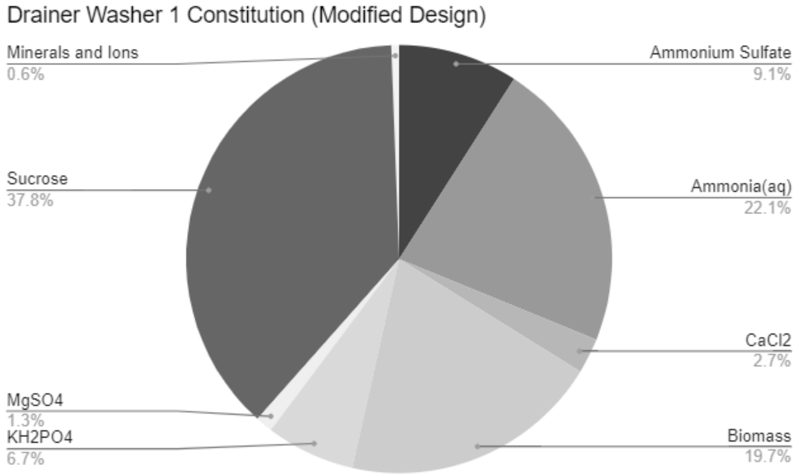


Figure 22. The solid composition of drainer washer 1 wastewater from the modified conceptual design. The total amount of water was 95.2 wt%

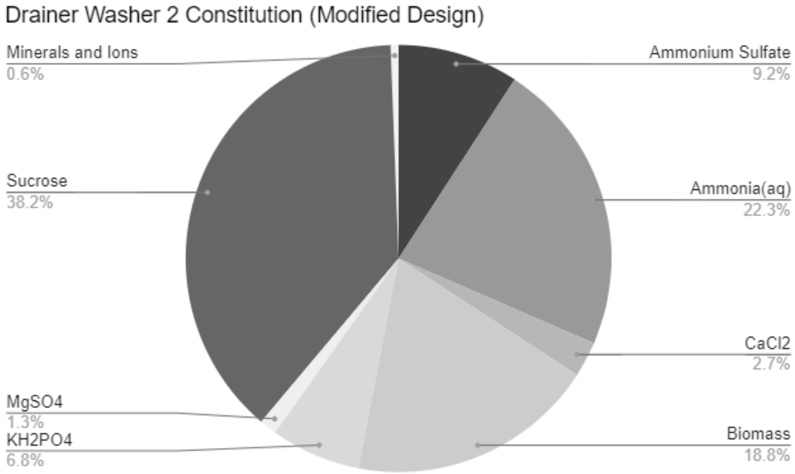


Figure 23. The solid composition of drainer washer 2 wastewater from the modified conceptual design. The total amount of water was 97.5 wt%

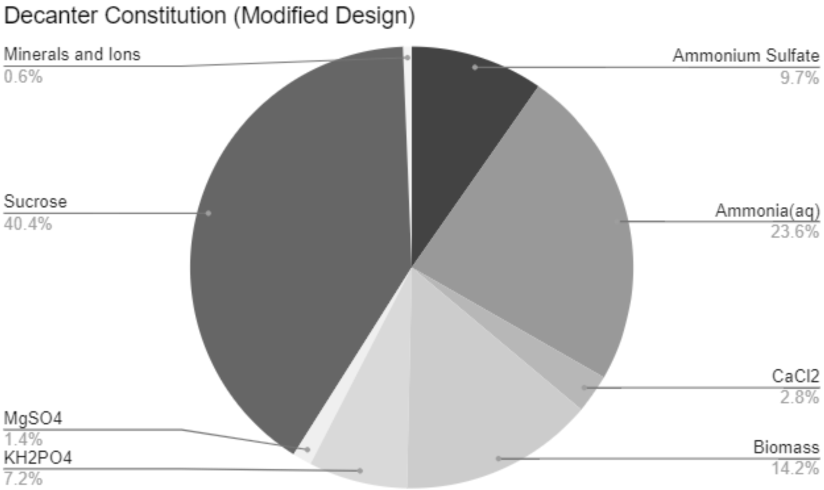


Figure 24. The solid composition of decanter wastewater from the modified conceptual design. The total amount of water was 97.7 wt%

Compared to the original wastewater streams, the new drainer washer streams contain a higher amount of biomass, while the decanter wastewater contains less and has a more diluted stream.

5 Discussion

5.1 Conceptual Design

While the conceptual design includes many assumptions, it could successfully be used to predict the contents of the wastewater. It can be seen that the fermenter is highly inefficient and that a lot of nutrients are barely used. This might partly be due to assumptions such as the fermentation having optimal yield, but it is known that the waste stream contains a lot of sucrose. (Ghanbari, 2024) Succeeding works would include the addition of CIP, the full upstream treatment, more industrial inputs for the units and cost factor as well as a kinetic reaction instead of stoichiometric. Another important factor that has been excluded from the simulation is that the downstream process is continuous. As the reactions are said to be stoichiometric, it does not make a difference when the product is taken out but for the real process, the constitution of the feed to the downstream might vary during the process. It should also be noted that heat treatment for Quorn is done at 68°C and not 85°C. The higher temperature in this process might create more leakage. All of the waste streams' environmental values were above the legal thresholds. While the current conceptual design is not detailed enough for good economic predictions, it gives a good base for future work.

5.2 Techno-Economic Analysis Framework

The following part discusses how well the proposed decision support framework worked and the extent to which it was followed. The first step was to determine the reason for doing resource recovery, which was to make the process more sustainable and profitable. In this step, the end objectives were also introduced, such as fertilizer and fish feed. No mathematical conditions were set as the study was more theoretical and based largely on assumptions. In the second step, the conceptual design was introduced to get a good idea of the stream composition and components that might be harmful to the end objective, such as ammonia to fish feed. In the following step, the environmental properties were determined but contrary to the suggestion of the framework, the cost of wastewater treatment was not considered and the potential profits were not calculated until the end. As several resource recovery options would be tested instead of trying to find the optimal one at first, the potential profits did not need to be calculated. In the case where there would be more options for resource recovery, potential profit should have been calculated beforehand.

After this, the framework suggests choosing the next step based on side-stream separation TRL, which in this case was only 2 (technology concept formulated). (*European Commission*, 2020) The next step was consequently process synthesis and thereafter process verification. This was done in the same way as suggested by the framework, that is by first determining the thermodynamic difference between the components and finding separation methods based on the largest differences in both physical and thermodynamic properties. A literature review was thereafter conducted to define separation values such as membrane rejection. The two last steps were to simulate the resource recovery and to do a techno-economic validation, which was executed in SuperPro Designer.

Overall the framework provides a good base for how to strategically find a resource recovery method. Its usefulness is more geared towards wastes where there are a lot of potential recovery options and the optimum is to be found quickly compared to this study, where there were few options and all were simulated. One improvement in the framework could be that the stream be defined before the end objective, as the objective can be difficult to know beforehand.

5.3 Resource Recovery

There were several different resource recovery strategies explored in this study. However, none yielded positive economic results. While the water and nutrient reuse clearly cannot compensate for its total costs, the fertilizer and fish feed production have a possibility of being profitable. Apart from increasing the selling price of the products, the economic model can also be modified. In the calculations, both yard improvements and building costs make up a high percentage of the total DFC, which then contributes to the facility's operating cost. One could argue, as this is an added downstream unit, that the building already should be a part of the conceptual design. Furthermore, the overall contribution of the FDC to the total operating cost (about 90%) seems a bit too high. The FDC cost depends on the DFC, so lowering DFC would also reduce operating costs.

The process simulated is far from optimum fermentation and a lot of nutrients are wasted. The most effective option for reducing waste would therefore not be resource recovery but more efficient nutrient use in the fermenter. Theoretically, all nutrients in the waste streams could be notably lowered. One option has already been explored, which is reducing the sugar, but it could be even more efficient, as both salts and ammonia are supplied in large excess. The biomass production and concentration might also become higher depending on how the change is done. An optimization of the process would therefore result in cleaner wastewater from the drainer washers, which would then increase the longevity of the membranes, probably increase the flux, and per se also decrease the membrane area. More water could also be recovered. The amount of biomass lost in the drain could potentially increase if the total biomass increases, which would make biomass recovery a more attractive option. As such, recovery of biomass is the most appealing of the treatment options presented in the study, not only because it will be positively affected by increased efficiency in the fermenter, but also because it has the lowest investment costs and is most likely to be profitable. It also recovers the most valuable and sought-after resource in the wastewater, protein, which is a scarce commodity.

As biomass can be combined with the other explored options, the total revenue could be increased by creating fertilizer as well. Neither option has a positive net present value in the simulations but could potentially be made more cost-effective. In addition, the total waste in communal wastewater facilities would be greatly lowered, which increases the sustainability of the process and might be worth the economic loss. The simulation did not account for wastewater treatment costs, but this would certainly also increase the profitability of these options.

It should be noted that not all resource recovery options were considered. As previously mentioned, the RNA from the biomass could act as an umami flavoring. Considering that the wastewater biomass from the decanters is mainly intracellular components, there is probably a larger proportion of RNA in the waste compared to the mycoprotein product. (Finnigan et al., 2017) Another use for the decanter stream could be as a gelling or foaming agent. Both options would require detailed knowledge of the wastewater contents.

5.3.1 Water Reuse and Fertilizer Creation

Reusing only water does not seem economically viable. The operating cost is a lot higher than the potential revenue and could not be compensated by the cost of water even in the part of the world where there is high water stress. The only imaginable situation where a company would agree to this process was if there was a legal limit on the amount of water a company has to reuse which is definitely a possibility in the future. However, at present time this treatment option would not be further considered if the concentration in the wastewater is not considerably decreased. It should also be noted that water reuse does not lower the amount of waste sent to wastewater facilities but makes it more concentrated, which might cause more issues with the legal specifications mentioned before. Of the wastewater treatment methods, water reuse has the lowest effect on the created waste and consequently lowest sustainability. For both the fertilization creation and water reuse process, membranes and resins are used. These are consumables that also create waste. The impact that this has on the environment compared to the benefits of resource recovery is difficult to approximate but still needs to be considered when mentioning sustainability. Likewise, the additional cleaning, and other consumables for all the treatments, will also affect the environmental sustainability.

A different method of recycling the water, which would be interesting to explore, would be through evaporation. Ammonia has a very low boiling point compared to the other components and could potentially be separated first followed by water, or alternatively, both could be separated together and recycled. This method was not tested in this report due to the difficulties in predicting the equilibrium of ammonia, which is dependent on pH, pressure, and temperature.

The simultaneous creation of water for reuse and fertilizer is a much more feasible way of recovering water and lowering waste. The fertilizer has a high nutritional value and can be sold for a fairly high price, which is the reason why it can bring in the highest revenue out of the different treatment options. The only downside is that the ammonia is present as aqueous ammonia and therefore has a higher volume than for example ammonium nitrate. This means that the fertilizer has to be used fairly close to the production site as transportation costs would otherwise be too high. In the simulation, water was also recovered as the process had already done several of the water treatment steps. If water recovery was to be removed, the fertilization capital cost would decrease by about 21%.

It should be noted that drainer washer 2 does not contribute a lot to the treated stream and depending on the process layout it might be cheaper not to include it in the treatment. The water recovery might also be increased in the RO but not in the NF, as the ammonium sulfate will precipitate. An additional change that might benefit both processes is sterilization without heat. The heat treatment makes up the largest cost for both processes. Sterilization methods that do not rely on heat have been mentioned previously but none of them exist in SuperPro Designer. Therefore, evaluation of the potential economic difference would have to be done by hand or using another program. Nonetheless, this change could positively impact the economy, especially for the fertilizer creation, where the revenue is not that far from the operating costs. In the simulation, RO, NF, and MF are used but whether it is necessary to have the MF can be questioned.

5.3.2 Biomass Recovery

The recovery of biomass could be done fairly easily and the technology is already well known within the company, which makes it the most realistic for resource recovery. The wastewater stream from this process is also more dilute than the original, making it more suitable for communal wastewater facilities. While the assumed selling price is too low to compensate for the operating cost, it has the highest potential out of the three to be profitable. Higher selling prices than the one assumed can, as previously mentioned, be found. If the add-on costs discussed above are also lowered, it could be possible to get a positive net present value. While the decrease in total waste is not very high, the potential indirect environmental benefits such as reduced use of small fish for fish feed, could be important.

The highest cost for biomass recovery is the evaporator. If it could be made more efficient or replaced by another drying method, both the operating and capital costs could be decreased.
A different way of increasing the economic feasibility is by looking into other animal feeds. Fish feed was primarily chosen as it is very forgiving when it comes to taste, appearance, and texture but the recovered biomass could very well be used for pet food if the actual composition is considered pet-safe and tasty by the animals. It is common for pet food to have animal parts that are not particularly appetizing for humans, so there is no reason why alternative meat cannot do the same. Furthermore, extra flavoring agents could be added if need be. As pet food, it could be sold for a lot more (about 6$/kg for cheap wet dog food) (*Hund & Hälsa,* 2024) and also have a higher water constitution, giving more total product. Furthermore, future work should among other things look deeper into the nutritional composition of the created fish feed, as the leaked intracellular components are not known.

5.3.3 Nutrient Recovery

The ion exchange is responsible for the impossible profitability of nutrient recovery. Other methods of demineralization have been presented and might be more economically viable but only ion exchange was possible to simulate in SuperPro Designer. The main contributors to the minerals in the wastewater stream are ammonium sulfate, monopotassium phosphate, and calcium chloride, which make up 32 wt% of the total minerals. Calcium does affect hyphal growth and thus needs to be removed. (McGeorge, 2019) Another option would be to lower the total amount of calcium chloride added. The best option would be to somehow monitor the amount of minerals left and only add new ones to fulfill the original recipe. The economic impact of monitoring and regulating in and outflows would need to be assessed. However, it would undoubtedly be more environmentally friendly than the use of ion exchange resins and regeneration liquid, and probably also better than letting the nutrients go to waste. It should be noted that nutrient recovery might not be as beneficial if the future process does not include such large nutrient use inefficiencies as it currently does.

5.4 Modified Conceptual Design

By having the heat treatment before the drainer washer, the total amount of pasteurization units is reduced given the previously mentioned assumption. It would, however, cause the streams to have a very similar composition, which might make separation of specifically water and biomass more difficult compared to the original design, where they are mainly present in the wastewater from drainer washer 1 and decanters respectively. As mentioned before, it might also be possible to kill the fungi through other methods that do not require heat, which could potentially be more effective and less costly.

5.5 Industry Relevance and Future Prospects

The conceptual design created in this study offers great potential for the evaluation of resource recovery and potential modification of the process. It can be easily changed to fit new recipes which makes it great for initial trials. While the theoretical wastewater is based on numerous assumptions, it gives a good overview of the potential constitutions. The options tested in this study show that there is a profit potential and that resource recovery for mycoprotein should be further explored. As previously mentioned, there is a great push towards a circular economy and the market for protein alternatives is undergoing enormous economic growth, which makes the treatment of mycoprotein wastewater all the more relevant. Industries doing semi-batch mycoprotein fermentation might also more easily expand to water-stressed areas if they employ water reuse.

Future work should include defining the wastewater stream based on actual lab-measured values as well as making the simulation semi-batch. As there is little information on the potential uses of the component found intracellularly, it would be interesting to study if there is something potentially valuable for further extraction. This study did not include all potential resource recovery options or separation methods as mentioned briefly in other sections. More simulations could definitely be made using other methods, such as evaporation for water recovery. Furthermore, actual lab tests would be useful in confirming the quality of the fish feed and fertilizer.

6 Conclusion

The framework and conceptual design created in this study were useful in determining resource recovery strategies. The most important change that could be made for the resource recovery and sustainability of the process, is to improve nutritional efficiency. The wastewater could then be used to create both fertilizer and fish feed, reducing its environmental impact remarkably while simultaneously yielding income. The separation methods used in this study were too costly for water or nutrient recovery. Further studies should be conducted to validate the composition of the wastewater streams, as they are currently purely theoretical.

References

Ahmad, M.I. *et al.* (2022) ‘A review on mycoprotein: History, nutritional composition, production methods, and Health Benefits’, *Trends in Food Science & Technology*, 121, pp. 14–29. doi:10.1016/j.tifs.2022.01.027.

Alkhadra, M.A. *et al.* (2022) ‘Electrochemical methods for water purification, Ion Separations, and energy conversion’, *Chemical Reviews*, 122(16), pp. 13547–13635. doi:10.1021/acs.chemrev.1c00396.

Ammonium sulfate china domestic price (2024-a) *ECHEMI*. Available at: https://www.echemi.com/productsInformation/temppid160705011796-ammonium-sulfate.html (Accessed:17 February 2024).

Anhydrous ammonia (no date) *Oklahoma State University*. Available at: https://www.nue.okstate.edu/N_Fertilizers/aqua_ammonia.htm (Accessed: 22 February 2024).

Aquarium Water Quality: Nitrogen Cycle (no date) *Florida Department of Agriculture and Consumer Services seal*. Available at: https://www.fdacs.gov/Consumer-Resources/Recreation-and-Leisure/Aquarium-Fish/Aquarium-Water-Quality-Nitrogen-Cycle (Accessed: 04 February 2024).

Aqueous Liquid Waste (no date) *UNM Health Sciences Center*. Available at: https://hsc.unm.edu/research/compliance/radiation-safety/aqueous-liquid.html (Accessed: 07 February 2024).

Awadalla, F.T., Striez, C. and Lamb, K. (1994) 'Removal of ammonium and nitrate ions from mine effluents by Membrane Technology', *Separation Science and Technology*, 29(4), pp. 483–495. doi:10.1080/01496399408002157.

Baratsas, S.G., Pistikopoulos, E.N. and Avraamidou, S. (2022) 'A quantitative and Holistic Circular Economy Assessment Framework at the Micro Level', *Computers & Chemical Engineering*, 160, p. 107697. doi:10.1016/j.compchemeng.2022.107697.

Bi, F. *et al.* (2014) 'Discussion on calculation of maximum water recovery in nanofiltration system', *Desalination*, 332(1), pp. 142–146. doi:10.1016/j.desal.2013.11.017.

Bridges, S. and Robinson, L. (2020) 'Centrifuges', *A Practical Handbook for Drilling Fluids Processing*, pp. 475–488. doi:10.1016/b978-0-12-821341-4.00021-x.

Calcium chloride fertilizer 50 lb. (2023) *Custom Hydronutrients*. Available at: https://customhydronutrients.com/Calcium-Chloride-fertilizer-50-lb_p_23114.html (Accessed: 16 February 2024).

Calcium Chloride Price Trend and Forecast (2024-b) *Chemanalyst*. Available at: https://www.chemanalyst.com/Pricing-data/calcium-chloride-1297(Accessed: 17 February 2024).

Calcium chloride 95-97% (2024) *Anorel*. Available at: https://www.anorel.net/en/products/calcium-chloride-94-98 (Accessed: 18 January 2024).

Choudhury, N.R. (2022) *Mycoprotein market*, *Future Market Insights*. Available at: https://www.futuremarketinsights.com/reports/mycoprotein-market (Accessed: 02 January 2024).

Circular economy action plan (no date) *European Commission*. Available at: https://environment.ec.europa.eu/strategy/circular-economy-action-plan_en (Accessed: 06 January 2024).

Craigh, S., Helfrich, LA. 2002. *Understanding Fish Nutrition, Feeds and Feeding, Viginia Coperative Extension Service*. Publication 420-256:1-4

Dekanter (no date-a) *Alfa Laval*. Available at: https://www.alfalaval.se/produkter/separering/centrifugalseparatorer/dekanter/ (Accessed: 15 December 2023).

Dhawan, G.K. (no date) *Back to basics: About ultrafiltration (UF), Applied Membranes*. Available at: https://www.appliedmembranes.com/back-to-basics-about-ultrafiltration-uf.html (Accessed: 21 February 2024).

Dunlop, M.V., Kilroe, S.P., Bowtell, J.L., Finnigan, T.J.A., *et al.* (2017) *Mycoprotein represents a bioavailable and insulinotropic non-animal-derived dietary protein source: A dose–response study: British Journal of Nutrition*, *Cambridge Core*. Available at: https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/mycoprotein-represents-a-bioavailable-and-insulinotropic-nonanimalderived-dietary-protein-source-a-doseresponse-study/EA83AC16BF75C659FD1058AE1D6AEEB7 (Accessed: 07 February 2024).

Durkin, A. *et al.* (2022) ‘Resource recovery from food-processing wastewaters in a circular economy: A methodology for the future’, *Current Opinion in Biotechnology*, 76, p. 102735. doi:10.1016/j.copbio.2022.102735.

Ethylenediaminetetraacetic acid, 99%, pure (no date) *Acros Organics*. Available at: https://www.uprm.edu/cms/index.php?a=file&fid=5899 (Accessed: 15 January 2024).

Ferreira, R.D.G., Misailidis, N. and Petrides, D. (2023) *Meat-Like Fungi Protein (Mycoprotein) Production via Fermentation – Process Modeling and Techno-Economic Assessment (TEA) using SuperPro Designer.* [Preprint]. doi:10.13140/RG.2.2.24718.13127.

Ferrous Sulfate 7782-63-0 Industrial Grade Ferrous Sulfate 98% (2024-b) *Global Sources*. Available at: https://www.globalsources.com/Inorganic-chemical/Chemical-1200718361p.htm (Accessed: 17 February 2024).

Finnigan, T., Needham, L. and Abbott, C. (2017) ‘Mycoprotein’, *Sustainable Protein Sources*, pp. 305–325. doi:10.1016/b978-0-12-802778-3.00019-6.

Fish feed: Why we need sustainable alternatives - eit food (2023) *Eitfood*. Available at: https://www.eitfood.eu/blog/fish-feed-why-we-need-sustainable-alternatives (Accessed: 23 January 2024).

Fiskfoder (2023) *Swedbait*. Available at: https://swedbait.se/produkter/fiskfoder/#prettyPhoto (Accessed: 21 February 2024).

Food - worldwide: Statista market forecast (no date) *Statista*. Available at: https://www.statista.com/outlook/io/manufacturing/consumer-goods/food/worldwide (Accessed: 02 January 2024).

Food waste and food waste prevention - estimates (2023) *European Commission*. Available at: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Food_waste_and_food_waste_prevention_-_estimates#Amounts_of_food_waste_at_EU_level (Accessed: 06 January 2024).

G. Technology Readiness Levels (TRL) (2020) *European Commission*. Available at: https://ec.europa.eu/research/participants/data/ref/h2020/wp/2014_2015/annexes/h2020-wp1415-annex-g-trl_en.pdf (Accessed: 15 December 2023).

Gao, S. *et al.* (2022) 'Exogenous sucrose supply regulates the physiological index levels before the flower blooming and fruit abscission stages in the biodiesel tree Sapindus Mukorossi Gaertn.', *HortScience*, 57(11), pp. 1397–1408. doi:10.21273/hortsci16626-22.

Ghanbari, H. (2024) Conversation and messages with Agnes Bjerborn, nov 2023 to march 2024.

Granulate Dryer: Working theory: Flexible installation (2023) *Yushunxin*. Available at: https://www.granulatorforfertilizer.com/granulate-dryer/ (Accessed: 17 February 2024).

Hashempour-Baltork, F. *et al.* (2020) 'Safety assays and nutritional values of mycoprotein produced by *fusarium venenatum* ir372c from date waste as substrate', *Journal of the Science of Food and Agriculture*, 100(12), pp. 4433–4441. doi:10.1002/jsfa.10483.

HFX – High Flow Ion Exchange (no date) *Water Innovations*. Available at: https://waterinnovations.net/high-flow-ion-exchange-systems/ (Accessed: 12 January 2024).

High Quality Boric Acid supplier high purity Boric acid flakes Boric Acid powder Boric Acid cas 10043-35-3 (2024-c) *Global Sources*. Available at: https://www.globalsources.com/Fine-chemical/Boric-Acid-1204071249p.htm (Accessed: 17 February 2024).

Huang, X. and Reardon, K.F. (2021) 'Strategies to achieve high productivity, high conversion, and high yield in yeast fermentation of algal biomass hydrolysate', *Engineering in Life Sciences*, 22(3–4), pp. 119–131. doi:10.1002/elsc.202100095.

Industrial Use Water Treatment 99% monohydrate/pentahydrate Copper Sulphate CAS 7758-98-7 Available at: https://www.globalsources.com/Inorganic-chemical/Ammonium-chloride-1200652022p.htm (Accessed: 17 February 2024).

Interactive: Platts Ammonia price chart (2024-f) *S&P Global*. Available at: https://www.spglobal.com/commodityinsights/en/market-insights/latest-news/energy-transition/051023-interactive-ammonia-price-chart-natural-gas-feedstock-europe-usgc-black-sea(Accessed: 17 February 2024).

Introduction to thecno-economic analysis (no date) *US Department of Energy*. Available at: https://www.energy.gov/sites/default/files/2022-01/2022-01-19 - Intro to TEA - Slides and Transcript_compliant_1_0.pdf (Accessed: 22 November 2023).

Ion Exchange, water demineralization & resin testing (no date) *Veolia Water Technologies & Solutions*. Available at: https://www.watertechnologies.com/handbook/chapter-08-ion-exchange (Accessed: 04 February 2024).

Jaksland, C.A., Gani, R. and Lien, K.M. (1995) ‘Separation process design and synthesis based on thermodynamic insights’, *Chemical Engineering Science*, 50(3), pp. 511–530. doi:10.1016/0009-2509(94)00216-e.

Kuzma, S., Saccoccia, L. and Chertock, M. (2023) *25 countries, housing one-quarter of the population, face extremely high water stress, World Resources Institute*. Available at: https://www.wri.org/insights/highest-water-stressed-countries (Accessed: 07 January 2024).

Levy, N.P. (2023) ‘Fertilisers and Manures’, *Lockhart and Wiseman’ s Crop Husbandry Including Grassland*, pp. 81–114. doi:10.1016/b978-0-323-85702-4.00014-5.

Lipnizki, F. (2019) ‘Membranes in food technology’, in *Handbook of Food Engineering.* 3rd edn. Boca Raton, Florida: CRC Press, Taylor & Francis Group.

Lonchamp, J. *et al.* (2022) ‘Mycoprotein as novel functional ingredient: Mapping of functionality, composition and structure throughout the quorn fermentation process’, *Food Chemistry*, 396, p. 133736. doi:10.1016/j.foodchem.2022.133736.

Magnesium Sulphate Price Trend and Forecast (2024-a) *Chemanalyst*. Available at: https://www.chemanalyst.com/Pricing-data/magnesium-sulphate-1249 (Accessed: 17 February 2024).

Manganese Sulfate/Sulphate Monohydrate Feed Grade/fertilizer Grade Food Grade CAS 10034-96-5 (2024-d) *Global Sources*. Available at: https://www.globalsources.com/Industrial-chemical/Manganese-Sulphate-Monohydrate-1205836349p.htm (Accessed: 17 February 2024).

McGeorge, J.,(2019) *The morphology, growth and metabolic state of Fusarium venenatum in cation modified media.* Doctoral thesis, Northumbria University

Monopotassium Phosphate MKP 99% White or colorless crystals (2024-b) *ECHEMI*. Available at: https://www.echemi.com/produce/pr2205031086-monopotassium-phosphate-mkp-99-white-or-colorless-crystals.html (Accessed:17 February 2024).

Morrow, J.L. *et al.* (2019) 'Additions of sugar and nitrogenous fertiliser affect plant nitrogen status and soil microbial communities', *Applied Soil Ecology,* 139, pp. 47–55. doi:10.1016/j.apsoil.2019.03.002.

Moszczyński, P. and Tabarowski, Z. (2018) 'Meal plans for Diabetics', *Nutritional and Therapeutic Interventions for Diabetes and Metabolic Syndrome*, pp. 403–427. doi:10.1016/b978-0-12-812019-4.00032-5.

Muller, O. (2005) 'Malnutrition and health in developing countries', *Canadian Medical Association Journal*, 173(3), pp. 279–286. doi:10.1503/cmaj.050342.

NF flat sheet (no date,-b) *Alfa Laval*. Available at: https://www.alfalaval.com/products/separation/membranes/flat-sheet-membranes/nf-flat-sheet/ (Accessed: 11 February 2024).

Patnaik, P. (2003). *Handbook of inorganic chemicals*. New York: McGraw-Hill. ISBN 0070494398. OCLC 50252041.

Pervez, Md.N. *et al.* (2021) 'Sustainable treatment of food industry wastewater using membrane technology: A short review', *Water,* 13(23), p. 3450. doi:10.3390/w13233450.

PLSC 210 Horticulture Science Name Fall Semester, 2010 Homework #3 (2010) *North Dakota State University*. Available at: https://www.ndsu.edu/pubweb/chiwonlee/plsc210/homework/homework%20%233.pdf (Accessed: 01 February 2023).

Potassium Iodide (2024-f) *Global Sources*. Available at: https://www.globalsources.com/Organic-chemical/Potassium-Iodide-1202776109p.htm (Accessed: 17 February 2024).

Prabhu , S. (2022) *Can a centrifuge separate salt from water?, Alfa Laval Centrifuge Systems - from Dolphin Centrifuge*. Available at: https://dolphincentrifuge.com/can-a-centrifuge-separate-salt-from-water/ (Accessed: 18 December 2023).

Promote nutrition yeast inactive brewer's yeast powder for fish (no date) *Alibaba*. Available at: https://www.alibaba.com/product-detail/Promote-Nutrition-Yeast-Inactive-Brewer-s_1600750777246.html?spm=a2700.7735675.0.0.6626dJAudJAuRf&s=p (Accessed: 21 February 2024).

Purolite (no date) *Mixed bed ion exchange resin, Purolite*. Available at: https://www.purolite.com/index/core-technologies/product-type/mixed-bed-ion-exchange-resin (Accessed: 12 January 2024).

Recovery of sugar (2019) *PCI Membranes*. Available at: https://www.pcimembranes.com/articles/recovery-of-sugar/ (Accessed: 21 February 2024).

Riktlinjer för den industrier och andra verksamheter anslutna till den allmänna VA-anläggningen (2021) *Mittskanevatten*. Available at: https://www.mittskanevatten.se/wp-content/uploads/2021/04/riktlinjer-for-industrier-och-andra-verksamheter.pdf (Accessed: 08 February 2024).

Risner, D. *et al.* (2023) ‘A techno-economic model of mycoprotein production: Achieving price parity with beef protein’, *Frontiers in Sustainable Food Systems*, 7. doi:10.3389/fsufs.2023.1204307.

Robinson, J.R., Isikhuemhen, O.S. and Anike, F.N. (2021) ‘Fungal–metal interactions: A review of toxicity and homeostasis’, *Journal of Fungi*, 7(3), p. 225. doi:10.3390/jof7030225.

2023 års taxestatistik (2023) *Svenskt vatten*. Available at: https://www.svensktvatten.se/globalassets/organisation-och-juridik/vass/taxa/sv_rapport_kommentar-2023-taxestatistik.pdf (Accessed: 02 February 2024).

Råd Vid Mottagande av Avloppsvatten från industri och annan verksamhet (2019) *Svenskt vatten*. Available at: https://www.svensktvatten.se/globalassets/avlopp-och-miljo/uppstromsarbete-och-kretslopp/p95-2019-rad-vid-mottagande-av-avloppsvatten-fran-industri-och-annan-verksamhet.pdf (Accessed: 08 March 2024).

Scaler (No date). Available at: https://scaler.bio/ (Accessed: 03 March 2024).

Schrödter, K. *et al.* (2000) ‘Phosphoric acid and phosphates’, *Ullmann’s Encyclopedia of Industrial Chemistry* [Preprint]. doi:10.1002/14356007.a19_465.

Silk, D. *et al.* (2020) ‘A decision-support framework for Techno-Economic-Sustainability Assessment of Resource Recovery Alternatives’, *Journal of Cleaner Production*, 266, p. 121854. doi:10.1016/j.jclepro.2020.121854.

Sjölin, M. *et al.* (2019) ‘Purification of sucrose in sugar beet molasses by utilizing ceramic nanofiltration and ultrafiltration membranes’, *Membranes*, 10(1), p. 5. doi:10.3390/membranes10010005.

Sodium hydroxide price list in global market (2024-c) *ECHEMI*. Available at: https://www.echemi.com/pip/caustic-soda-pearls-pd20150901041.html (Accessed: 21 February 2024).

Solubility table of compounds in water at temperature (no date) *Sigma-Aldrich*. Available at: https://www.sigmaaldrich.com/SE/en/support/calculators-and-apps/solubility-table-compounds-water-temperature (Accessed: 07 February 2024).

Soong, C.J. (2016) 'Nutrients requirements and composition in a grouper fish feed formulation', *European Proceedings of Social & Behavioural Sciences* [Preprint]. doi:10.15405/epsbs.2016.08.10.

Spraytorkning (no date-b) *Tetra Pak Sweden*. Available at: https://www.tetrapak.com/sv-se/solutions/integrated-solutions-equipment/processing-equipment/spray-drying (Accessed: 17 February 2024).

Sridhar, A. *et al.* (2021) 'Conversion of food waste to energy: A focus on sustainability and life cycle assessment', *Fuel*, 302, p. 121069. doi:10.1016/j.fuel.2021.121069.

Sucrose (no date) *National Center for Biotechnology Information. PubChem Compound Database*. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Sucrose#section=MeSH-Entry-Terms (Accessed: 12 February 2024).

sugar prices per producers and regions (2024) *European Commission*. Available at: https://agriculture.ec.europa.eu/system/files/2023-03/sugar-prices-producers-regions_en_0.pdf (Accessed: 14 February 2024).

Sulphuric Acid Price Trend and Forecast (2024-d) *Chemanalyst*. Available at: https://www.chemanalyst.com/Pricing-data/sulphuric-acid-70 (Accessed: 16 February 2024).

Tetra Pak® drainer washer 2 for cottage cheese curd (no date-a) *Tetra Pak*. Available at: https://www.tetrapak.com/solutions/integrated-solutions-equipment/processing-equipment/draining-washing-blending/drainer-washer2 (Accessed: 15 December 2023).

Tetrasodium EDTA Price Trend and Forecast (2024-c) *Chemanalyst*. Available at: https://www.chemanalyst.com/Pricing-data/tetrasodium-edta-1413 (Accessed: 17 February 2024).

The use of fish meal in animal feeds (2001) *ACAF*. Available at: https://acaf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/ACAF00106.pdf (Accessed: 09 February 2024).

Theoretical oxygen demand (thod) calculator (2023) *Aropha*. Available at: https://www.aropha.com/thod-calculator.html (Accessed: 02 February 2023).

Thomas, A.B. *et al.* (2017) ‘Employing central composite design for evaluation of biomass production by fusarium venenatum: In vivo antioxidant and antihyperlipidemic properties’, *Applied Biochemistry and Biotechnology*, 183(1), pp. 91–109. doi:10.1007/s12010-017-2432-5.

Tomasula, P.M. *et al.* (2011) ‘Pilot-scale crossflow-microfiltration and pasteurization to remove spores of bacillus anthracis (Sterne) from milk’, *Journal of Dairy Science*, 94(9), pp. 4277–4291. doi:10.3168/jds.2010-3879.

Upcraft, T. *et al.* (2021) ‘Protein from renewable resources: Mycoprotein production from agricultural residues’, *Green Chemistry*, 23(14), pp. 5150–5165. doi:10.1039/d1gc01021b.

Vom Og Hundemat active Proteinrikt Fullfoder (2024) *Hund & Hälsa*. Available at: https://www.hundochhalsa.se/product/vom-og-hundemat-active-proteinrikt-fullfor-500-gr (Accessed: 20 February 2024).

Wen, G. *et al.* (2020) ‘Inactivation of fungal spores in water using ozone: Kinetics, influencing factors and mechanisms’, *Water Research*, 185, p. 116218. doi:10.1016/j.watres.2020.116218.

Wiebe, M. (2002) ‘Myco-protein from fusarium venenatum : A well-established product for human consumption’, *Applied Microbiology and Biotechnology*, 58(4), pp. 421–427. doi:10.1007/s00253-002-0931-x.

Wikandari, R., Hasniah, N. and Taherzadeh, M.J. (2022) ‘The role of filamentous fungi in advancing the development of a sustainable circular bioeconomy’, *Bioresource Technology*, 345, p. 126531. doi:10.1016/j.biortech.2021.126531.

Zinc-Sulfate-Heptahydrate (2024-a) *Global Sources*. Available at: https://www.globalsources.com/Organic-chemical/Zinc-Sulfate-Heptahydrate-1189357808p.htm (Accessed: 17 February 2024).