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Bioprocessing of Lignin into Valuable Lipids

A Techno-Economic Evaluation based on Modelling in ASPEN PLUS

Master's thesis in Innovative and Sustainable Chemical Engineering

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Abstract

To keep up with the increasing energy dependency and at the same time manage the global environmental crisis a development of renewable green energy is required. A renewable resource that is of particular interest is lignocellulose, which is the most abundant biogenic carbon resource in nature. The second most abundant polymer in nature is lignin, that is a polymer in the cell wall of lignocellulose that can be hard to utilize and do not have many areas of application yet. Therefore, lignin is a promising feedstock for biofuel production. About 97% of all vehicles in Sweden run on gasoline or diesel and even though electrical engines is in the upcoming it will be hard to make all vehicles electrical. Especially long traveling and heavy-duty transportation. Therefore, is it important to develop biofuels that these existing vehicles and engines can run on to decrease the dependency of oil and other fossil fuels.

This master thesis is performed in collaboration with Preem, which is Sweden´s biggest fuel company. Preem count into some of Europe's most modern and climate efficient refineries and they have a goal to be climate neutral by the year 2035. This goal is in line with the goal of the European Union, that aims to have an economy that is climate neutral by the year 2050. This thesis aims to investigate if there are some bioprocesses available that could convert lignin into valuable products. The products are aimed to be used as a future feedstock for biofuel-production.

The technical assessment of this project has scaled up an experimental result from literature with the simulation software Aspen Plus. The results from the simulation have further been used in a techno-economical evaluation, to generate total operating cost and fixed capital cost of the process and to understand what sensitivities the process have. There are some uncertainties in the project because a lot of assumptions has been made and because some information about reactions and results were missing. The results showed that the investment cost for this process would be approximately 1 355 MSEK and the operating cost would be approximately 233 MSEK. It showed that the most expensive compounds are the bacteria and the media for pre-cultivation and fermentation. To make the process profitable the price of the lipids would have to be 5 409 USD/ton of lipids, which is more than double the price the lipids costs today. The results also showed that it is of significant interest to invest in a pre-cultivation process because the already pre-cultivated bacteria are more expensive and affects the price of the lipids a lot. While a higher efficiency of the pre-treatment of lignin did not have a significant change on the price of the lipids. It also showed that a higher concentration of lignin monomers in the fermentation process has the largest impact on the price of and is the most significant sensitivity for this process.

From the results of the technoeconomic simulations it could be concluded that this experimental technology still is novel, and a lot more experimental research needs to be done, for the technology to be mature enough to invest in for industrial scale. It indicates that the concentration and yields of lipids is one of the major things that would need to be investigated and developed more in the future to truly make this process competitive and worth investing in for industrial scales. It was also concluded that a secondary use of the bacterial remains would be of large interest to truly make the process profitable and sustainable.

Keywords: Lignin, bioconversion, biofuel, green, sustainable, scale-up, economy, lipids, microorganism

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Contents

1. Introduction

The increasing energy dependency and the increasing environmental impacts that fossil fuels consumption have caused, have called for a development of renewable green energy (1). Converting renewable biomass into fuels and chemicals is an attractive method for making greener chemicals and more sustainable products. Lignocellulose is one of the most abundant resources in nature and it consists of cellulose, hemicellulose, and lignin. Lignin is the second most abundant polymer after cellulose in nature. Both cellulose and hemicellulose can be degraded and used as various products and by-products. Among these different components in biomass, lignin can be hard to utilize efficiently because of its complicated aromatic heteropolymer structure and the carbon-carbon bounds it makes with cellulose and hemicelluloses in the cell wall. It is most often used as an energy supply or discarded as waste. Therefore, lignin is a promising future feedstock for production of biofuels and biochemicals (2).

Since lignin has high carbon-oxygen ratio and skeleton rich in aromatics it is an interesting feedstock for several industries, like for example for biodiesel, biopolymers, biochemicals among others. But to really exploit this amorphous heteropolymer it is in an urgent need to investigate and understand more about the degradation process and develop pathways for conversion (2). Lignin valorisation is also very important to fully make lignocellulosic biorefineries sustainable and profitable, since lignin is a major waste stream from biorefineries and paper and pulp industry. If lignin would be utilized further it would improve CO_2 mitigation, waste management, net energy gain and even cost effectiveness of the biofuels that the biorefinery produces. A big challenge with the lignin valorisation is that content and composition of the molecule varies significantly among different plants. Even though many processes have been tried for lignin depolymerization, they involve high pressure, high temperature, high energy cost and they generates inhibitors for microbial growth which makes them not ideal. But enzymatic depolymerization of lignin might be an option for the future since it has milder conditions and even potentially fewer inhibitors for microbial growth. Unlike the enzymatic cellulose degradation, enzymatic lignin degradation depends a lot on the redox reactions, which relies on electron transfer from a proper donor to the target. This is thus often done by molecule oxidants produced by enzymes like laccase (3).

In this project a bioconversion process has been evaluated, where a pre-treatment unit would degrade kraft lignin with the enzyme laccase to solubilized lignin monomers. These monomers are feed into a fermentation unit with the oleaginous bacterium *Rhodococcus opacus* are consuming them and accumulating lipids. The lipids are extracted from the bacteria and then they can be used as a feedstock for biofuel production. To evaluate the maturity of this process a techno-economic evaluation is investigating operation costs and capital costs, as well as investigating what parts of the process is most contributing to the cost of the products, the lipids.

1.1. Background

This master thesis is made in collaboration with Preem refinery in Gothenburg. Preem has two refineries one in Gothenburg and one in Lysekil. The refineries have a capacity to produce 18 million cubic meters per year, they are producing and refining fuel and other products for both companies and private persons. Preem is today Sweden's biggest fuel company, they count into some of Europe´s most modern and climate efficient refineries. The refineries have greenhouse gas emissions far below the existing legislations and demands. In year 2015 about 97% of all vehicles in Sweden run on either gasoline or diesel, so if Sweden is going to lower its demand on oil, other more renewable fuels will have to be developed, that still can be used in the existing engines. This is not only important for Sweden but for the whole world and all types of engines, maybe even more important for the long traveling transports like aviation and heavy-duty trucks

that will be hard to make electrical. Preem produced 300 000 cubic meter biodiesel year 2021, that can be used in the existing diesel vehicles. Preem has a vision to lead the transformation towards a more sustainable society and has set the goal to be climate neutral by year 2035. The company has therefore a careful thought about what raw materials they choose to invest in and wants to invest in sustainable feedstocks like by-products from forestry and agriculture. They take an active decision to not invest in raw materials that can contribute to environmental problems like for example the contribution of deforestation of rainforests (4).

It is not only Preem that has goals about being climate neutral in the future, but European Union also aims to be climate-neutral by the year 2050. Meaning that they aim to have an economy within the union that have net-zero emissions from greenhouse gases. This is the objective of the European Green Deal and in line with the commitment of the global climate action that lies under the Paris Agreement, that has the objective to keep the increase in global temperature below 2°C. EU can impact the way that the transition goes by investing in realistic technological solutions, adjust action in areas like industrial policy, research, and finance (5).

1.2. Aim

The master thesis aims to investigate if there are any available bioprocesses that can breakdown and convert lignin into valuable products. The products are aimed to be used as a future feedstock for biofuel production. A bioprocess for this purpose was evaluated through a techno-economic analysis by using Aspen Plus software. This techno-economic analysis provides information about how the process could be installed on industrial scale and provide technical and economical details.

1.3. Scope

The master thesis has a time limitation of approximately 20 weeks: Performing experiments was not in the scope of this thesis, so instead results found in the already existing experimental literature was scaled up to an industrial level. In the techno-economic evaluation a block flow diagram was drawn of a proposed process that was modelled in an industrial scale in Aspen Plus and an economic evaluation was performed by using Aspen Plus. This thesis mainly focuses on the valorisation of lignin into a feedstock that later can be further processed into biofuels. There is a gap in literature for a complete process of lignin valorisation using biochemical methods, so therefore this report will assume the process on industrial level by using experimental processes that already exist in literature.

2. Theory

With the growing demand for energy, chemicals and fuels combined with the growing global concerns about the environmental problems caused using fossil fuels, lignocellulosic biomass is viewed as a promising raw material to replace fossil fuels and thereby slow down the climate crisis. The cell wall of biomass consists of cellulose, hemicellulose, and lignin with different ratios of the polymers depending on type of biomass (6). Lignin is the second most abundant polymer in nature after cellulose, it can be found in all vascular plants mainly in the cell walls of the plants. The lignin polymer is bound by chemical linkages to the other polysaccharides in the cell wall, making it very hard to separate the polymers (7). How it can look like can be seen in Figure 1, and as can be seen the lignin is mainly located in between cellulose layers and bound to the other polysaccharides in the cell wall, which makes it so hard to separate out individually (8).

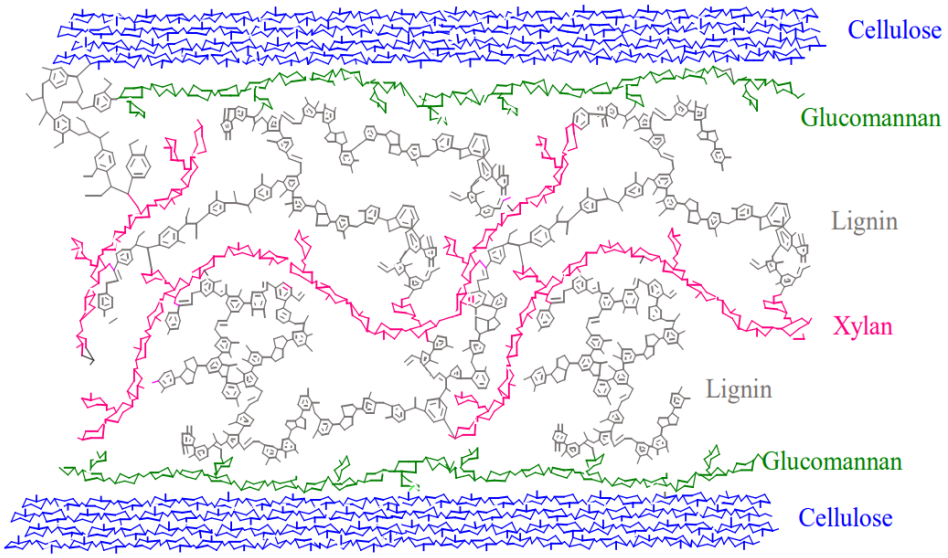

Figure 1, a model of the cell wall structure showing the components and where they are in the cell wall (8).

2.1 Materials

To produce a feedstock for biofuel production from lignin a lot of materials are needed, and they will be presented and explained in this section of the report.

2.1.1 Biofuels

Biofuels are a renewable and sustainable energy carrier that can be produced from microbes, vegetable oil, organic waste, or different types of biomasses. The biofuels emit lower amounts of carbon dioxide than the conventional fuels, since the carbon dioxide that the biofuels emit is a part of the carbons natural cycle and the fossil carbon dioxide is adding extra carbon to the atmosphere that is not a part of the natural cycle. Thereby biofuels play a big role in lowering of the greenhouse gas emissions overall (9). Because of a continued increase in demand for fuels and the emerging global warming upshots, the development of alternatives is prioritised in the development and research area. The huge need for alternative resources to produce sustainable fuels, is because of the effects that the greenhouse gas emissions have to our nature. For example, the greenhouse gases affect the biodiversity, raise in seawater levels and the increase in weather variations. The tremendous increasing demand for fossil fuels is additionally affecting the global economy and an escalating pressure on the crude oil production has been seen (9).

Biofuels can be classified into four different generations, where the first generation includes biofuels produced from vegetable oils and crop plants. So, biofuels from this generation can

impact the food security and similar. Second generation biofuels are produced from agricultural waste and non-edible crops and the main products are bioethanol and biogas. For the third-generation biofuels the feedstock is seaweed, microorganisms, marine reserves and cyanobacteria and the products can be bioethanol and biobutanol. And lastly for the fourth generation of biofuels the feedstock is mainly photosynthetic microorganisms and non-arable land, and the products can be solar and electro fuels (9).

The cheapest and often seen as the best source for biofuel production is lignocellulosic substrates, but these raw materials are complex and difficult to degrade. So, a challenging task in the biofuel production is to treat the complex polymers and convert them into more straightforward compounds. One method to do this is by enzymatic treatments, that is a green approach that requires little energy and provides high specificity. The enzymes convert the lignocellulosic substrates into products that later can be fermented by various microorganisms to produce biofuels (9). Developing renewable, biodegradable substitute fuels that will have similar properties as the already existing petroleum diesel, will be important so that the fuels will be compatible in the existing transportation vehicles. Currently prices of fuels fluctuate depending on many different factors but the price of producing biofuel can be assumed to be in the range between $105 -$115 per barrel, while the crude oil is sold at approximately $45 per barrel. Therefore, it is very important to continue to develop strategies and platforms of producing biofuels from lignocellulosic biomass to be able to reduce the cost of refining biomass into biofuels (10).

2.1.2 Lignin

Lignin is an important component in the cell wall of plants to generate structure and stiffness, it is hydrophobic and thereby insoluble in both alcohol and water. It is a complex polymer that is constructed of monolignol units and has been studied extensively because of its significance in the paper and pulp industry (6), where cellulose rich product was produced by eliminating lignin from the pulp. The lignin in the wood biomass is mainly bound to polysaccharides and in woody plant about 15-40% of the biomass is constituted of lignin. Lignin is mainly built up of three different monolignols, sinapyl alcohol (syringyl), coniferyl alcohol (guaiacyl) and p-coumaryl alcohol (hydroxyphenyl), see Figure 2, that are linked by carbon-carbon or ether bonds (6,11,12).

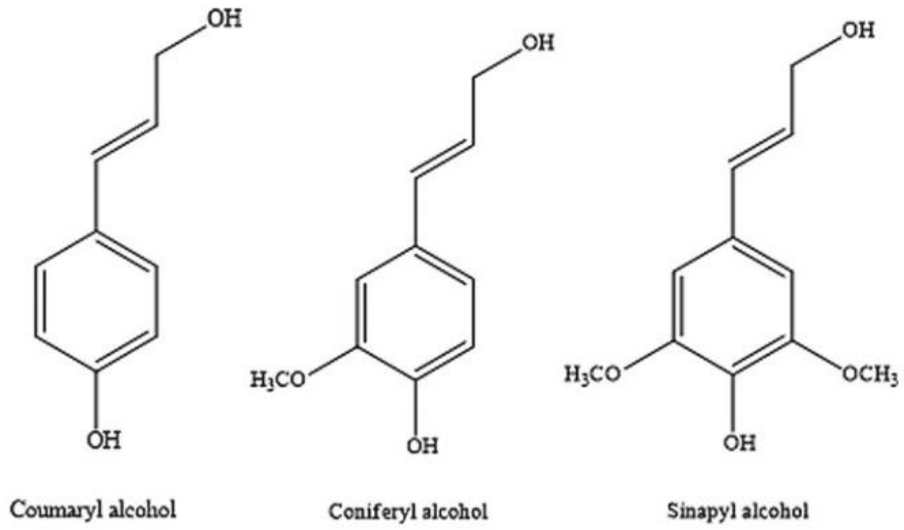

Figure 2, the three monolignols that lignin is mainly constructed of and their chemical structures (6).

It is also important to mention that the structure of native lignin is still not fully known because of its amorphous nature. A simple picture of how the common chemical structure of lignin often is showed can be seen in Figure 3, where it also can be seen how the monolignols are bound to each other. There are also some other non-conventional monolignols identified in the lignin structure, but the amounts are negligible. The amount of the different monolignols differs depending on the type of plant (6,11,12).

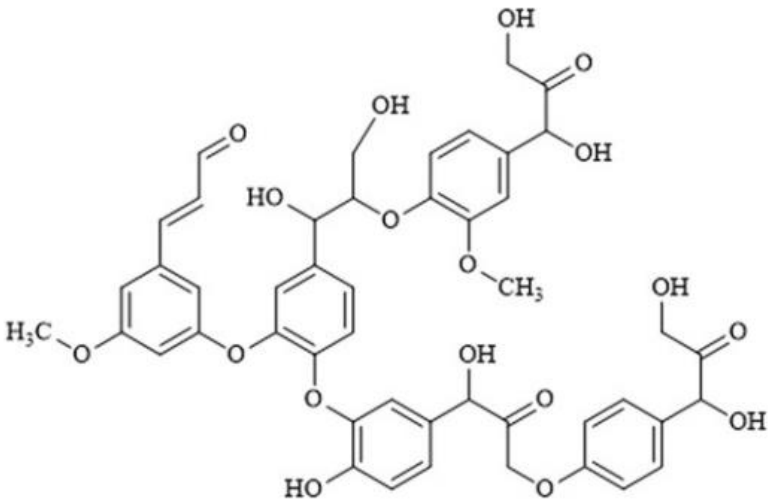

Figure 3, a common model of a simple lignin molecule (6).

Today about 80 million tons of lignin is produced only as a by-product from both paper and pulp industry and lignocellulosic biorefineries. Only a small part, about 2%, of this is used in the industry and the rest is mainly waste, dumped or burned. This is a wastage of resources, hence valorisation of lignin through different technological developments is necessary for a more sustainable use of resources (6,12). To separate lignin from the other biomass and how that is performed will have a significant influence on the structure, purity, and properties of the lignin (11). The lignin that is obtained from the Kraft process, a specific paper and pulp process, can only be water soluble in alkaline solutions. This type of lignin is available commercially, but the production is still quite small compared to what it could be, because the demand for such lignin is still very small. This even though research has been conducted for decades on how to use these types of lignin as carbon fibres, phenol-formaldehyde plastics, adhesives, and others (7). Since lignin is a part of biomass that is a renewable resource that can generate several different products, it has a relatively stable price through seasons and years. The price of the lignin generated from the Kraft process has a range from 260-500 USD/MT (11). For several years there has only been one source of commercial Kraft lignin, that has been Indulin AT, a Kraft lignin that is obtained through a two-step acidification process. More recently a new process for isolation of Kraft lignin has been developed, this process isolates the Kraft lignin from black liquor and is more adapted to the modern Kraft process. This new process is called LignoBoost, and the Kraft lignin product is quite pure and has a low content of residuals, ash and carbohydrates, the product can be obtained as a solid powder (7).

Kraft lignin differs from native lignin in chemical structure, the Kraft lignin only has a small number of lignin-carbohydrate linkages left after the pulping process. A lot of the predominant linkage inside the lignin molecule, the aryl ether linkage (beta-O-4) is cleaved in the Kraft process which results in fragmentation of the lignin molecule (7). Even though many processes to depolymerize lignin have been developed, most of them includes high pressure, temperature, energy cost and generates inhibitors for microorganisms' growth. Therefore, enzymatic

depolymerization of lignin might be beneficial due to the fewer inhibitors for microbes it generates and the milder condition that it demands for (3).

2.1.3 Laccase

Laccase is a promising enzyme that can act as a biocatalyst for degradation of lignin into fragments that later can be upgraded to valuable bioproducts and biofuels. Since the laccases have a broad substrate ability and a natural green chemistry, they are being used in pharmaceutical, food, pulping and bleaching industries among others (13).
Laccase is a multi-copper dependent enzyme, also called blue multicopper oxidases (MCO) (14), the catalytic site of laccase contains three to four copper atoms. One of the copper atoms is located at the surface of one of the active sites of the enzyme where it can interact directly with the substrate, lignin, or mediator. The enzyme is functioning by overlapping electrons between the copper atoms to catalyse a sequence of four electrons that will oxidise the substrate and at the same time a reduction of dioxygen will form two water molecules (13). Depending on how many copper ions that exist in the active site of laccase it will give different colours, white, blue, or yellow. The blue laccase is often referred to as true laccase, this is since it has all four copper ions. The white laccase commonly contains one copper ion and the yellow laccase do not have a type I copper atom at all. Both the white and the yellow laccase are referred to as non-true laccases since they contain other metal ions like iron or zinc instead of the copper ions (9).

The enzyme can catalyse the oxidation of several different substrates, including phenolic compounds, it can also in the presence of a mediator molecule oxidise non-phenolic compounds. This mediator could for example be 1-hydroxybenzotriazole (HBT). Laccases has been found in many organisms including fungi, bacteria, insects, and different plants, and are involved in both polymerisation and depolymerisation of lignin. This is because the enzymes can generate radicals by extracting one electron from the phenolic substrate (14). As mentioned above so are laccase on itself not suitable for degradation of lignin, since there are only a small number of phenolic groups in the entire lignin structure. Therefore, it is important to use a redox mediator in combination with the laccase enzyme to be able to oxidate also the nonphenolic lignin structures. This combination is called a laccase mediator system (LMS) (15).

One big drawback with the laccase enzyme is that there is no technology for recycling the enzyme available today in industry. This will impact the economic viability of the different processes. For a full commercialization it would require that a high production and purity of the enzymes would be done at affordable price, and that the mediators as well are cheap and intensive to help increasing the redox potential. The enzymes are under active development towards being affordable commercially, by continually developing purification processes and increasing the stability and the choice of substrate for the enzymes (13). One thing that is being investigated to reduce the production cost of laccases is homologous and heterologous hosts to create the enzymes, one example of such a host is *Aspergillus* sp. A well-known industrial host that has an ability to produce several g/l which potentially could reduce purification process (13). There are also some factors that can affect the enzyme expression and durability, that can for example be the different inhibitors that are produced during lignin degradation. One example of such inhibitors can be syringaldehyde (9).

2.1.4 HBT

1-hydroxybenzotriazole also known as HBT is a common reagent in chemistry labs and the reports of its use in organic synthesis is increasing every year ((16). HBT is a coupling reagent that often is used for synthesizing of amides by a condensation reaction, and as a racemization

suppressor for synthesis of peptides. It is often used as a condensation reagent to prepare different esters, acids, and other derivatives. It is a dry solid powder that contains a low amount of water, approximately 10% (17). The HBT can as have been mentioned before being used as an electron mediator, meaning that it has a redox moiety that can help facilitate transfer of electrons or redox potential. This is done from a donor to an acceptor during a reaction. These reactions can help enzymes to break bonds that needs a higher redox potential than the one they can have on their own (3).

2.1.5 Lignin monomers

It has been a lot of research on how to process lignin, including studies on how to depolymerize lignin into monomers by different means. Then these monomers are in the hope of being upgraded to industrial relevant chemicals and products (18). From the experimental report that this project is going to be following, they have detected the following monomers after the laccase-HBT treatment of lignin: Vanillic acid, Vanillin, Phenol, acetovanillone, 4-hydroxybenzoic acid, dehydroabietic acid, 4-hydroxybenzaldehyde, cinnamic acid and several more monomers. These mentioned here are the ones that they had detected in a larger amount (3). These monomers can be used as feedstock for biopolymer, biochemical and biofuel productions among others, vanillin can also be valuable and sold as it is in monomer form (18).

It has been shown previously that bioconversion of lignin and lignin monomers from physical or chemical methods has led to a very limited production of bioproducts using biochemical methods compared to the production when model compounds such as vanillin has been used. This is one of the reasons why enzymatic lignin fragmentation is of particular interest since its products is potentially less toxic and inhibitory to bioconversion organisms (3).

2.1.6 Bioconversion organisms

All living microorganisms are composed of lipids, typically around 6 to 8 % of their total dry cell weight is lipids. Some microorganisms can produce larger amounts of lipids that is typically used for energy storage inside the organism, but if they produce more than 20% of their dry cell weight the organisms are called oleaginous. The lipids that they produce are often called single cell oils (SCOs) and these lipids are a promising feedstock for biofuel production (19). Some types of oleaginous microorganisms can for example be fungi, bacteria, yeast, and microalgae, most of these will produce triacyl glycerides (TAG) that is the main component in Hydro Vegetable Oil (HVO) and biodiesel production. The TAG is being mixed with an alcohol in the presence of a catalyst that most often is an alkaline or acid. When the TAG and the alcohol react, they will produce fatty acid methyl esters (FAME) that can be used as a component in diesel blending, biofuel feedstock etc. This process is often known as transesterification (10).

The oleaginous soil bacterium *Rhodococcus opacus* (*R.opacus*) has been studied a lot in the recent years because of its great ability of accumulate intracellular lipids in great amounts. Some strains of the bacterium have been shown to accumulate lipids over 80% of the organism's dry cell weight, the strains have also been shown to degrade aromatic compounds in the absence of glucose (10). The Rhodococcus is often used as a model organism for lignin valorisation, because of its ability to tolerate and adapt to inhibitory compounds like phenols that often is produced in depolymerization of lignin. Some reports have demonstrated that if the lignin first is converted into aromatic compounds it will increase the lipid titre in the R.opacus (10).

The ability to tolerate and metabolize on aromatic compounds is something several different microorganisms can do, and it is likely an evolutionary advantage since the large availability of

lignin in natural environments (20). The R.opacus is storing the carbon as TAGs in the cell as an energy storage, in R.opacus acetyl-CoA is produced during several catabolic pathways including glycolysis and also some aromatic degradation pathways like the β-ketoadipate pathways. When the bacteria feed on only aromatic compounds the lipid production is reduced, but it has been seen that it still can reach up to 44% TAGs in cell dry weight. The lipid production in R.opacus has been demonstrated on different carbon feedstocks, when kraft lignin that has been pre-treated with laccase was used as carbon source it could produce 0,145 g/l of lipids. When R.opacus was grown with glucose and glycerol as carbon source it could generate 2.4 g/l lipids, and since glycerol is a common by-product of the transesterification process it could be beneficial to recirculate this back to potentially reduce costs (20). When a kraft lignin pre-treated with a laccase-mediator system was used as carbon source for fermentation with R.opacus it was shown that the cell growth was increased dramatically compared to pure kraft lignin growth. The dry cell weight reached 2.79 g/l and the lipid titre reached 1.02 g/l lipids (3).

2.1.7 Lipids

The lipids that the bioconversion organism is producing can differ some depending on the organism and the feedstock, but in general they produce some type of TAGs that can be used for further valorisation and biofuel production (21). TAGs can also be commonly found in animal fats and vegetable oils, and as mentioned before transesterification is a common method to make biofuel from these TAGs by reacting them with an alcohol to make FAMEs or fatty acid ethyl esters (FAEEs). Depending on the oil that is used as feedstock it will affect the properties of the final product, such as viscosity and cetane number which can have an impact on the performance of the biofuel. Biofuel from soybeans is one of the main alternatives in the US, and that reduces greenhouse gases and air pollutants by approximately 41% compared to the use of conventional diesel (21).

For this project it has been assumed that the production of lipids is as follows: 42.9% steric acid, 36.6% oleic acid and 20.5% palmitic acid. These acids have been shown to be like soybean oil and other types of vegetable oils that has been used for biofuel production (22).

2.1.8 Medias

To grow, ferment and process biomaterials the type of medias that are used differ a lot and there are several different medias that can be used, and they have different advantageous and disadvantageous. Researchers have discussed a lot about the nutrients that are needed in the media for cultivation and fermentation of organisms and how they can affect the lipid production. There are some focuses on carbon nitrogen ratio in the media, one factor that is known to increase the lipid accumulation in organisms is to have a limiting amount of nitrogen (23).

In this project the medias have been assumed to be like the ones used in the experimental report (3), so for the lignin pre-treatment a phosphate buffer of 113 g/L K_2HPO_4 and 47 g/L KH_2PO_4 is used. And for the fermentation and pre-cultivation processes the media has been assumed to be the same phosphate buffer as for the pre-treatment process, a trace element solution that contains: $FeSO_4$, $ZnSO_4$, $MnSO_4$, H_3BO_3, $NiCl_2$, EDTA, $CoCl_2$ and $CuCl_2$. The media also contains $(NH_4)_2SO_4$, $MgSo_4$ and CaCl. All these components are needed to ensure that the organisms get all nutrients they need to grow and thrive, then the lignin monomers are the sole carbon source in the fermentation process (3).

2.2 Aspen Plus

Aspen Plus is a flowsheet simulation computer software, that is most often used to model process plants, especially chemical process plants. Starting from a raw material and going to a finished

product, simulation tools like Aspen Plus give the ability to predict behaviors of processes by implementing basic engineering relationships. Simulation tools can optimize plants to increase profitability, designing and predicting revamps or like in this project starting from a rough research or idea to give a first understanding into what will be important for a novel process to continue to optimize and develop (24).

2.2.1 Aspen Plus components

Aspen Plus property database has a lot of components in them but of course there are some that they do not have. But it is still possible to implement such components in Aspen Plus by user define them, one example for such component is lignin. Depending on what is going to be done to the component some physical properties need to be known and implemented into Aspen Plus. The minimum physical properties that Aspen requires are enthalpy and density of the component (25).

2.2.2 Property package

Another key decision in Aspen Plus is the choice of property package, a property package or property method is a set of models that are used to calculate kinetic, transport and thermodynamic properties (24). For this project the NRTL (non-random two liquid) property package was chosen, this package includes the Henry´s law for dissolved gases, liquid activity model, Redlich-Kwong-Soave equation, it also uses ideal gas at 25 °C. Therefore, Aspen Plus needs input for the heat of formation of the components at 25 °C. Other things than the minimum physical properties that Aspen requires when the NRTL method is used are, critical pressure, critical temperature, vapor pressure, heat of formation and heat of vaporization. The NRTL property package is chosen for this project because it is preferred when having highly non-ideal mixtures and low operating pressures (25).

2.3 Assumptions

For modelling in the software Aspen Plus and to make the techno-economic calculations some assumptions were needed to be made and they will be presented and explained in this section.

2.3.1 Assumptions for Aspen Plus

As mentioned before not all components were available in the Aspen Plus database so then they either needed to be user defined or a model compound was needed to be used instead. There was also some equipment that could not be used since too little information was known about reactions kinetics.

The enzyme Laccase was not found in the Aspen database so therefore, the enzyme Ubiquinone was chosen instead to simulate Laccase as a model compound, this enzyme has a similar chemical composition and molecular weight as laccase. See appendix A1 for exact chemical structures and molecular weight for the two enzymes. Unfortunately, later when the enzyme was supposed to be used in the simulation it did not work, this was because too little information was known in the database about the structure of the enzyme. The same problem occurred for the mediator HBT. Since no reactors could be used in the program, the reactions were not simulated but rather the volume of the reactor was simulated with a tank and the components consumed and produced in the reactions are simulated with a component splitter and a mixer. So, therefore it was decided not to implement the components laccase and HBT, instead they were simulated as lignin to ensure that the mass flow of the pre-treatment unit still would be accurate for calculations of equipment.

The bacteria R.opacus was also not available in the Aspen Plus database, and unfortunately no bacteria or other microorganisms were found in the database at all. So instead, it was assumed that the bacteria R.opacus could be simulated as the ketone Fenchone, since this was the molecule with a chemical structure similar to the bacteria that was found in Aspen Plus database, see appendix A1 for more information. Neither was Kraft lignin available in the Aspen Plus database so therefore lignin was user defined as three different components, carbon rich, oxygen rich and hydrogen rich lignin. This lignin is like native lignin that exist in biomass like for example wood, and it was defined with data from Gorensek M, Shukre R & Chen C´s report (26).

It was assumed that the products from the pre-treatment of lignin only consists of 6 major products, phenol, cinnamic acid, vanillin, 4-hydroxyaldehyde, 4-hydroxybenzoic acid, methyl salicylate and that the other compounds are in such small amount that they are neglected in the Aspen model. The product 1,4-cyclohexadiene was assumed to be the only monomer produced after the fermentation with the bacteria, according to the results from the experimental report (3). It was also so that some of the compounds was not available in the Aspen database, like 7-oxo-dehydroabietic acid for example and then it was excluded from the model as well. There was no information about exact amounts of each of these compounds but there were a heatmap that showed the abundance of the different aromatic monomers before and after fermentation (3). From this heatmap an assumed mass fraction between the compounds were made, see appendix A3 for assumed mass fraction of the different monomers.

In the report it did not mention the time of the periodically outtake of bacteria (3), so this was assumed to be done every 24 h, and it is assumed that the reported result is reached after 48 h of fermentation. This time is based on other similar experiments that has discovered maximum lipid accumulation after 48 h(27,28). Since no kinetics of the fermentation is known and the bacteria is accumulating the lipids, the reactor is simulated as a tank at constant temperature of 28°C in Aspen Plus, since all available reactors need to have some information about the kinetics.

2.3.2 Assumptions for techno-economic calculations

As mentioned above there were a lot of assumptions made first in the simulation of the process and that has also ended up in some simplifications of the process. That will result in some assumptions here as well.

There were also some assumptions made of the price of the different raw materials and products. The prices of the different medias were assumed similar as the medias that was used in bioethanol production. The price of Laccase was assumed to be the same as the cellulase enzyme used in bioethanol production and from that the mediator HBT was assumed to be 1/3 of the enzyme price. This because it was found on Sigma Aldrich that the HBT cost was approximately 1/3 of the cost of Laccase. The price of bacteria was assumed to be like the price of biological enzyme, both for buying already pre-cultured bacteria, that was assumed to be like buying already cultured biological enzyme, and for buying bacteria seeds for pre-cultivation process, that was assumed to be similar as buying proteins for enzyme production. The price of hexane was assumed to be like the price of crude oil + 15 USD/barrel and the price of the lipids was assumed to be approximately 2000 USD/ton. All assumed prices can be seen in Table 9 in Chapter 3.

3. Process

To understand how this process of converting lignin into lipids with a bioconversion organism would look like and what different process units that would be needed a schematic picture of the process was made. The schematic picture of the process from kraft lignin to lipids can be seen in the Figure 4 below. This process is made based of the results from Xie, S et.al 2016 report about *Advanced Chemical Design for Efficient Lignin Bioconversion* (3). It is a batch process, but to be able to simulate it in Aspen all flows have been recalculated to fit a continuous process. The process was also upscaled from experimental levels to industrial scale, this was done to produce about 10 000 m^3 lipids per year. All calculations and numbers for the upscaling can be seen in Appendix A1. Since there was not mentioned anything about carbon dioxide production during the fermentation, it is being assumed that 40% of the carbon source (lignin monomers) is converted to carbon dioxide during fermentation. It is also assumed that hexane is the only chemical used in the extraction process as a simplification.

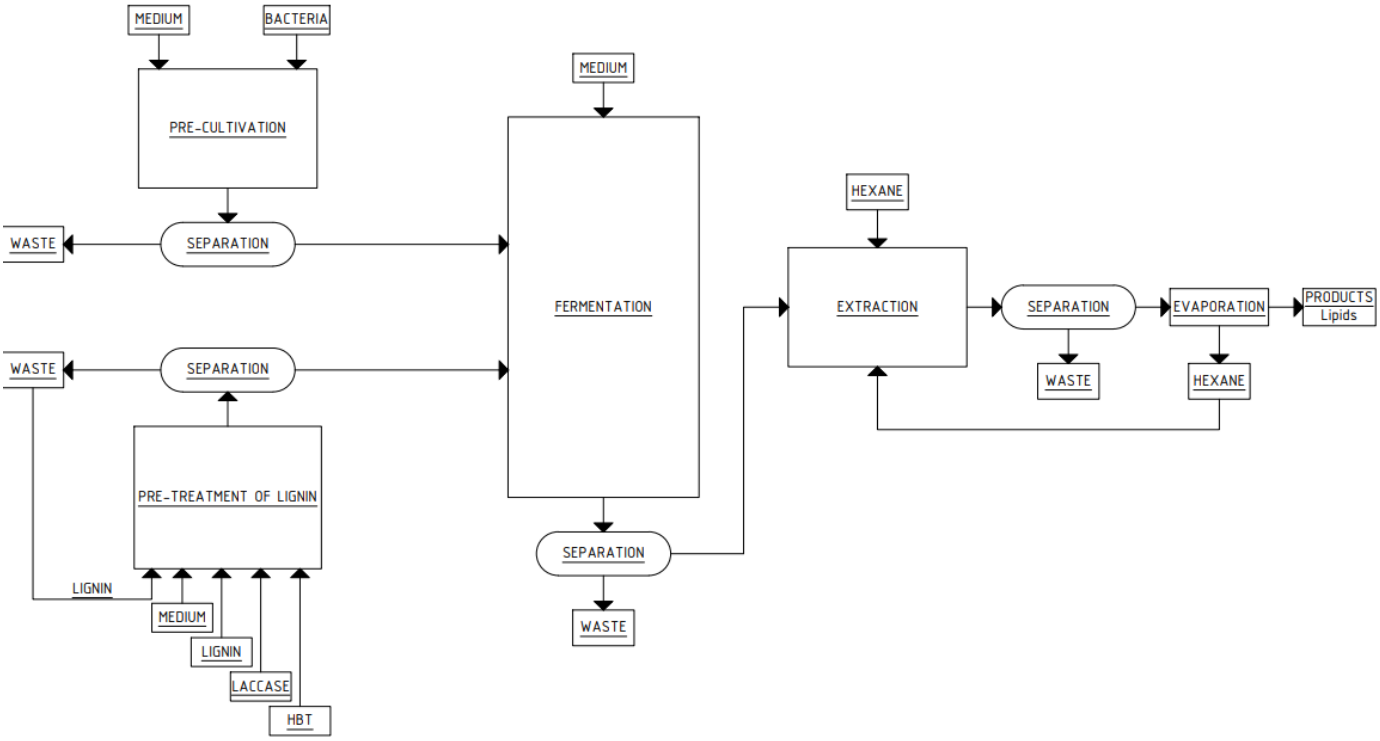

Figure 4, a schematic picture of how the process was assumed as, this is a very simple first sketch of the process and what is needed in it.

The process described in Figure 4 was modelled in Aspen Plus software, version 12.1. There have been some simplifications in this model and some assumptions have been made, these were explained in Chapter 2.3. This was done both because of the time limit of the project and because some features and compounds were not available in the Aspen Plus database or because the information was not known for the process.

The template for batch Pharmaceuticals was chosen because it was found that this option was supposed to be good when working with biological feedstock and to simulate living organisms like yeast or bacteria and with biological enzymes like laccase. Then the NRTL (non-random two-liquid) property package was chosen as the base property model because it is good when having highly non-ideal mixtures and low operating pressure. Most of the components were available in Aspen Plus database, but some of the components needed was not available. Lignin was one of

the compounds that was not available so, this was user defined by Gorensek M, Shukre R & Chen C´s report (26). The bacteria of choice R.opacus PD630 and the enzyme Laccase was not available in any Aspen Plus databank, there was not found any report or data on how to user define any of these compounds in Aspen Plus. Instead, model compounds were chosen for these two, see Appendix A1.

3.1. Pre-treatment of lignin

To be able to produce a feedstock, lipids, for biofuel production from kraft lignin, first the lignin needs to be treated and degraded into smaller compounds and monomers. This step is important for the effectiveness of the later fermentation step, where it has been shown that the microorganisms easier consume smaller lignin fragments and that results in higher yields of lipids. The pre-treatments are performed by incubating the enzyme laccase, that is being bought, with the kraft lignin that has been dissolved in a phosphate buffer. The mix is incubated in a batch reactor for about 24 h in 50°C. Where 0.015 gram laccase per gram of lignin together with a ratio of 3:5 (laccase:HBT) of HBT mediator is breaking bonds in the lignin molecules into smaller fragments like vanillin, phenols etc.

The pre-treatment of lignin was simulated as can be seen in Figure 5. Here lignin, laccase and HBT is mixed with a phosphate buffer. With a weight percent of 6% (w/V) of lignin per volume buffer, then they are heated up to 50°C. Then the stream enters the tank, after the tank a component splitter is simulating the depolymerization of the lignin by removing 35% of the lignin, since the efficiency of the degradation of lignin is 35%. Then a new stream is mixed in to simulate the lignin monomers that is being produced. So, everything inside the blue rectangle is what has been simulated as the pre-treatment reactor. After the "reactor" a centrifuge is separating the non-reacted lignin from the solubilized, the solid lignin is transferred back to the mixer and reused again. While the solubilized mixture of lignin monomers is going on to the fermentation unit, this feed is 38 743 l/d.

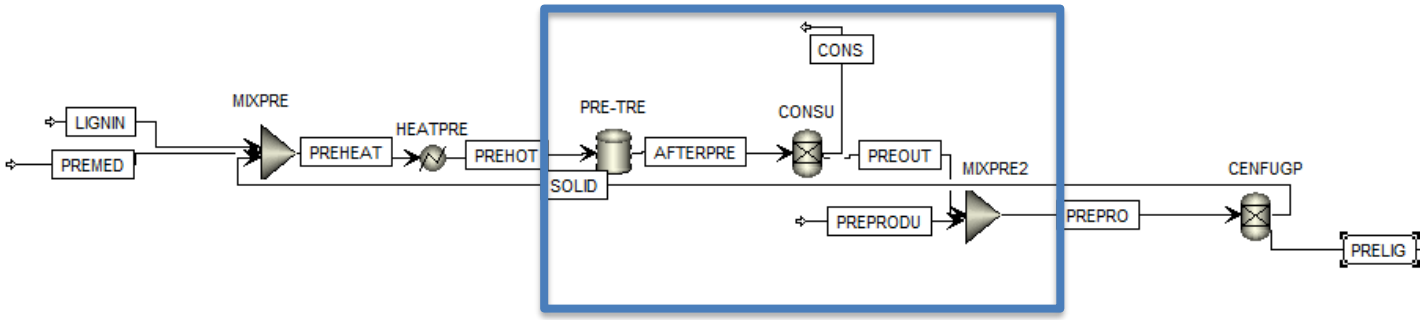

Figure 5, lignin pre-treatment process, by using the enzyme laccase and mediator HBT to convert lignin into solubilized monomers. Modelled in Aspen Plus.

3.2. Pre-cultivation of bacteria

At the same time as the pre-treatment process is taking place, the microorganism, Rhodococcus opacus, is being pre-cultured for 24 h in 28°C, this so that it can consume as much feed as possible and accumulate it to as much lipids as possible. It is also important to get viable cells that can withstand more stress.

When the pre-culturing of bacteria was simulated, this process can be seen in Figure 6. Bacteria seeds with a feed of 26 108 g/d, media with a feed of 44 179 l/d and glucose with a feed of 221 l/d get mixed in all at room temperature and then heated up through the heater to 28°C. Then

here like the other processes it can be seen in the blue rectangle what has been simulated as the pre-cultivation reactor, the mixture of bacteria, media and glucose is mixed into a tank and then a component splitter is simulating the media and glucose being consumed and the stream "PRODUCB" is adding in what is assumed being produced. After the pre-cultivation the bacteria is being separated out by a centrifuge, the bacteria are further then being used in the fermentation unit while the remaining liquid that mainly consists of water is transferred to a water treatment facility and then recirculated back to the process.

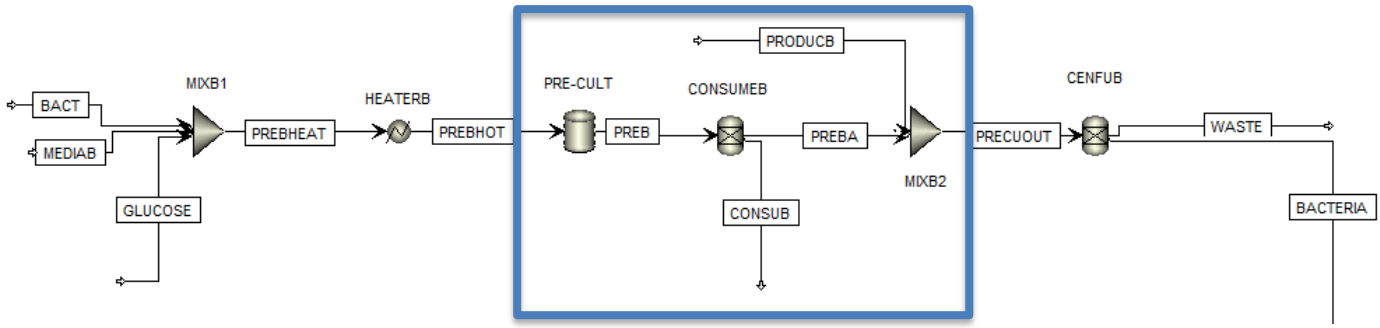

Figure 6, pre-cultivation process step for production of bacteria from seeds, simulated in Aspen Plus.

3.3. Fermentation

In the main process, the fermentation stage, the lignin monomers from the pre-treatment and the microorganisms from the pre-cultivation together with medium is mixed in a batch reactor. Where the microorganisms consume the lignin monomers and convert them into lipids that will be accumulated inside the cell. The reactor will hold a temperature of 28°C with a stirring speed of 200 rpm for 48 h.

The fermentation of the lignin monomers with the bacteria was simulated and can be seen in Figure 7 below. Here the pre-cultured bacteria, with a flow of 44 250 l/d are first mixed with the fermentation media with a flow of 24 750 000 l/d, where the bacteria stream is 28°C and the other stream is assumed to be room temperature, 25°C, before it gets mixed with the lignin monomers from the pre-treatment process that have a flow of 38 743 l/d, this stream is 50°C. When all streams have been mixed, they are going through a heater to assure that they have the temperature of 28°C before entering the reactor. Since the bacteria is not producing lipids but rather accumulating them, meaning that the bacteria is consuming the lignin monomers and converts them into lipids that are stored inside the bacteria as an energy resource. It was hard to simulate this with a reactor in Aspen, since the bacteria was not available in the Aspen database or was able to be user defined a model compound needed to be used. Since this model compound do not fully acts as the bacteria and the reactions of the bacteria was not fully known it could not be simulated as a reactor. Therefore, the reactor was simulated as a tank with following component separator that simulate the monomers that is being consumed in the fermentation and an extra stream that is being mixed in to add the products, to simulate what is being produced during fermentation, it is estimated that 46 137 152 gram of CO_2, 69 205 728 gram of bacteria and 14 819 gram of lignin monomers is produced every day. The equipment that is simulated as a reactor can be seen in the blue rectangle in Figure 7.

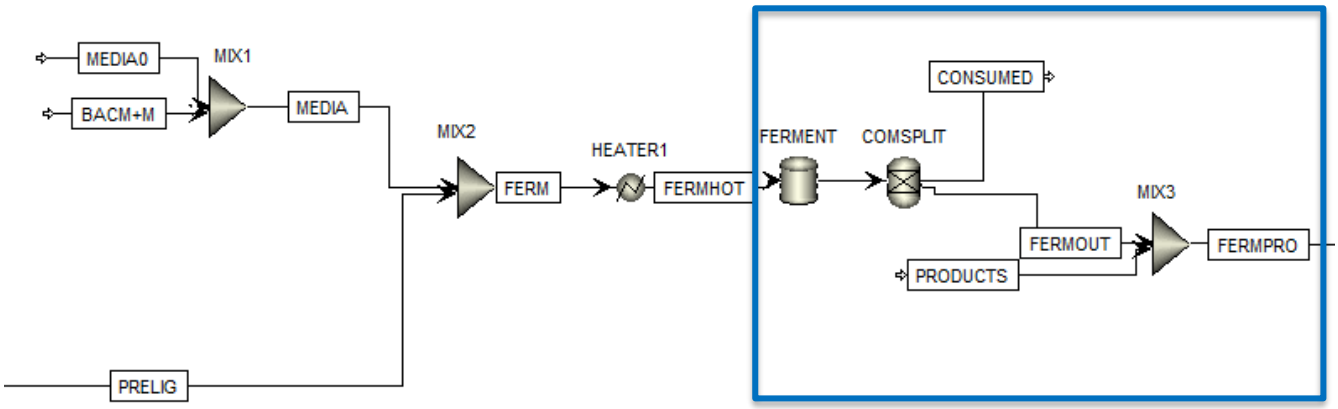

Figure 7, the simulated fermentation process of bacteria that is consuming solubilized lignin monomers, simulated in Aspen Plus.

3.4. Extraction

Then when the fermentation process is done, the microorganism needs to be separated through centrifugation. The cells that are harvested then needs to go through extraction to harvest the lipids that is accumulated inside the bacteria. So, that the lipids later can be used as a feedstock for biofuel production.

The extraction process can be seen in Figure 8. The stream going out of the fermentation tank was led into a centrifuge. This separated out the "bacteria", that was further processed, and the liquid was further transported to a water treatment facility to clean the water so it could be re-used in the process again. The bacteria were mixed with hexane, with a flow of 4 000 000 l/d and heated up to 65°C in a tank for approximately 5 min to extract the lipids out of the bacteria cells. Due to the lack of information about the bacteria in Aspen database, lipids needed to be added manually, this was added in as a stream at the same time as the hexane and the composition was estimated to be 10 866 421 g/d steric acid, 9 270 653 g/d oleic acid and 5 192 579 g/d palmitic acid. The slurry then gets centrifuged, to separate the hexane and lipids from the remains of bacteria cells. The hexane and lipid liquid are collected and furthered processed in two evaporators, to remove hexane from the lipids, the lipids are after this collected and sent to storage tanks. While the hexane is recirculated back to mixer4 for recycling, in this stream there are also be some traces of lipids, and there would also be some traces of hexane in the lipid stream. The bacterial remains, seen named as Bremain in Figure 8, would be discarded as waste, or burned as energy resource and this flow is approximately 43 950 000 g/d.

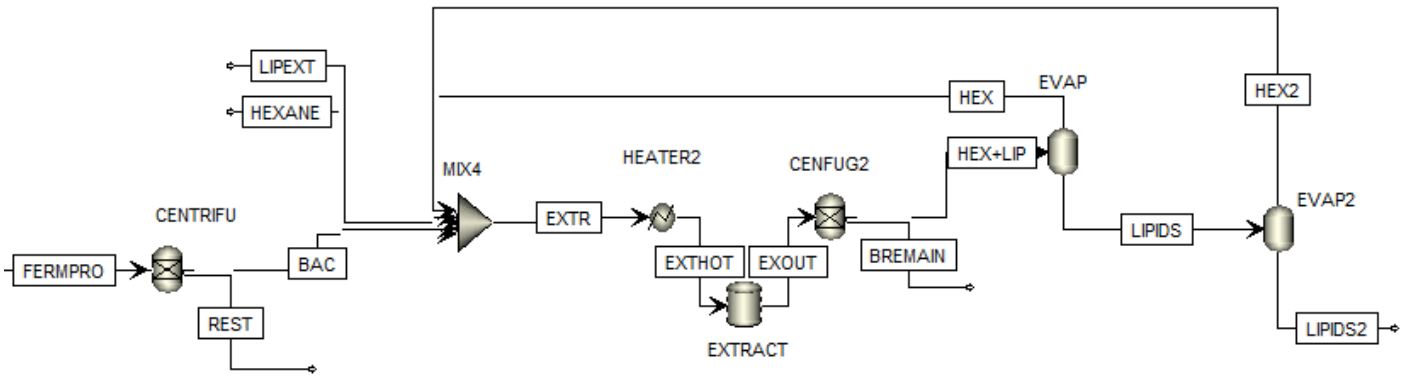

Figure 8, the extraction process to separate out the lipids from the bacteria with hexane, and evaporators. Modelled in Aspen Plus.

3.5 Techno-economic evaluation

For the techno-economic evaluation the equipment was assumed to be like the ones simulated in Aspen Plus, meaning that there would be tanks, centrifuges, extraction towers, evaporators, heaters, and pumps in between all significant equipment, see Appendix A for specification of what all equipment in Aspen Plus is assumed to be. The techno-economical evaluation will also have some assumptions and simplifications, all of them can be seen in Chapter 2.3.2 Assumptions for techno-economic calculations. In this project it is being assumed that all heat in the process would be produced from a fired heater, that uses natural gas as feed source. No heat exchangers were assumed to be used but would of course be beneficial for the overall process.

3.5.1 Equipment cost

The equipment cost was calculated according to tables and equations from *Chemical Engineering Design* by R.K Sinnott and G Towler, 2013(29). The equation that was used for the calculation of equipment cost was:

$$C = a + bS^c \quad (1)$$

Where C is the cost, a, b, and c are constant cost variables that represent type of equipment and material. S is the size of the equipment; the unit of S can differ from equipment to equipment. What the unit of S is supposed to be and the size range of it together with the cost constants is listed in table 7.2 in the book mentioned above. Example calculations of equipment costs can be seen in Appendix B.1.

3.5.1.1 Tanks

The size of the tanks is in cubic meter (m^3), so for this it was checked in Aspen Plus how big of a flow it would go into the tank for two days. The biggest size of a tank with a floating roof is 10 000 m^3 so the volume that would be needed was divided with 10 000 to see how many tanks that was needed for the process. The values a, b and c for the different tanks can be seen in Table 1 below. A floating roof tank was chosen to simulate the reactors, since this type of tank is a little bit more expensive which a reactor probably would be.

Table 1, equipment cost variables for tanks.

Equipment	a	b	c
Tank with floating roof, “reactor”	113 000	3 250	0.65
Storage/product tanks	5 800	1 600	0.7

3.5.1.2 Pumps

The size of the pumps was needed in the unit m^3/h, this was also something that was taken from Aspen Plus for all the pumps. The values a, b and c for the cost calculations can be seen in Table 2 below.

Table 2, equipment cost variables for pumps.

Equipment	**a**	**b**	**c**
Pump	8 000	240	0.9

It was assumed that there would be twice as many pumps as would be needed in the process, to always have one as backup, if one breaks down or needs maintenance etc.

3.5.1.3 Evaporators

The size of evaporators was in area (m^2), to calculate this was checked in Aspen Plus what volume the evaporators needed to fit for one batch and then a hight and diameter was calculated out to fit this volume, and the same height and diameter was used to calculate the area of the evaporators. The values for a, b and c for the cost calculations can be seen in Table 3 below.

Table 3, equipment cost variables for evaporators.

Equipment	**a**	**b**	**c**
Evaporator	330	36 000	0.55

3.5.1.3 Centrifuges

The size of the centrifuges was the diameter (m), here also the volumetric flow wash checked in Aspen, same as for the pumps. Then a length was assumed, and the diameter was changed until it fit the volume needed. The values for the centrifuge cost calculations a, b and c can be seen in Table 4.

Table 4, equipment cost variables for centrifuges.

Equipment	**a**	**b**	**c**
Centrifuge	57 000	480 000	0.7

3.5.2 Capital cost

When building a process plant, it is important to have an estimation of what the plant capital cost would be. This cost consists of both working capital, capital that is needed to get the production running, and fixed capital cost. These two together is the total capital cost and that is also what is referred to as CAPEX.

3.5.2 Operating cost

The operating costs was calculated based on prices of all raw materials (30–33) and how much that was needed every year, in Table 5 all prices of the different raw materials can be seen. In this project it is being assumed that all heat is produced by a fire heater that uses natural gas as fuel. This is because so little is known about the processes so no further assumptions on heat exchangers were made.

Table 5, raw materials and their prices.

Raw material	Price
Media fermentation	0.564 $/kg
Media pre-treatment	0.482 $/kg
Media pre-cultivation	0.564$/kg
Process water	19 SEK/m^3
Bacteria pre-cultivation	1.25 $/kg
Lignin	380 $/ton
Hexane	0.562 $/kg
Laccase	1.99 $/kg
HBT	0.663 $/kg
Heat demand	350 SEK/MWh

The fixed operating cost covered maintenance, wages, management, supervision, taxes, and insurance and these were calculated based on the values that can be seen in Table 6 below (29).

Table 6, fixed operating cost based on some estimations, the estimations and what type of fixed operating cost they relate to.

Type of fixed operating cost	Estimation
Maintenance	0.05*fixed capital cost
Staff	15 persons
Allowances	1.45*salary*12 months*staff
Lab	0.2*allowances
Supervision	0.2*allowances
Management	0.5*allowances
Rate of capital	0.1*fixed capital cost
Insurance	0.01*fixed capital cost
Taxes	0.02*fixed capital cost
General OH and R&D	0.05*(maintenance + subtotal fixed operating cost)

The total operating cost is also what is referred to as OPEX.

3.5.3 Investment calculations

The investment calculations of this process plant are based on an assumed lifetime of 10 years, with two years added to build it. The investment cost is divided into three years, where it is assumed that 40% of capital investment is paid in the first year (year 0), 50% in the second year (year 1) and the last 10% is paid in third year (year 2). The first two years is only building the plant and production starts in the third year. Meaning that there will not be any working capital cost during these years, the working capital starts in the third year when production starts but is assumed to be recovered in the end of the plant´s lifetime. The production is assumed to be running at 70% capacity during the third year (year2), 85% the next year and then 100% in the year after that and further to the end of the lifetime. It is also assumed that sales income and operating costs increases with 2% in price every year, as well as a discount rate of 10%, see all costs and revenues for the base case in Appendix B2.

From the operating cost, equipment cost and some assumptions of the price of lipids the rate of return (ROR), net future worth (NFW) and the net present worth (NPW) was calculated.

4. Results

The results from the techno-economic evaluation are presented in this section, this will include the Aspen Plus simulation, all input and output flows, process integration and cost calculations.

4.1 Process simulation

The process was simulated in Aspen Plus and the finished process with all parts included can be seen in the figure below, Figure 9 shows the process, from inlet of pre-cultivation and pre-treatment processes, the fermentation process to the extraction process, where Lipid2 is the finished product that can go to storage and be sold as a feedstock for biofuel production.

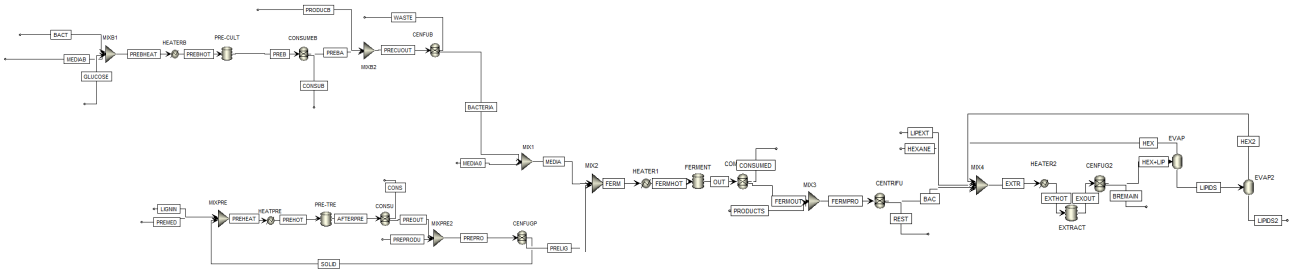

Figure 9, from right side is the inlet of both pre-cultivation at the top and inlet of pre-treatment at the bottom. Then they mix togheter before entering the fermentation process and at the end is the extraction process and out comes the prodct, the lipids. For a larger picture see Appendix C.

4.2 Costs

The different costs that are bound to this whole process were the capital cost, CAPEX, and the operating cost, OPEX will be shown in this section.

4.2.1 Equipment cost

In the capital cost the different equipment costs are included, all equipment cost and what equipment and how many units of each equipment that has been used for each process can be seen in Table 7-10. In Table 7 is the equipment cost for the pre-treatment of lignin process, Table 8 is for the pre-cultivation of bacteria process, Table 9 is for the fermentation process and Table 10 is for the extraction process, respectively.

Table 7, equipment specifications for the pre-treatment of lignin process.

Equipment	Number of units	Real equipment	Size per unit	Cost per unit SEK	Total cost SEK
Tank	1	Reactor	50 m^3	2 713 706	2 713 706
Centrifuge	1		1 m^3/h	4 289 680	4 289 680
Small pumps	10		10 m^3	151 259	1 512 592
Storage tank	4		100 m^3	808 707	3 234 830
Total cost					**11 750 808**

Table 8, equipment specifications for the pre-cultivation of bacteria process.

Equipment	Number of units	Real equipment	Size per unit	Cost per unit SEK	Total cost SEK
Tank	1	Reactor	200 m^3	3 776 315	3 776 315
Centrifuge	1		4 m^3/h	4 289 680	4 289 680
Small pumps	8		5 m^3/h	146 347	1 170 775
Storage tank	2		100 m^3	808 707	1 617 415
Total cost					**10 854 185**

Table 9, equipment specifications for the fermentation process.

Equipment	Number of units	Real equipment	Size per unit	Cost per unit SEK	Total cost SEK
Tank	6	Reactor	10 000 m^3	24 738 513	148 431 078
Centrifuge	6		180 m^3/h	6 125 077	36 750 464
Large Pumps	4		1 050 m^3/h	838 482	3 353 930
Small Pumps	2		200 m^3/h	297 564	595 128
Product tank	2		300 m^3	1 626 853	3 253 707
Total cost					**192 384 307**

Table 10, equipment specifications for the extraction process.

Equipment	Number of units	Real equipment	Size per unit	Cost per unit SEK	Total cost SEK
Tank	1	Extraction	5 000 m^3	16 486 103	16 486 103
Centrifuge	2		180 m^3/h	6 125 077	12 250 155
Evaporator1	4		180 m^2	10 881 592	43 526 368
Evaporator2	1		20 m^2	3 383 723	3 383 723
Large pumps	2		1 050 m^3/h	838 482	1 676 965
Small pumps	12		200 m^3/h	297 564	3 570 765
Total cost					**80 894 079**

As can be seen in the Tables 9-10 both large and small pumps are needed here, this is because the volumes of the flows both for the fermentation unit and extraction unit is quite large and therefore larger pumps are needed. But there are still some smaller flows as well in the processes, for the fermentation unit the bacteria flow after the centrifuge is smaller so here only a small pump is needed. For the extraction unit small pumps is needed for the lipid product, since this flow is smaller than the other flows in the process.

In Figure 10 below it can be seen that it is the fermentation process that is the most expensive process of the four. This is because this unit needs 6 tanks (reactors), that is one of the most expensive equipment units, to process the large amounts of media, bacteria, and lignin and because of the large volumes that are handled in this process unit it will need 6 centrifuges that are quite expensive as well. Then the extraction process and lastly the pre-treatment and pre-cultivation processes almost cost the same to build.

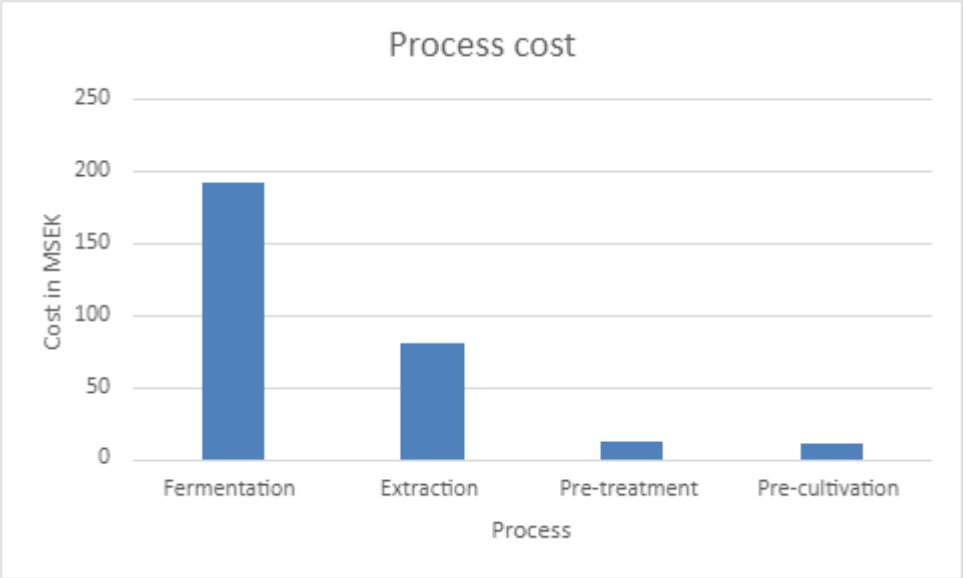

Figure 10, cost per process in MSEK.

To summarize the cost of all equipment that would be needed for the total process, see Table 11. In Table 11 it is shown that in total, it will be needed 68 units of different equipment and all these units together would cost approximately 312 MSEK to buy. The total fixed capital cost, that includes the construction of all units, electricity, instruments etc., see Appendix B1 for all the factors used for estimation of fixed capital cost, ended up to approximately 1 355 MSEK.

Table11, total Equipment, total numbers of units of each equipment, total cost per type of equipment, total cost for all equipment's and total fixed capital cost that includes everything around the process like electricity, instruments etc.

Total equipment	**Total number of units**	**Total cost per equipment**	**Percentage of total fixed capital cost**
Tank	9	171 407 200 SEK	12.7%
Storage/product tank	8	8 105 952 SEK	0.6%
Pumps	38	11 880 153 SEK	0.9%
Evaporators	5	46 910 092 SEK	3.5%
Centrifuges	10	57 579 979 SEK	4.3%
Furnace	1	925 872 SEK	0.07%
Total cost	68	**312 164 223 SEK**	
Fixed capital cost		**1 354 792 728 SEK**	

In Figure 11 the most expensive equipment is the tanks, this include both storage tanks and tanks that are used as reactors. Even though it can be seen in Table 6 that the largest number of units is for pumps, this equipment cost is far from most expensive. What also can be seen in Figure 11 is that the cost for centrifuges and evaporators is quite high, this is both because quite many of these are needed for all the processes and because they are a more expensive type of equipment. It can also be seen that the initial buy of hexane is the third most expensive capital cost, this is because it is a large volume of hexane that is needed for the first batch of extraction.

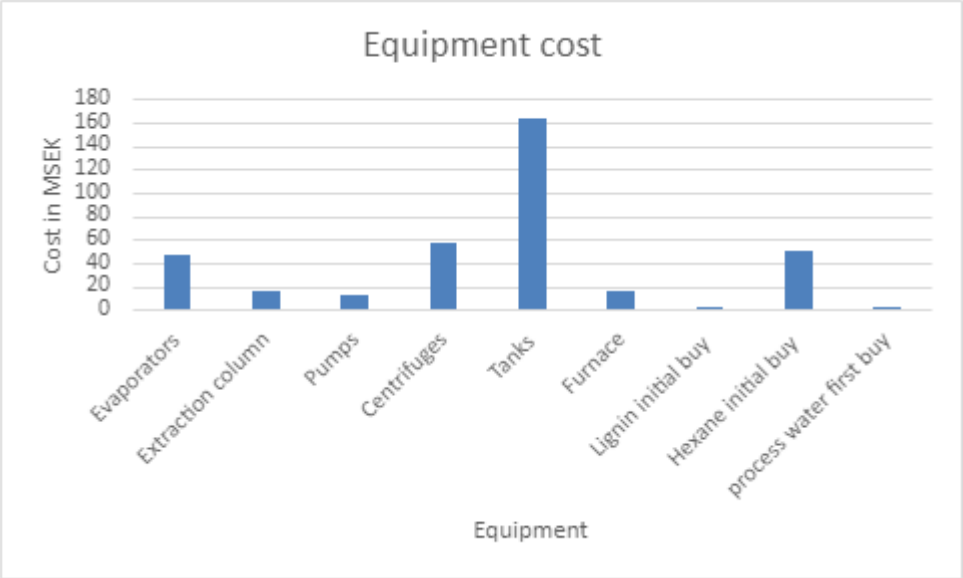

Figure 11, equipment cost divided per type of equipment.

4.2.2 Operating costs

Since these processes are dependent on raw materials, the operating cost will have a big influence on the total cost to run these processes. The operating costs of all the processes can be seen in Table 12, including the type of raw material, price of each material, how much of each material will be needed, the cost per year in SEK and the total cost of all raw materials, which ended up being approximately 233 MSEK/year. The total fixed operating cost, that includes maintenance, wages, insurance, taxes, lab, R&D etc. is approximately 102 MSEK/year. So, then the total operating cost per year ended up being approximately 335 MSEK/year. Beyond this an extra initial cost for hexane, lignin, and water of approximately 52 MSEK was added to the capital cost, this is larger volumes that would be needed initially to start the process and before the recirculation flows get into motion.

Table 12, all raw materials needed in the process and their prices and flows.

Raw material	Price	Flow	SEK/year (2022 currency)	SEK/kg lipids
Media fermentation	0.564 $/kg	1 030 kg/h	57 461 132	6.22
Media pre-treatment	0.482 $/kg	111 kg/h	5 241 721	0.57
Media pre-cultivation	0.564$/kg	708 kg/h	39 461 260	4.27
Process water initial	19 SEK/m^3	49 396 m^3	938 526	-
Process water continuous	19 SEK/m^3	103 m^3/h	17 128 078	1.85
Bacteria pre-cultivation	1.25 $/kg	627 kg/h	77 461 760	8.38
Lignin initial	380 $/ton	0.38 ton/batch	1 614	-
Lignin continuous	380 $/ton	24 ton/year	103 143	0.01
Hexane initial	0.562 $/kg	8 000 000 l	50 879 651	-
Hexane continuous	0.562 $/kg	244 l/h	13 557 267	1.47
Laccase	1.99 $/kg	68 kg/h	7 406 651	0.8
HBT	0.663 $/kg	113 kg/h	13 328 806	1.47
Heat demand	350 SEK/MWh	0.6 MWh	1 852 548	0.2
Total variable cost/year			**232 982 367**	**25.2**
Total fixed operating cost			**102 246 989**	11.06
Total operating cost			**335 229 356**	
Extra initial cost			**51 819 791**	

To show what contribution each variable has to the price of lipids, see Figure 12. In the figure the total variable cost can be seen to have the largest contribution to the price of lipids, approximately 25 SEK/kg lipid. Then the bacteria and the medias are the variables that has large contribution to the price of lipids.

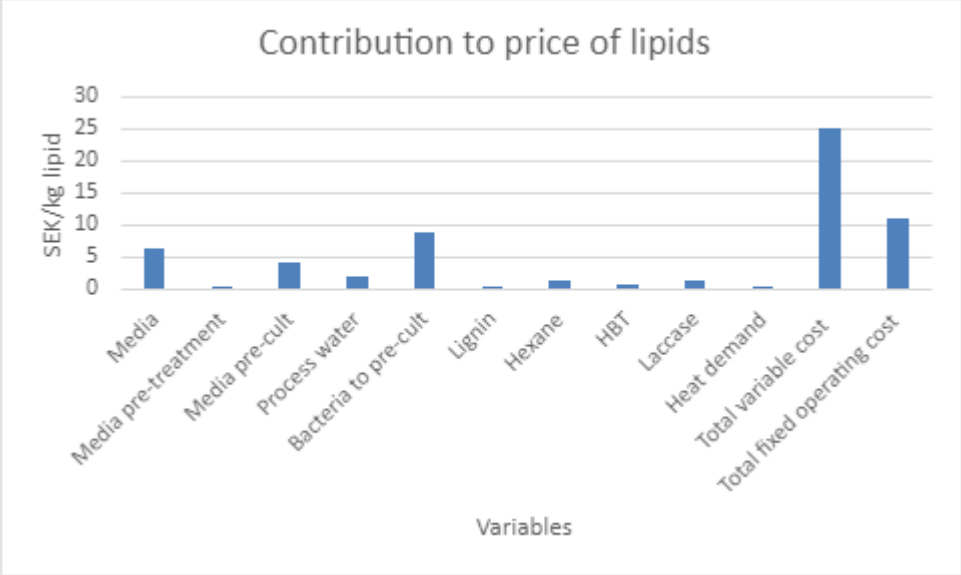

Figure 12, each variables contribution to the price of lipids.

4.2.3 Investment costs

The price of lipids was assumed to be 2 000 USD/ton which is approximately 22 580 SEK/ton, and the process produces approximately 9 200-ton lipids every year. In Table 13 all investment cost for the process can be seen if the price of lipids is 2 000 USD/ton.

Table 13, the economic values of the process when the price of lipids is 2 000 USD/ton, and 9 200-ton lipids is produced annually.

Economic value	Value
Net future worth	-2 787 764 976 SEK
Net present worth	-2 095 260 102 SEK
Rate of return	-19.8 %

As can be seen in Figure 13, at the current price of lipids the process will continually lose money.

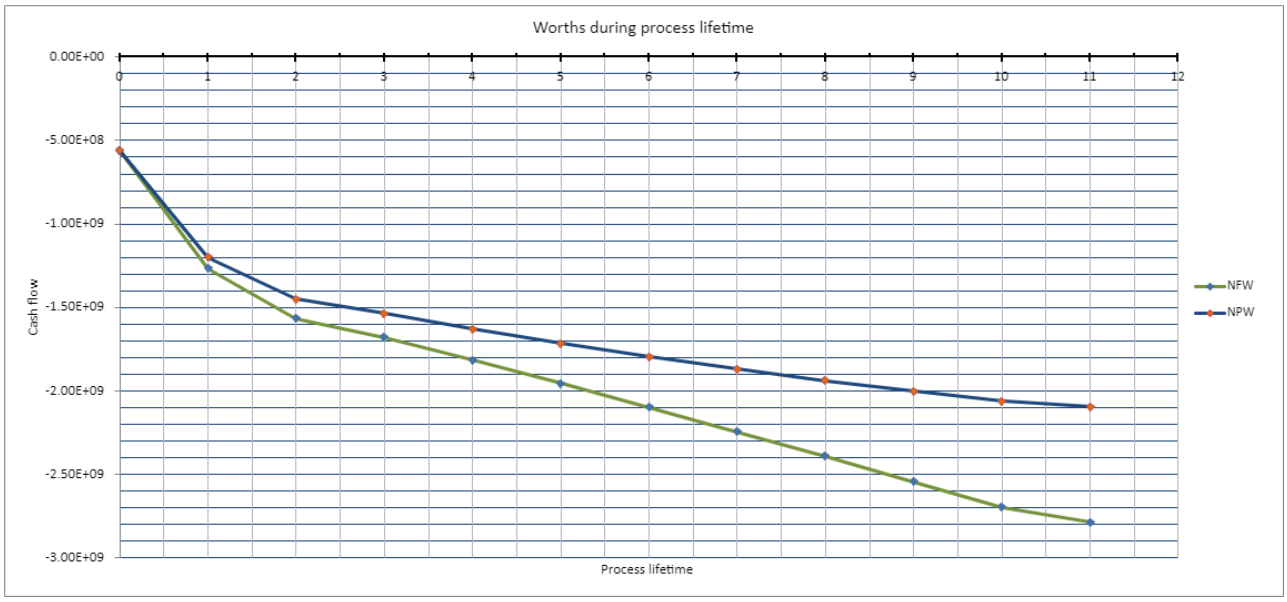

Figure 13, graph over the NFW and NPW over process lifetime, years at the x-axis and cash flow at y-axis.

If the price of lipids would be 5 409 USD/ton or higher both the NFW and NFP would start being positive and that would mean that the process would be economically feasible. The values of the NFW, NPW, ROR and PBT at the price 5 409 USD/ton lipids can be seen in Table 14.

Table 14, economic values when the price of lipids is 5 409 USD/ton and the priocess produces 9 200-ton lipids annually.

Economic value	Cost
Net future worth	1 097 918 639 SEK
Net present worth	8 967 SEK
Rate of return	7.8 %
Pay-back time NFW	7.3 year
Pay-back time NPW	11 years

Then as can be seen in Figure 14 the process will in the beginning lose money but when the process starts to produce lipids, we can see a turning point. This is what we see after 2 years, then the process starts to get positive NFW after year 7 and a positive NPW after year 11.

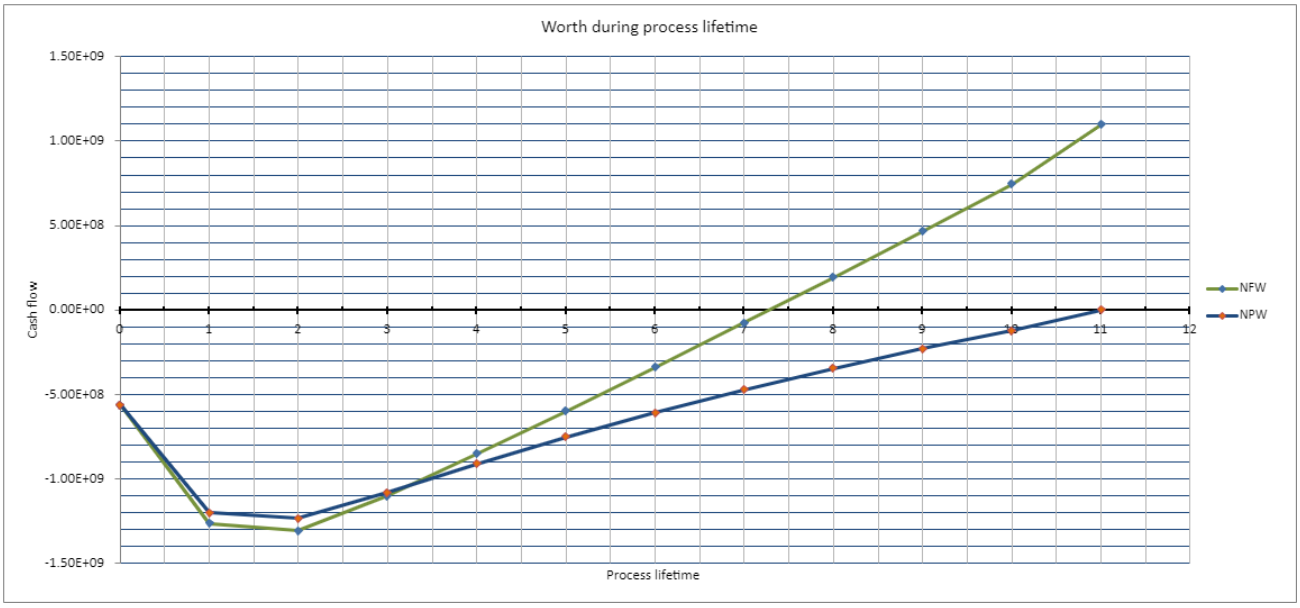

Figure 14, graph over NPW and NFW, over process lifetime, years on the x-axis and cash flow on the y-axis.

4.2.4 Sensitivity Analysis

To evaluate how the economics of the process is dependent on different factors a sensitivity analysis was conducted. The parameters that were investigated was the pre-cultivation process, the efficiency of the pre-treatment of lignin and the concentration of lignin in the fermentation process.

4.2.1.1 No pre-cultivation unit

When the pre-cultivation process was investigated, it was investigated if the pre-cultivation process could be skipped, and if the bacteria instead could be bought already pre-cultivated and see what effect on the economics that would have. Then the bacteria would be bought at a higher price, that is assumed to be 5.07 USD/kg, based on prices of similar compounds. This would make the equipment cost cheaper, thereby the fixed and working capital costs cheaper, the CAPEX would then be approximately 1 308 MSEK, but the annual operating cost would instead be more expensive, the OPEX would then be approximately 1 200 MSEK/year. See Table 15 for all values.

Table 15, investment costs for the process without pre-cultivation unit, bacteria cost 5.07 USD/kg.

Economic Values	
Total equipment cost	301 304 096 SEK
Fixed Capital cost	1 307 659 776 SEK
Total operating cost	1 199 647 228 SEK/year
Production	9 245 ton/year
Net future worth	-12 180 756 098 SEK
Net present worth	-7 140 344 047 SEK
Rate of return	-89.6%
Price needed to earn money	13 617 USD/ton lipids
Pay-back time NFW	7.3 years
Pay-back time NPW	11 years

In Figure 15 a comparison of CAPEX, OPEX and price of lipids between the base case and the no pre-cultivation case. Here it can be seen that the CAPEX differs a little while the OPEX is much higher for the no pre-cultivation case than it is for the base case, and the same can be seen for the price of lipids.

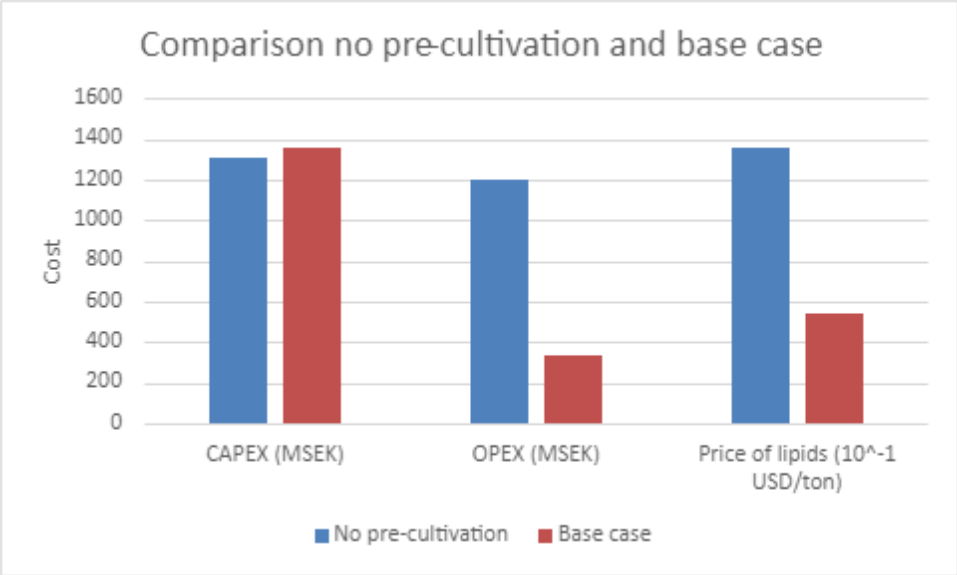

Figure 15, comparison of CAPEX and OPEX for base case and for no pre-cultivation unit.

As can be seen in Table 15, the price of the lipids would need to be 13 617 USD/ton or higher to make the process earn money and then the pay-back time would be 5.6 years. This is more than double the price needed to make the process with a pre-cultivation unit profitable.

4.2.1.2 Higher efficiency on pre-treatment unit

When the efficiency of the pre-treatment of lignin was investigated, it was tested what would happen to the economics of the process if the efficiency of the pre-treatment of lignin was higher than 35%. In this case the effects were checked for 40%, 45%, 50%, 55% and 60% efficiency. Here the outlet of the pre-treatment unit, also the same as the inlet to the fermentation unit, was kept the same and only the inflows to the pre-treatment unit was changed, meaning the volume of lignin, laccase, HBT and media. In Table 16, the main economic values for all the efficiencies can be seen, the total equipment cost, fixed capital cost, total operating cost, production amount, net future worth, net present worth, rate of return, pay-back time and at what price the lipids would need to be sold at to make the process start earning money and the pay-back time for that price. It can be seen in Table 16 that even though the operating cost decreases and the price of lipids decreases, the price only decreases little. It goes from costing 5 409 USD/ton lipids for the base case 35% efficiency to cost 5 306 USD/ton lipids for 60% efficiency.

Table 16, investment cost for efficiencies on pre-treamtnet unit 35% up to 60%.

Economic value	35%	40%	45%	50%	55%	60%
Total equipment cost	312 164 223 SEK	312 141 795 SEK	312 129 544 SEK	312 127 576 SEK	312 125 970 SEK	312 114 683 SEK
Fixed Capital cost	1 406 612 519 SEK	1 406 514 910 SEK	1 406 461 532 SEK	1 406 452 824 SEK	1 406 445 713 SEK	1 406 396 616 SEK
Total operating cost	335 229 356 SEK/year	332 520 338 SEK/year	329 869 867 SEK/year	327 722 120 SEK/year	325 966 393 SEK/year	324 494 157 SEK/year
Production	9 245 ton/year	9 245 ton/year	9 245 ton/year	9 245 ton/year	9 245 ton/year	9 245 ton/year
Net future worth	-2 787 764 976 SEK	-2 758 082 753 SEK	-2 729 084 141 SEK	-2 705 620 344 SEK	-2 686 439 308 SEK	-2 670 312 234 SEK
Net present worth	-2 095 260 102 SEK	-2 079 213 458 SEK	-2 063 554 089 SEK	-2 050 898 068 SEK	-2 040 552 141 SEK	-2 031 835 267 SEK
Rate of return	-19.8%	-19.6%	-19.4%	-19.2%	-19.1%	-19.0%
Price needed to earn money	5 409 USD/ton	5 383 USD/ton	5 357 USD/ton	5 337 USD/ton	5 320 USD/ton	5 306 USD/ton
Pay-back time NFW	7.3 years	7.3 years	7.3 years	7.3 years	7.3 years	7.3 years
Pay-back time NPW	11 years	11 years	11 years	11 years	11 years	11 years

In Figure 16 below the different OPEX costs can be seen, and the trend of the OPEX being cheaper for a higher efficiency can clearly be seen here.

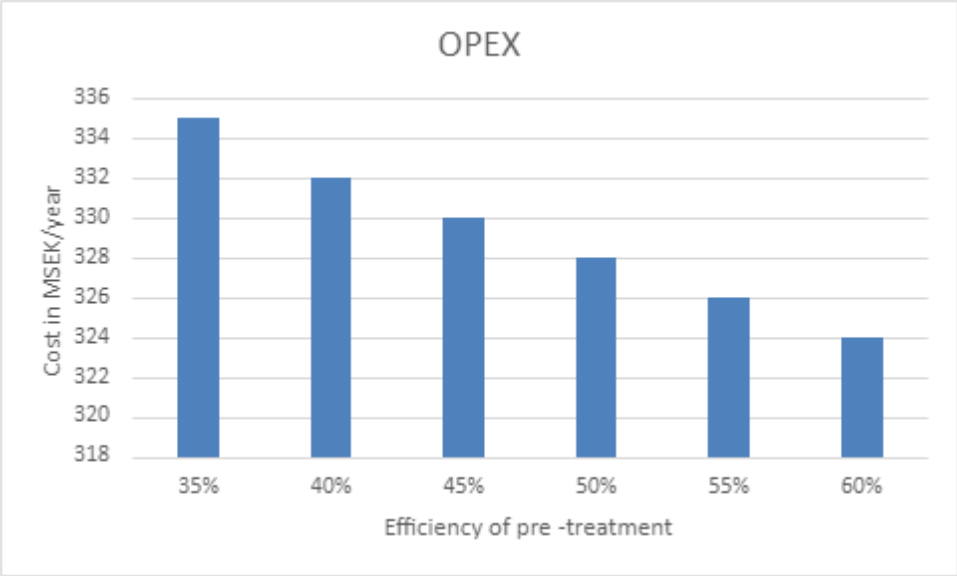

Figure 16, OPEX for the different efficiencies.

And in Figure 17 also the price of lipids is decreasing with a higher efficiency on the pre-treatment process.

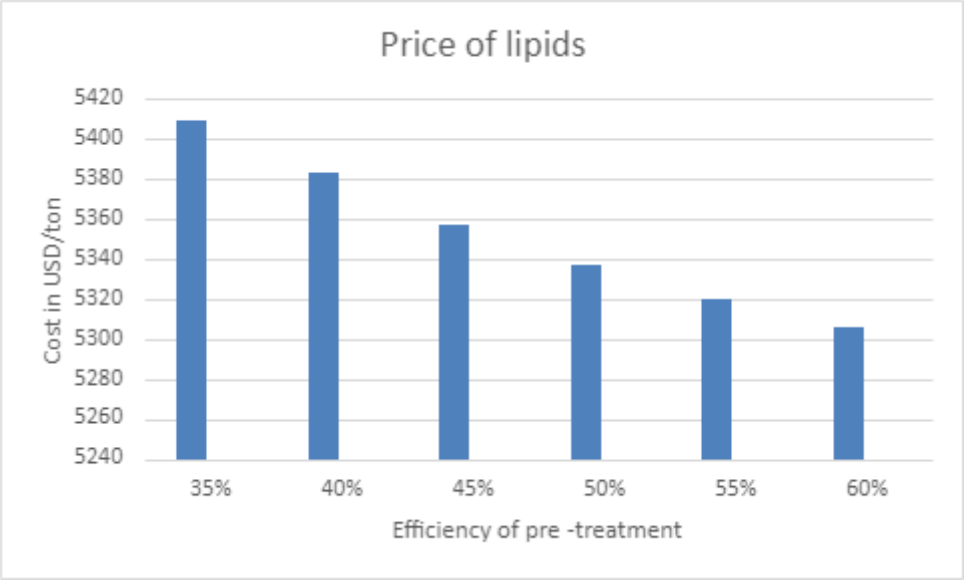

Figure 17, the price of lipids for the different efficiencies.

4.2.1.3 Higher concentration of lignin into fermentation

When the concentration of lignin into fermentation was investigated, it was tested what effect it would have on the economics of the process if the concentration of lignin into fermentation was increased. In this evaluation the concentration of lignin into fermentation was doubled, tripled, and quadrupled, it was also assumed that the increase of lignin concentration would affect the growth of bacteria and production of lipids in a linear manner. Meaning that it was assumed that if the concentration of lignin doubled, so did also the concentration of bacteria and lipids, meaning that the production rate would be increased linearly. But the volume of media into the fermentation was kept the same for all cases. In Table 17 all the main economic values can be seen for each increase of lignin concentration, what can be seen is the total equipment cost, fixed capital cost, total operating cost, production of lipids, net future worth, net present worth, rate of return, pay-back time and at what price the lipids would need to be sold at to make the process start earning money and the pay-back time at that price.

Table 17, investment cost for concentrations of lignin into fermentation from base case to 4 times the base case concentration.

Economic value	x1	x2	x3	x4
Total equipment cost	312 164 223 SEK	313 115 176 SEK	315 338 393 SEK	317 120 729 SEK
Fixed Capital cost	1 406 612 519 SEK	1 410 743 623 SEK	1 420 394 155 SEK	1 428 132 361 SEK
Total operating cost	335 229 356 SEK/year	453 008 299 SEK/year	570 445 628 SEK/year	698 949 568 SEK/year
Production	9 245 ton/year	18 552 ton/year	27 921 ton/year	37 353 ton/year
Net future worth	-2 787 764 976 SEK	-1 783 079 215 SEK	-746 942 835 SEK	149 733 227 SEK
Net present worth	-2 095 260 102 SEK	-1 555 247 734 SEK	-1 010 310 342 SEK	-520 355 073 SEK
Rate of return	-19.8%	-12.6%	-5.4%	1%
Price needed to earn money	5 409 USD/ton	3 261 USD/ton	2 544 USD/ton	2 210 USD/ton
Pay-back time NFW	7.3 years	7.3 years	7.3 years	7.3 years
Pay-back time NPW	11 years	11 years	11 years	11 years

In Figure 18 both CAPEX and OPEX can be seen for each concentration, here it can be seen that the CAPEX only increases slightly while the OPEX is increasing more for every time the concentration is increased. This is because the media volume is kept the same for the fermentation process and therefore the equipment only changes some for the pre-treatment and pre-cultivation processes that need to process larger volumes. While the OPEX is increasing more since more raw materials would be needed to be bought, this increase can be seen to be very close to linear.

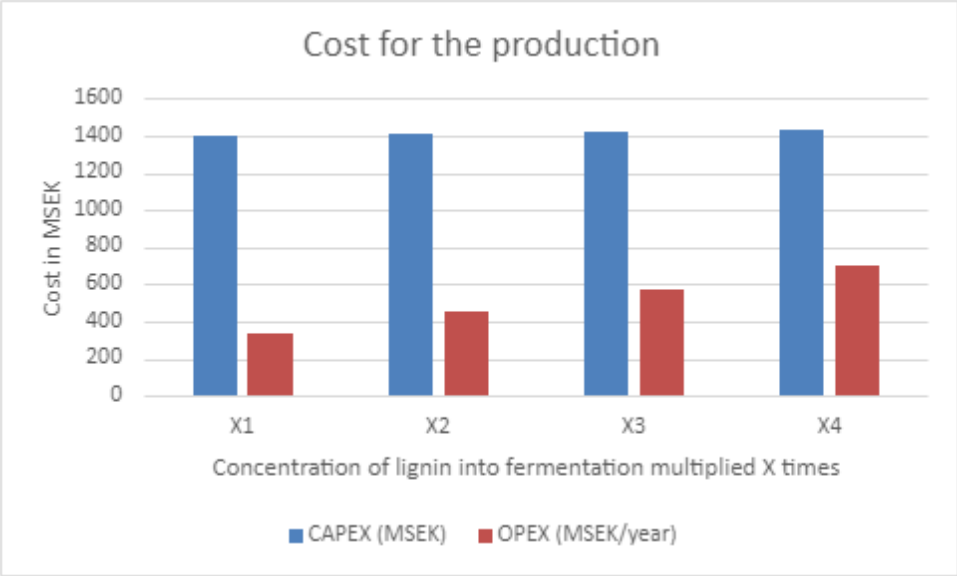

Figure 18, CAPEX and OPEX for the different concentrations of lignin into fermentation.

The price of the lipids for each concentration can be seen in Figure 19 below, here it can be seen that the increasing of lignin concentration would result in cheaper prices on lipids and the quadrupled one is the cheapest one.

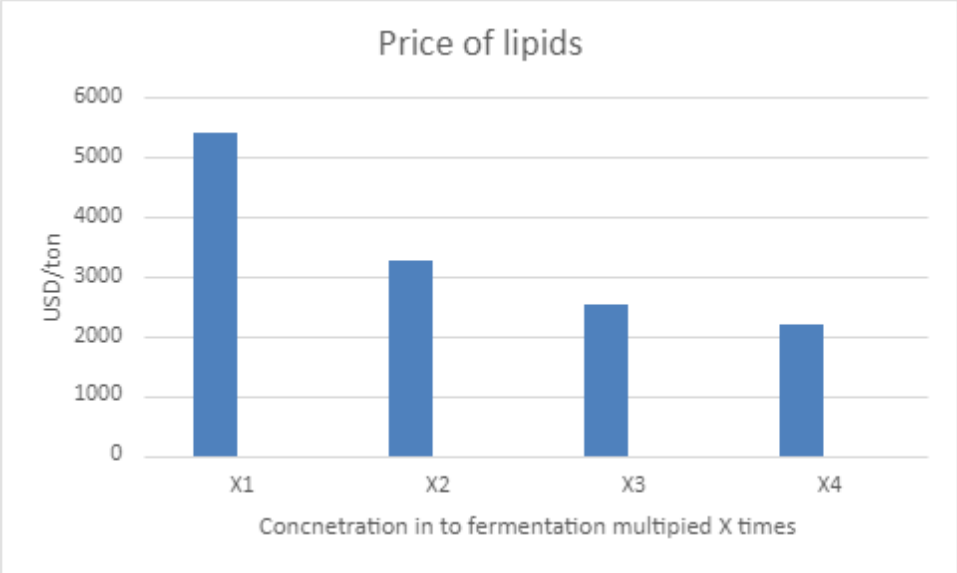

Figure 19, the price of lipids for the different concentrations of lignin into fermentation.

So, the cheapest price is obtained when the lignin concentration in is quadrupled, then the price of the lipids needs to be 2 210 USD/ton or higher while for the base case the price needs to be 5 409 USD/ton or higher, see why in Table 12.

5. Discussion

In this part of the report the results, both from the Aspen Plus modelling and the techno-economical evaluation will be discussed.

5.1 Aspen Plus evaluation

It has been instructively and interesting to learn more about the software Aspen Plus, it is a good tool for making flowsheets and model chemical processes. For this project it has been some disadvantages to use Aspen Plus, one of them was that the bacteria, laccase and HBT was not available in Aspen Plus property database. Since it could not be found any literature on how to user define these components, model components were chosen instead. It is not clear how much this have affected the results, but since a lot of things has been assumed and this result mainly should be seen as an estimation, it is acceptable. In the future it would be interesting to see if Aspen might have some solution for biocomponents and for bioprocessing overall. Unfortunately, I did not have enough knowledge about Aspen and not enough time to investigate all possible solutions that Aspen Plus provides further for this project. Another solution to this problem would be to investigate other simulation programs and see if there might be someone that are more adapted towards bioprocesses.

Another thing that could have made the model even better, would have been if more were known about the reactions that is taking place in the different processes. If the reaction rate and the stoichiometry of the reactions would have been known it could have been simulated the process with a reactor instead of the tank, component splitter and mixer as it is now. This problem could be solved by performing physical experiments to investigate the reaction rates etc. For this project it has been assumed that the scale up is linear but that is rarely the case, so the results from this project would probably differ to reality. But the main core of the process would most likely be like the one in this project, so this is a good starting point to understand what other factors that would need to be investigated further in the future.

5.2 Economic evaluation

For the economic evaluation there are several different aspects to take into consideration and all these parts will be discussed in this section.

5.2.1 Equipment cost

The techno-economic evaluation of the process showed that for the base-case process approximately 68 equipment units would be needed and most of that would be pumps (38 pieces). The equipment unit that ended up being the most expensive was the tanks, that is representing reactors, they stand for 12.7% of the total capital cost. This cost might have been underestimated to what it could be, depending on where the reactors would be placed and what specifications they would need. The tanks were chosen to simulate a bioreactor since it was understood that a bioreactor main function is to receive the feed components, keep a constant temperature and have a mixing inside it. This feature was assumed to be more like the cost of a tank than the cost of a reactor, as they specify in literature. Another thing that could be seen in the equipment cost is that the most expensive process was the fermentation process, since this is the main process to produce the products, meaning the lipids, it would be adequate that this is the most expensive process. The second most expensive process is the extraction process and that might be because of the extra equipment this process would need, here the evaporators that is needed to separate the hexane from the lipids. The cost for this process is something that could be optimized if another type of extraction process would be investigated. Like for example some type of physical separation method to extract the lipids from the bacteria. But that was not something that was within the scope of this project.

In the equipment cost it can also be seen that the most expensive expenditure was for the installation cost, that is including pipes, electricity, design, instrument etc. This cost is hard to avoid but it will depend on the cost of all the equipment's since it is a value that is multiplied with the equipment cost. Important to remember here is that in this project it has been assumed that this cost do not includes wages for the construction workers, so in the end this would be slightly more expensive than these estimations but that is most probably also depending on the amount of equipment and the complexity of the process plant. It would probably also depend on if the company would have inhouse competence and personal that would contribute to the construction or if everything would be built on contract.

5.2.2 Operating cost

For the operating cost the second most expensive part is the fixed operating cost, that includes wages, maintenance R&D etc. This is a cost that is hard to cut down on, it depends on the working capital cost which is approximately 5% of the capital cost. And it is something that would be valuable for the company to invest money on, since it is the workers in the company that sort of represents the company to the communities in surrounding. It is also important to invest in maintenance to ensure the standards of the plant and so that it can operate for a long time, and to invest in R&D to continue to optimize processes and develop new process and other parts of the business to ensure competitiveness in the market.

The most expensive variable cost is the bacteria, this can both be seen in Table 7 and is illustrated in Figure 12. This is probably because the bacteria cannot be reused, since the lipids is accumulated in the bacteria it would in the existing extraction process be destroyed to get out the lipids. So, one solution to this problem could be to find some type of secondary use of the bacteria remains since this flow will be quite large it would be beneficial if this could either be used in the process somehow or sold to generate an extra income. One thing that would be interesting to investigate further is if these bacterial remains could be used as some type of fertilizer or as a substrate for biogas production, to produce both biomethane and other valuable products, since the bacteria contains mostly organic matter and will contain a lot of nutrients this could be a solution and lower the amount of waste from the process. If not a secondary use for the bacteria remains would be found it could at least be burned to generate energy, this would at least lower the cost for the energy utilities and could perhaps even generate energy and heat for adjacently plants or buildings. But this is something that would need to be investigated further and calculated on. Another thing that would be interesting to investigate further would be the possibilities to use the bacterial remains in pyrolysis to produce pyrolysis oil. Another solution to the problem would be to investigate the possibilities to use another organism that would produce lipids rather than accumulate them. Then the ability to reuse the organism would be more probable than it is for the organism used today. I have not found such an organism in my investigation but I have understood that it is a lot of research going on in the field so it is not impossible that it could exist such an organism.

The second most expensive variable is the different medias, as can be seen in Figure 12 the media for fermentation is the third highest bar in the graph. Here the price of the media has been assumed to be the same as media for bioethanol production, this is also something that probably could be optimized further. This price and media are used in industry today, but it is possible that there are other medias that could be used for this purpose that is cheaper. Another approach to optimize the cost for the media could be if less media would be needed, this could be the case if the processes did not need to be as diluted as they are today. If that could be the case, it could possibly also lower the cost for process water.

5.2.3 Investment cost

For the investment costs it was showed that to make both NPW and NFW reach positive values before the process lifetime ended the price of lipids needed to be 5 409 USD/ton or higher, se Figure 14 for a graphical picture of the NFW and NPW. This price is more than double the price that has been assumed to be today, 2 000 USD/ton. This big difference in price makes it hard to think that this is a process that is a good invest today. What we have seen recently in fuel prices is that is has been very unsteady and the prices has risen a lot lately. So, it is not impossible that it could be feasible in the future, but as it is right now it would not be very likely. For this scenario it is calculated that the process would produce approximately 9 200-ton lipids every year. Another investment cost that has not been evaluated in this project is the question of investing in land for the process plant. That is something that would need to be evaluated and investigated further in the future and the availability and price of the land would also depend on where the process plant would be built.

5.2.4 No pre-cultivation of bacteria process

To see what aspects of the process that has significant effects of the costs and revenues a sensitivity analysis was conducted, one thing that was checked here was the significance of the pre-cultivation process. This is a process that could be skipped and instead the bacteria could be bought already pre-cultured and then put directly into the fermentation process, but then the price of the bacteria would be higher per kilogram. What could be seen from this investigation was that if the pre-cultivation process would be skipped the capital cost would be lower, as can be seen in Table 10. But instead, the operating cost would be higher because of the higher price of bacteria. This resulted in that the lipids would be needed to be sold at a price of 13 617 USD/ton or higher to make both the NPW and NFW values positive, and thereby earning money on the process. From this it can be concluded that it would be more expensive to not have the pre-cultivation unit, even if it saves money on investment the operating cost would be so much higher that it would not be profitable and therefore not probably a good solution. This would on the contrary also make the workload at the plant lower, so maybe less workers would be needed. From the calculations and evaluation that has been done in this project it would be recommended to still have a pre-cultivation unit. Since it is affecting the cost significantly.

5.2.5 Higher efficiency on pre-treatment of lignin

When the efficiency of the pre-treatment unit was investigated, it showed a trend that the higher the efficiency the lower the price of the lipids needed to be. This can be seen in Table 16 in the results. The price of the highest efficiency, 60%, was 5 306 USD/ton lipid while the price of the efficiency of today, 35%, would need a price of 5 409 USD/ton lipid. As can be seen the price change is not that much, so this is not seen as a significant change and therefore it would not be recommended to be a focus for future studies.

5.2.6 Higher concentration of lignin into fermentation

When the concentration of lignin into the fermentation unit was investigated, it was seen that a higher concentration of lignin into fermentation would lower the price of lipids. It can be seen in Table 17 that the price would need to be 2 210 USD/ton or higher to make the quadrupled process economically feasible, while the base case would need a price of 5 409 USD/ton. This change is significantly lower than the base case and indicates that the increasing concentration of lignin has a significant effect on the price of lipids. Even though the price on the lipids after a quadrupled concentration of lignin into fermentation is significantly lower it is still a little bit higher than the price of lipids today. So, it would probably need to be increased a little bit more to fully be economically feasible. Another thing one could investigate further would be the yield

of lipids that is obtained, this would probably show the same trend as the concentration of lignin did.

6. Conclusion

This master thesis provides the economic and technical performance of production of biolipids from lignin through a bioconversion with bacteria and pre-treatment of lignin with enzymatic degradation. Due to some simplifications and assumptions, such as a linear upscaling, the results of this project might differ some to the results of real-life production. This is still a novel research area and there is still a lot that needs to be done and further developed for the bacterial conversion of lignin monomers to be a feasible option for production of biolipids. For the use of the simulation tool Aspen Plus, there needs to be known more about the reactions that are taking place in the processes to fully make a good simulation of the process.

Further it can be concluded that some secondary use for the bacterial remains would need to be found, to further make the process more profitable and sustainable. It is more profitable to have a pre-cultivation process than to buy the bacteria already pre-cultivated, the efficiency of the pre-treatment of lignin does not have a significant value to the economic cost of the entire process. Focus should instead be on higher concentrations and yields of lipids from bacteria. Since it was shown that a higher concentration of lignin into the fermentation process has a significant effect on the price of lipids. So, for future investigations it would be beneficial and interesting to look at higher yields of lipids from the bacteria and investigate further how to optimize the cost of bacteria, media, and water since these three are the main contributors to the cost of lipids.

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Appendix A

In Appendix A, data that concerns the Aspen Plus program will be presented, description of streams, components etc.

A.1 Components

The components that was not available in Aspen Plus databank needed to be user defined, and the values that was used for this can be seen in Table A1.1.

Table A1.1, User defined components and all the properties that was defined in Aspen Plus, where DHSFRM describes the solid heat of formation at 298,15 K, VSPOLY the molar volume of solid, CPSPO1 the solid heat capacity, MW the molar mass and DNSTML solid molar volume.

Component	DHSFRM [kJ/kmol]	CPSPO1 [J/kmol-K]	MW [g/mol]	DNSTML [kmol/cum]
LIGO	-1,8475e+06	26828,9	422,389	3,5986
LIGH	-1,7227e+06	27722,6	436,459	3,4826
LIGC	-759390	16404,8	258,274	5,8852

There were some compounds that could not be found in Aspen Plus database, neither could be user defined. These compounds got a model compound instead, what those compounds were and what model compound that was chosen can be seen in Table A1.2. The model compounds that were chosen were already existing in the Aspen Plus database, so no further properties were needed to be implemented for the compounds.

Table A1.2, the compound that could not be found in Aspen Plus databank, their chemical composition and molar mass, the model compound that were chosen, their chemical composition and molar mass.

Compound	**Chemical composition**	**Molar mass**	**Model compound**	**Chemical composition**	**Molar mass**
Laccase	C44H69N11O20	1072.1 g/mol	Ubiquinone	C59H90O4	863.3 g/mol
R.opacus PD630	CH1.8O0.5N0.2		Fenchone	C10H16O	152.23 g/mol

The streams in Aspen Plus and what process units they are connected to can be seen in Table A1.3 below.

Table A1.3, all streams from Aspen Plus, their name in Aspen Plus, were they come from, where they are going to and what they are containing.

Stream name	**Coming from**	**Going to**	**Containing**
Bact		MixB1	Bacteria seeds
MediaB		MixB1	Pre-cultivation media
Glucose		MixB1	Glucose
PreBheat	MimB1	HeaterB	Bacteria, glucose, media
PreBhot	HeaterB	Pre-cult tank	
PreB	Pre-cult	Splitter consumeB	
ConsuB	Splitter consumeB		Media-water, glucose
PreBa	Splitter consumeB	MixB2	Bacteria, water
ProducB		MixB2	Bacteria, CO2
PrecuOut	MixB2	Splitter cenfuB	Bacteria, water, CO2

Waste	Splitter cenfuB		Water, CO2
Bacteria	Splitter cenfuB	Mix1	Bacteria
Lignin		Mixpre	LIGO, LIGH, LIGC
Premed		Mixpre	Pre-treatment media
Preheat	Mixpre	Heatpre	
PreHot	Heatpre	Pre-tre	
Afterpre	Pre-tre	Splitter consu	
Cons	Splitter consu		35% lignin,
PreOut	Splitter consu	Mixpre2	65% lignin, media
PreProdu		Mixpre2	Lignin monomers
PrePro	Mixpre2	Splitter cenfugP	Lignin, lignin monomers, media
Solid	Splitter cenfugP	Mixpre	Lignin
Prelig	Splitter cenfugP	Mix2	Lignin monomers, media
Media0		Mix1	Fermentation media
Media	Mix1	Mix2	Fermentation media, bacteria
Ferm	Mix2	Heater1	Media, lignin monomers, bacteria
Fermhot	Heater1	Tank ferment	
Out	Tank ferment	Splitter comsplit	
Consumed	Splitter comsplit		Media-water, lignin monomers
Fermout	Splitter comsplit	Mix3	Water, bacteria, lignin monomers
Products		Mix3	Bacteria, CO2, monomers
Fermpro	Mix3	Splitter centrifu	Water, bacteria, monomers, CO2
Rest	Splitter centrifu		Water, monomers, CO2
Bac	Splitter centrifu	Mix4	Bacteria
Lipext		Mix4	Oleic, steric, palmitic acid
Hexane		Mix4	Hexane
Extr	Mix4	Heater2	Bacteria, hexane, lipids
Exthot	Heater2	Tank extract	
Exout	Tank extract	Splitter cenfug2	
Bremain	Splitter cenfug2		Bacteria
Hex+Lip	Splitter cenfug2	Evaporator evap	Hexane, lipids
Hex	Evaporator evap	Mix4	Hexane, trace of lipids
Lipids	Evaporator evap	Evaporator evap2	Lipids, hexane
Hex2	Evaporator evap2	Mix4	Hexane, trace of lipids
Lipids2	Evaporator evap2	Storage	Lipids, trace of hexane

All process units that are used in Aspen Plus and what they would represent in a real industry can be seen in Table A1.4.

Table A1.4, all equipment from Aspen Plus and what they represent in Aspen and in real life.

Equipment in Aspen Plus	What it represents
Mixers	A simple T-pipe or simplification, might be mixed into ex reactor
Heater	Some type of heat exchanger or heating equipment
Tank	Bioreactor
Splitter	centrifuge
Evaporators	Evaporators
Extraction tank	Extraction tower

A.2 Calculations on needed flows, concentrations etc

To know what to put into all the streams first a calculation of everything that was known was done, in Figure A.2.1 below a print from a excel sheet can be seen and in that picture everything that was known from the experimental report can be seen. It has here been assumed that 60% of the carbon feed into fermentation will go to lipid accumulation and the rest will go to production of CO_2. It has also been assumed that the lignin concentration in the fermentation would be the same as the lipid concentration divided by 0.6, the amount of carbon (lignin) that goes to lipid production.

			flow	yield experiment					
	g/l		l/d	g/d		g per experiment	g per day	Liquid per day ml	Density g/l
Lipid production (g/l*2d)	1.02			0.02805		0.0561	0.02805	0.0025	
Bacteria after fermentation (g/l*2d	2.79			0.076725		0.15345	0.076725		
Bacteria before ferment (g/l)	1.77		0.0025			0.09735	0.048675		1100
60% of carbon -> bacteria	0.6					0.033	0.0165		
lignin in fermnetation (g/l)	1.7		0.00025			0.0935	0.04675		1206.666667
	mass frac			density g/l					
Phenol	0.1		0.00025	1070					
Vanillin	0.225		0.00025	1060					
cinnamic acid	0.15		0.00025	1250					
4-hyaldehyde	0.225		0.00025	1230					
4-hyacid	0.15		0.00025	1460					
Methyl salicylate	0.15		0.00025	1170					
	konc	volume	mass	volume in fermentation	konc in ferm	flow			
	g/l	l	g	l	g/l	l/d			
(NH4)2SO4	14	0.9628	13.4792	0.0495	272.3070707	0.02475			
MgSO4	1	0.9628	0.9628	0.0495	19.45050505	0.02475			
CaCl2	0.15	0.9628	0.14442	0.0495	2.917575758	0.02475			
K2HPO4	113	0.0352	3.9776	0.0495	80.35555556	0.02475			
KH2PO4	47	0.0352	1.6544	0.0495	33.42222222	0.02475			
FeSO4	0.5	0.001	0.0005	0.0495	0.01010101	0.02475			
ZnSO4	0.4	0.001	0.0004	0.0495	0.008080808	0.02475			
MnSO4	0.02	0.001	0.00002	0.0495	0.00040404	0.02475			
H3BO3	0.15	0.001	0.00015	0.0495	0.003030303	0.02475			
NiCl2	0.01	0.001	0.00001	0.0495	0.00020202	0.02475			
EDTA	0.25	0.001	0.00025	0.0495	0.005050505	0.02475			
CoCl2	0.05	0.001	0.00005	0.0495	0.001010101	0.02475			
CuVl2	0.005	0.001	0.000005	0.0495	0.00010101	0.02475			
Total flow (l/d)	0.0275								

Figure A.2.1 picture of everything that was known about the experiments from an excel sheet.

From this the flows into fermentation were calculated in l/d and m^3/d. What can be seen in the first column in Figure A.2.2 is the flows in l/d that was used in the experiment from literature and what can be seen in green is the upscaled values (10^9) to produce approximately 10 000 m^3/year lipids and that is the values that have been used in Aspen Plus.

Upscale	l/d	l/d	m3/d	l/d	m3/d	m3/y
Media	0.02475	247500000	247500	24750000	24750	9033750
Bacteria	0.00004425	442500	442.5	44250	44.25	
lignin monomers	3.87431E-05	387430.9392	387.4309392	38743.0939	38.74309392	
Total flow	0.024832993	248329930.9	248329.9309	24832993.1	24832.99309	

Figure A.2.2 flows into fermentation, the ones in green were the ones that was used in Aspen Plus.

Then to see how much bacteria and lipids that would be produced in this upscaled process, the calculations in Figure A.2.3 was made. Here the concentrations from Figure A.2.1 was multiplied with the total flow that can be seen in Figure A.2.2. then these results were converted to lipid ton/year and to know what extra flows to put into Aspen Plus, that is what can be seen in yellow in Figure A.2.3. These extra flows are the products that are produced during reactions that are added in manually with new streams and mixers.

Results	g/d	m3/d	m3/year	g/y	kg/y	ton/y	kton/y		
Bacteria product	69,284,050.73			25,288,678,517.20	25,288,678.52	25,288.68	25.2886785		
Lipids	25,329,652.96	28.25919		10,314.60					
lipid ton/y	9,245.32								
Bacteria feed	78,322.50			28,587,712.50	28,587.71	28.59	0.02858771		
Bacteria product in	69,205,728.23				densitet	steric acid	oleic acid	palmitic acid	medel
co2	46,137,152.15				kg/m3	941.00	895.00	853.00	896.33
TOTAL					g/m3	941,000.00	895,000.00	853,000.00	896,333.33
					molmassa g/mol	284.48	282.47	256.40	274.45

Figure A.2.3 from the upscaling, the results of bacteria, lipid production and CO_2 production as well as the density of the assumed lipids.

For the pre-treatment unit some further calculations were needed, it was known from previous calculations, Figure A.2.2 how much lignin monomers that were needed into fermentation, seen in orange in figure, then it was known that only 35% of the solid lignin feed into pre-treatment would be solubilized. So, the flow of lignin was divided by 0.35 to get how much lignin that were needed in the feed into pre-treatment, see value in orange in Figure A.2.4. Then the ratio between laccase and HBT was known from the experimental report, 0.015 g laccase/g lignin and a ratio of 3:5 (laccase:HBT) was used here. And the values can be seen in Figure A.2.4 below in yellow. It can also be seen in Figure A.2.4 how the volume of the media was calculated, since only the weight percent was known, results can be seen in orange in the figure.

out flow of solution l/d	38,743.09		
lignin monomers conc in this solution g/l	20.32		
lignin monomers in this solution g/d	787,259.67	65,863.26	
lignin monomers solution ton/year	287.35	24.04	
lignin feed in g/d	2,249,313.34	188,180.74	
lignin feed in ton/year	821.00	68.69	
volume feed in l/d	37,488.56		
volume feed in m3/d	37.49		
Laccase g/d	33,739.700	2,822.711	
Laccase ton/year	12.315	1.030	
HBT g/d	56,232.833	4,704.519	
HBT ton/year	20.525	1.717	
			formel viktprocent 6% (w/V)
volym media l/d	(173,207.52)	(14,490.79)	(mM-mL)/(VM-VL)=0,06
volym media m3/d	(173.21)	(14.49)	assume density media same as water
			1000 Vm- mL=0.06Vm-0.06VL
			Vm=(mL-0.06VL)/999.94

Figure A.2.4 picture of the calculated feeds for pre-treatment, solid lignin, lignin monomers, laccase, HBT and media.

For the extraction process it was known the volume that they had in the experiment and then this was scaled up to same size as the rest of the process and it was remade to fit into the continuous flow in Aspen Plus, see Figure A.2.5 for the values for hexane in blue. In Figure A.2.5 in orange it can be seen the values for the assumed lipid production for the extraction process. Here the percentage was multiplied with the total volume of lipids out, that can be seen in Figure A.2.3. For the flows of the pre-cultivation process, what can be seen in yellow in Figure A.2.5, it was known how much bacteria it would need to produce. But here not much else were known, so it was assumed that the volume of bacteria would be tripled during the pre-cultivation. The ratio of media was assumed to

be the same as for the fermentation process and the glucose was known to be 0.5% of the solution, results of these calculations can be seen in yellow in Figure A.2.5.

Extraction				
Bacteria	Experiment	l/d	upscale l/d	m3/year
Hexane	8 ml/d	0.004	4,000,000.00	1460000
Lipids			gram/day	
steric acid	42.9%	0.429	10,866,421.12	
oleic acid	36.6%	0.366	9,270,652.98	
palmitic acid	20.5%	0.205	5,192,578.86	
Pre-cultivation				
	l/d	g/d	l/d	
Bacteria in		26,107.50		
Bacteria out	44,250.00	78,322.50	71.20	
pre-cult media	44,178.80			
glucose % 0.5	220.894			

Figure A.2.5 picture of the hexane volume needed for extraction, lipid feed in per type of lipid, pre-cultivation feed calculations of bacteria, media, and glucose.

A.3 Mass-fraction of monomers before and after fermentation

In the report that was followed for the fermentation production there were two heatmaps that showed the abundance of different monomers, from this a mass fraction was assumed of the monomers used in this model. The assumed mass-fractions before fermentation can be seen in Table 3.1 below.

Table A3.1, monomers before fermentation and the mass-fraction of each of them, the total of the mass-fractions end up being 1.

Monomers	**Mass-fraction**
Phenol	0.1
Methyl salicylate	0.15
4-hydroxybenzoic acid	0.15
Cinnamic acid	0.15
4-hydroxybenzealdehyde	0.225
Vanillin	0.225
Total	**1**

The assumed mass-fraction of the monomers used in the model can be seen in Table 3.2 below.

Table A3.2, monomers after fermentation and the mass-fraction of each of them, the total of the mass-fractions end up being 1.

Monomers	**Mass-fraction**
Methyl salicylate	0.25
Vanillin	0.25
1,4-cyclohexadine	0.5
Total	**1**

A.4 Mass balance

A mass balance over the whole process, everything that is going into the process and not is being recirculated in any way and everything that is going out of the process.

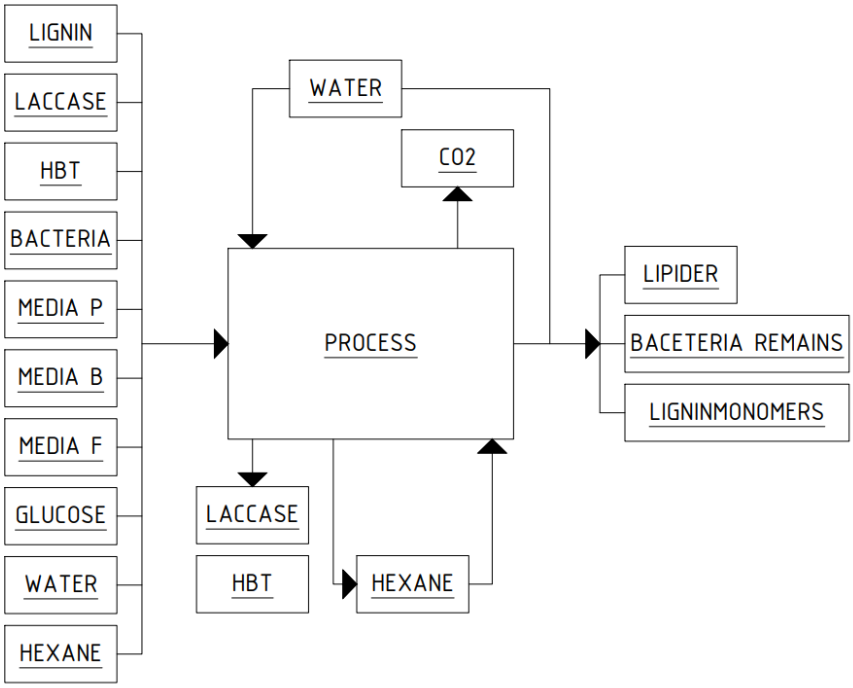

Figure A.4.1 picture over the overall flows in and out of the process.

Lignin + Laccase + HBT + Media pre-treatment + Bacteria + Glucose + Media pre-cultivation + Media fermentation = Laccase + HBT + Lignin monomers + CO2+ Bacteria remains + Lipids

Everything that is put into the process.

Table 7, all components going into the process and their mass flows.

Component	**Kg/h**
Lignin	7.84
Laccase	1.406
HBT	2.343
Media pre-treatment	111
Bacteria	626.6
Glucose	14 358
Media pre-cultivation	706.9
Media fermentation	1030
Total	16 844.089

Everything that is going out of the process and not is being recirculated.

Table 8, all components going out from the process and their mass flows.

Component	Kg/h
Laccase	1.406
HBT	2.343
Lignin monomers	0.617
CO2	3824.3
Bacteria remains	7282.6
Lipids	5732
Total	16 843.3

As can be seen in Table 13 and Table 14 the total in is 16 844.089 kg/h and the total out is 16 843.3 kg/h this is not completely equal but very close. There could be some rounding errors.

Appendix B

Data that was needed for calculating economics.

B.1 Example calculations equipment cost

For the calculation of all equipment the equation (1) in the process chapter of the report was used, and the values that are presented for each equipment can be seen in the tables in the process chapter. So, for example for the calculation of equipment cost for a pump, it was checked in Aspen Plus what the flow would be for the pump. Then it was calculated according to:

$$C = a + b \cdot S^c$$

$$C = 8\,000 + 240 \cdot 200^{0.9} = 36\,257.8\,USD$$

Then this cost was multiplied with an index since the literature is from 2010, so it was multiplied with cpe-index 2022/cpe-index 2010, this value was 1.558. Then the price was converted to SEK, this was done with the exchange rate of 2022, 11.29 SEK/USD. To include all the construction cost of the equipment there needed to be some assumptions for these things. In this project the factors for estimation of fixed capital cost that was used can be seen in Table B1.1.

Table B1.1, the factors needed for estimation of fixed capital cost, major equipment's, the factors that affect them, the estimated values PCE and the equations for calculating the PCE*, the value used for estimating the fixed capital cost.

Major equipment	PCE	Equation
F1 Equipment erection	0.4	
F2 Piping	0.7	
F3 Instrumentation	0.2	
F4 Electrical	0.1	
F5 Buildings, process	0.15	
F6 Utilities	0.2	
F7 Storage	0.15	
F8 Site development	0.05	
F9 Ancillary buildings	0.15	
Total physical plant cost, PPC	3.1	PPC=PCE*(1+F1+F2+…+F9) =PCE*
F10 Design and engineering	0.3	
F11 Contingency	0.1	
Fixed capital	**4.34**	PPC*(1+F10+F11) =PCE*

B.2 Costs and revenues

In the Figure B.3.1 below, all cost and revenues from the base case excel sheet can be seen. The NFW is the sum of all net cash flows, and the net cash flows are:

$$net\ cash\ flow = gross\ profit\ - capital\ investmnet - working\ capital$$

And the NPW is the sum of the discounted cash flow. The discounted cashflow is:

$$\frac{(Net\ cashflow\ )}{(1 + discount\ rate)^{year}}$$

Economic evaluation of the project

				Fixed capital	1,406,612,519 SEK	From Capital cost tab
				Working capital	67,739,636 SEK	From Capital cost tab
Predicted price of MEK	22,580.00			Annual operating cost	335,229,356 SEK/year	From Operating cost tab
Production, m3/year	0-40000	40000-90000	90000-140000	Production	9,245 ton/y	From Hysys
Price of Lipids, SEK/ton	22,580.00	22,580.00	22,580.00			

1) Investment divided so that 40% paid 2023, 50% 2024 and 10 % 2025

2) Working capital is needed as long as the plant is running, i.e. it has to be added at the production start 2025, but will be recovered in the end of the lifetime at 2034

Summary of costs and revenues — 70% of full capacity 2025, 85 % 2026 and 100 % 2027 and onwards

Year	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	
Capital investment, SEK[1]	562,645,008	703,306,260	140,661,252										
Working capital[2]			67,739,636									-67,739,636	
Operating costs, SEK	0	0	244,140,835	302,385,863	362,863,036	370,120,297	377,522,702	385,073,157	392,774,620	400,630,112	408,642,714	416,815,569	
Production, m3/y	0	0	6,472	7,859	9,245	9,245	9,245	9,245	9,245	9,245	9,245	9,245	
Sales income, SEK/y	0	0	152,035,242	188,306,506	225,967,808	230,487,164	235,096,907	239,798,845	244,594,822	249,486,719	254,476,453	259,565,982	
Gross profit, SEK/y	0	0	-92,105,594	-114,079,357	-136,895,228	-139,633,133	-142,425,795	-145,274,311	-148,179,797	-151,143,393	-154,166,261	-157,249,586	
Net cash flow, SEK/y	-562,645,008	-703,306,260	-300,506,482	-114,079,357	-136,895,228	-139,633,133	-142,425,795	-145,274,311	-148,179,797	-151,143,393	-154,166,261	-89,509,950	
Cumulative cash flow, SEK	-562,645,008	-1,265,951,268	-1,566,457,750	-1,680,537,106	-1,817,432,334	-1,957,065,467	-2,099,491,262	-2,244,765,573	-2,392,945,371	-2,544,088,764	-2,698,255,026	-2,787,764,976	NFW
Discounted cash flow, SEK/y	-562,645,008	-639,369,327	-248,352,464	-85,709,509	-93,501,283	-86,701,189	-80,395,648	-74,548,692	-69,126,969	-64,099,553	-59,437,767	-31,372,691	
Cumulative DCF, SEK	-562,645,008	-1,202,014,335	-1,450,366,799	-1,536,076,308	-1,629,577,591	-1,716,278,781	-1,796,674,429	-1,871,223,121	-1,940,350,090	-2,004,449,644	-2,063,887,411	-2,095,260,102	NPW
(Year no)	0	1	2	3	4	5	6	7	8	9	10	11	
Discount rate	0.1												

Rate of return, ROR	-0.198189973
Simple Payback time	-10.18439927 years

Figure B.3.1 excel sheet overall revenues and cost for the base case process over the process lifetime.

Appendix C

The whole process, from Aspen Plus simulations

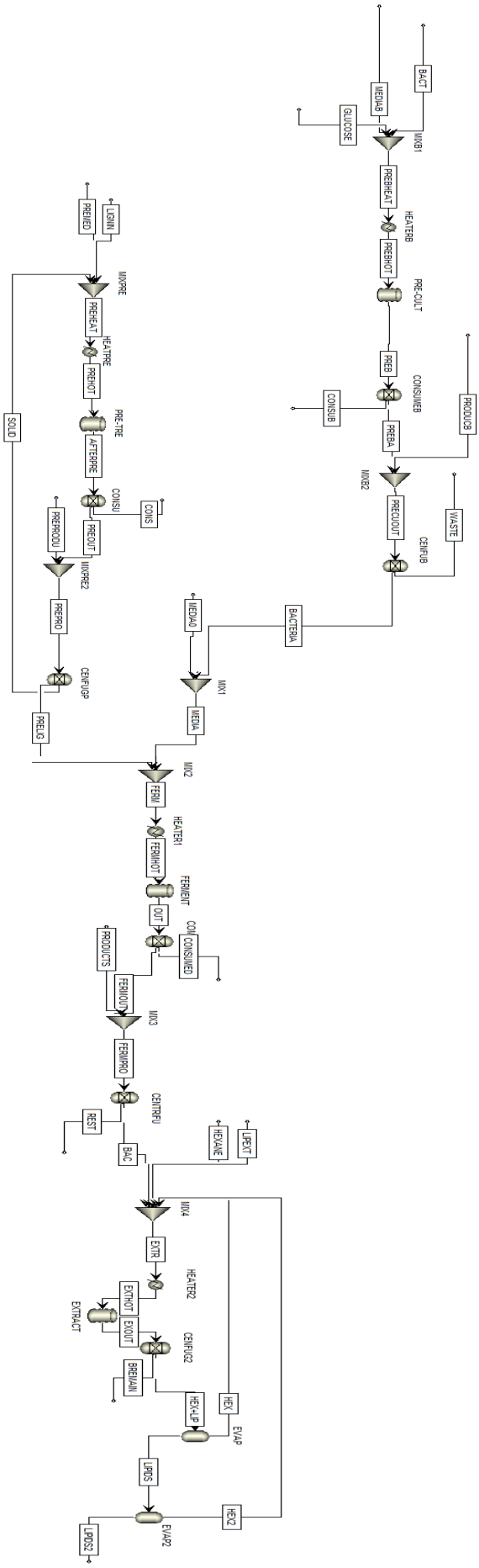

Figure C.1. from right side is the inlet of both pre-cultivation at the top and inlet of pre-treatment at the bottom. Then they mix togheter before entering the fermentation process and at the end is the extraction process and out comes the prodct, the lipids

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