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Preliminary modelling of a process for winter and aviation biofuels production from mannosylerythritol lipids

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Abstract

This thesis aims at preliminary evaluation of a process for production of winter and aviation biofuels using mannosylerythritol lipids (MEL) as a precursor. The lipid moiety of MEL is comprised of short alkyl chains with 8 to 12 carbons, with the appropriate size to provide fuel molecules mixtures appropriated for jetfuel and winter diesel. These specialized fuels require freezing points lower than conventional (bio)diesels, which are comprised by molecules with more than 16 carbons. Two main types of reactions were experimentally performed to convert MEL in fuel molecules: (i) transesterification aiming to obtain short chain methyl esters and (ii) hydrotreatment aiming to obtain short chain alkanes. MEL transesterifications were carried out under both acid and alkaline conditions (sulfuric acid and sodium methoxide catalysed reactions respectively), resulting in maximum yields of 95% and 65%, respectively. The best reaction performance was observed at 5 % wt MEL, catalyst to substrate molar ratio of 8 and 60ºC for 4 h or 24 hours reaction times for acid or alkaline catalysed reactions, respectively. Hydrotreating reactions were performed at 240ºC and 55-60 bar, using a 7% Ni/SAPO-11 bifunctional catalyst. A glucose fed-batch strategy was followed to increase MEL production by *Moesziomyces antarcticus*. Experimental results from this thesis and literature were combined with assumptions into hypothesized scenarios. Process simulations were performed using SuperPro Designer® v8.5 for a preliminary cost and environmental assessment. The production of fuel was modelled considering (i) the bioprocess for production of 1000 units/year MEL and (ii) chemical conversion of such MEL into the fuel molecules.

Keywords: Mannosylerythritol lipids (MEL), transesterification, hydrotreating, process simulation

Resumo

Esta tese tem como objetivo a avaliação preliminar de um processo de produção de biocombustíveis de inverno e aviação, utilizando manosileritritolípidos (MEL) como precursor. A fração lipídica de MEL é constituída por cadeias alquilo de curto cumprimento com 8 a 12 carbonos, com o tamanho apropriado para proporcionar misturas de combustíveis adequadas para aviação e diesel de inverno. Estes combustíveis especializados necessitam de pontos de congelamento inferiores ao (bio)diesel convencional, composto por moléculas com mais de 16 carbonos. Dois tipos principais de reações foram realizadas experimentalmente para converter MEL em moléculas de combustível: (i) transesterificação com o objetivo de obter metil esteres de cadeia curta e (ii) hidrogenação com o objetivo de obter alcanos de cadeia curta. As transesterificações de MEL foram realizadas em condições ácidas e básicas (catalisadas com ácido sulfúrico e metóxido de sódio), resultando em rendimentos máximos de 95% e 65%, respetivamente. O melhor desempenho da reação foi observado com 5 % em massa de MEL, uma proporção molar de catalisador/substrato de 8 a 60ºC durante 4 h e 24 horas em condições de catálise ácida e básica, respetivamente. Reações de hidrogenação foram realizadas a 240ºC e 55-60 bar, utilizando um catalisador bifuncional Ni / SAPO-11 a 7%. Seguiu-se uma estratégia de *fed-batch* com glucose para aumentar a produção de MEL por *Moesziomyces antarcticus*. Os resultados experimentais desta tese e dados da literatura foram combinados com suposições em cenários hipotéticos. As simulações de processo foram realizadas recorrendo ao *software* SuperPro Designer® v8.5 para a determinação de um custo preliminar e avaliação ambiental. A produção de combustíveis foi modelada considerando (i) o bioprocesso para produção de 1000 unidades/ano de MEL e (ii) conversão química de tal MEL em moléculas de combustível.

Palavras-chave: Manosileritritolípidos (MEL), Transesterificação, Hidrogenação, Simulação de processo

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Nomenclature

- **MEL** - Mannosylerythritol Lipids;
- **SBO** – Soybean Oil;
- **CDW** – Cell Dry Weight;
- **HPLC** – High Performance Liquid Chromatography;
- **GC** – Gas Chromatography;
- **IEA** – International Energy Agency;
- **GDP** – Gross Domestic Profit;
- **OECD** - Organisation for Economic Co-operation and Development;
- **GHG** - Greenhouse Gases;
- **OPEC** - Organization of the Petroleum Exporting Countries;
- **USA** – United Sates of America;
- **FAME** - Fatty Acid Methyl Esters;
- **FAEE** - Fatty Acid Ethyl Esters;
- **FT** - Fischer-Tropsch;
- **BtL** – Biomass to Liquid;
- **HVO** - Hydroprocessed Vegetable Oils;
- **HEFA** – Hydroprocessed Esters and Fatty Acids;
- **CtL**– Coal to Liquid;
- **GtL**– Gas to Liquid;
- **CP** – Cloud Point;
- **CFPP** - Cold Filter Plugging Point;
- **PP** - Pour Point;
- **FP** – Freezing Point;
- **SMR** - Steam Methane Reforming;
- **SPK** - Synthetic Paraffinic Kerosene;
- **HDO** - Hydrodeoxygenation;
- **HDC** – Hydrodecarboxylation;
- **DCO** – Hydrodecarbonylation;
- **WHSP** – Weight Hourly Space Velocity;
- **LHSV** - Liquid Hourly Space Velocity;
- **DMDS** - Dimethyl disulphide;
- **TLC** - Thin Layer Chromatography;
- **$NaNO_3$** – Sodium Nitrate;
- **KH_2PO_4** – Potassium Phosphate;
- **$MgSO_4$** – Magnesium Sulfate;

- **PYCC** - Portuguese Yeast Culture Collection;
- **H_2SO_4** – Sulfuric Acid;
- **$Ni(NO_3)_2 \cdot 6H_2O$** – Nickel (II) Nitrate Hexahydrate;
- **CH_3NaO** – Sodium Methoxide;
- **AcCl** – Acethyl Chloride;
- **CS** – Carbon Steel;
- **SS316** – Stainless Steel 316;
- **NaCl** – Sodium Chloride;
- **DCM** – Dichloromethane.

1. Introduction

1.1. Motivation

In today´s society the ever-increasing fossil fuels depletion, price fluctuations and environmental pollution associated with their consumption have urged researchers around the world to find feasible, renewable and sustainable energy, specialized chemicals and fuel alternatives.

The incentive for research in biofuel production comes from their additional benefits, regarding high energy density and ease in process utilization. Current biofuel technologies are not economically feasible since they require government subsidies to be lucrative to the producers and affordable to the users. This is mainly due to: (i) high feedstock costs and (ii) energy-intensive process steps involved in their production.

Furthermore, second generation biofuels are now currently desired. Lignocellulosic waste has received special attention, since it ensures both security of supply and environmental sustainability, key factors in order to truly evaluate the feasibility of a biofuel or its production method. Lignocellulosic materials are comprised by a lignin fraction and a sugar rich fraction in glucose (cellulose) and pentose (hemicellulose) based natural polymers.

While power generation for industry and households can rely on renewable energy sources (such as wind, hydro, nuclear and solar energy) to generate electricity; vehicles can use fuel engines or electricity to operate and trains can be electric-powered; such is not the case in the aviation industry, where energy requirements, among other specifications, limit the type of biofuel that it can use.

The development of biofuels for aviation is mainly restricted to a few attempts using algae or vegetable oils from very specific sources and most alternatives are far from achieving full-scale industrial implementation and fuel certification. The main challenge in the production of alternative bio jetfuel is the requirement for target molecules that have an energy of combustion high enough (around 40 MJ/kg) to power the aircraft, but a freezing point low enough (-47ºC) to remain in the liquid state at high altitudes. These properties correspond to hydrocarbons chains comprising 8 to 14 carbons.

This thesis focuses in drop-in biofuels, defined as “liquid bio-hydrocarbons that are functionally equivalent to petroleum fuels and are fully compatible with existing petroleum infrastructure” [1]. In this context, an alternative strategy for a sustainable biofuel production for land and air transportation was discussed, exploring the natural ability of unconventional yeasts, *Moesziomyces antarcticus* and related strains to yield extracellular short alkyl chain glycolipids, mannosylerythritol lipids (MEL), that already possess an ideal chain length for adequate fuel properties.

1.2. Research questions and objectives

This thesis intends to address the following issues:

1) Is it possible to convert MEL by transesterification into methyl esters?
2) What are the optimal reaction conditions (substrate/catalyst load, concentration, reaction time and temperature) to maximize reaction yield?
3) Is it possible to convert MEL by hydrotreatment into short chain hydrocarbons with adequate chain length to meet jet biofuel standards?
4) What are the ideal reaction conditions for hydrotreatment (substrate/hydrogen ratio, catalyst, temperature and pressure)?
5) What is the overall process cost and energy requirements to produce MEL from glucose?
6) How much energy is required to produce 1 unit of fuel?
7) What strategies can be addressed to reduce the production cost?

1.3. Research Methodology

To achieve the above-mentioned objectives different research methodologies were followed:

Substrate production: MEL was produced by *Moesziomyces antarcticus* JCM 10317T, grown from glucose. Soybean oil (SBO) is reported in the literature as the preferred carbon source for MEL production, leading to higher titres. However, product recovery from oil containing broths results in a more complex downstream processing with high MEL losses and the use of edible vegetable oils raises questions regarding process sustainability. In this context, lignocellulosic materials represent an exciting and available renewable feedstock.

Moesziomyces antarcticus strain was employed in all experiments as it was identified as the best MEL producer from xylose and pentoses [2].

Biomass growth was measured by cell dry weight (CDW) and sugar consumption was quantified by HPLC (High performance liquid chromatography). MEL lipidic chains were quantified by GC (gas chromatography) on the basis methyl esters with carbon alkyl chains of 8 to 14 carbons obtained by complete transesterification reaction with a mixture of methanol/acetyl chloride, followed by extraction with hexane and water. [3]

Transesterification reactions: Transesterification has emerged as the method of choice for biodiesel production from vegetable oils. Hydrogenation of vegetable oils is increasing popularity because it yields fuel molecules chemically identical to the alkanes found in fossil driven diesel. However, transesterification is still the method preferably selected by industry considering mild conditions of such reaction. While a variety of reaction conditions can be used, chemical transesterifications under both acidic or alkaline conditions with methanol/ethanol are the most popular choices.

MEL transesterification was studied using methanol and sulphuric acid and sodium methoxide catalysis. Several experiments were performed to conclude about the best reaction conditions regarding substrate/catalyst ratio, concentration, temperature and reaction time.

Hydrotreating reactions: Hydrotreating reactions generally employ bi-functional sulfided catalysts, such as $NiMoS_2$ and $CoMoS_2$ [4]. A low acidity bi-functional Ni / SAPO-11 catalyst with 7% metal loading, reported to yield alkanes from vegetable oils, was prepared by incipient impregnation and used as a hydrotreating catalyst. The resulting product was analysed for identification [5].

Process Simulation: With the purpose of evaluating the feasibility of a biofuel production process from MEL (regarding process cost and energy requirements) a process simulation was performed, with the support of SuperPro Designer® v8.5. Preliminary production costs and energy requirements for the production of MEL and fuel were estimated and several hypothesized scenarios combining experimental results from this thesis, literature data and a series of assumptions were discussed.

1.4. Thesis outline

This thesis is divided in 5 chapters.

- Chapter 1 – Introduction;
- Chapter 2 – State of the art and Theoretical Background;
- Chapter 3 – Materials and Methods;
- Chapter 4 – Results and Discussion;
- Chapter 5 – Conclusions and Future Work.

Chapter 1 describes the motivation behind the topics approached in this work, summarizing the overall goals and objectives, as well as the research methodology followed and overall thesis structure.

Chapter 2 contextualizes the experimental work performed. A short introduction is included to discuss three major themes: (i) the role of biofuels in the search of an alternative for fossil based fuels, (ii) transesterification technology and (iii) hydrotreatment technology.

Chapter 3 presents a series of methods used for MEL production, quantification and metabolites analyses, as well experimental protocol followed for each type of reaction performed.

Chapter 4 gathers all experimental data collected, including fermentation, reactions/conditions tested and yields obtained. Discussion of the results is presented. It also describes the process simulation performed with SuperPro Designer® v8.5 with the goal of estimating cost and energy requirements for MEL and fuel production by transesterification.

Chapter 5 includes the main conclusions and suggestions for future work.

A list of figures, tables and abbreviations used is also available.

2. State of the Art and Theoretical Background

2.1. Biofuels

2.1.1 Biofuels as an alternative to fossil fuels

Forecasts from the International Energy Agency (IEA) predict that energy demand is expected to keep growing by around 50% until 2030. This growth is mainly caused by population and economic growth, resulting in an increased standard of living. By 2035 the world´s population is projected to increase by 23%, up to around 8.7 billion people (Figure 1). Over the same period, gross domestic product (GDP) is likely to more than double, with non-OECD (Organisation for Economic Co-operation and Development) Asia (including India and China) being the main driver for this growth [6, 7].

The transportation sector represents the main contributor for energy consumption, accounting for 25% of total world delivered energy consumption in 2012. The use of energy for transportation is expected to increase by 1.4%/year from 2012 to 2040 [8].

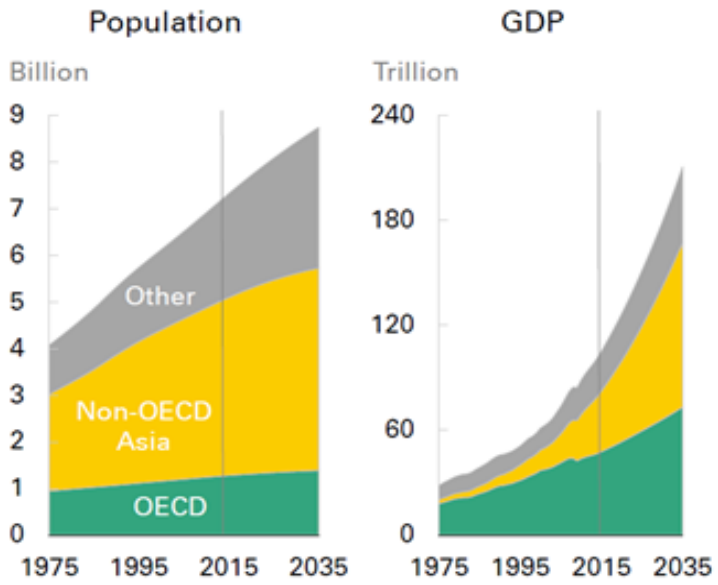

Figure 1 - Population and GDP evolution from 1975 to 2035 [7].

The urgency to shift from fossil based fuels to a more sustainable alternative is mainly due to: (i) the foreseeable scarcity of fossil fuel reserves, (ii) the need to effectively diminish greenhouse gases (GHG) emissions, (iii) the volatility of oil prices (particularly in the transportation sector) and (iv) political uncertainty in addition to geopolitical conflict in supplier countries [6, 8].

The unpredictability of oil prices has long been a major incentive in the search for fossil based transportation fuels alternatives. Since the oil crises in the 1970's, the OPEC (Organization of the Petroleum Exporting Countries) encouraged several countries to promote alternative for fossil fuels. While investment in biofuels dropped in the 1980's as oil prices decreased (Figure 2), countries like Brazil and the United States of America (USA) continued to try to commercialise biofuels, mainly bioethanol (produced from sugars and starches) and biodiesel (from plant crop seed oils). Later, in the 2000´s, investment in second generation biofuels (produced from non-food feedstock) increased due to a growing concern regarding CO_2 emissions, climate change and an increasing dependence on crude oil imports in Europe and North America. More recently, despite the discovery of new unconventional oil and gas resources (such as shale oil, shale gas and oil sands), the IEA forecasts that the price of oil will continue to increase in the next decades [1, 9].

According to IEA's most recent annual energy report, the price of oil is expected to keep rising up to $130/barrel by 2040, according to the new policies scenario (Figure 2). However, this scenario takes into account wide range strategies, including commitments to reduce GHG emissions and plans to minimize fossil based energy subsidies, even if the measures to implement these commitments have yet to be either identified or announced.

Furthermore, if no new policy action is implemented ("current policies" scenario), oil price is projected to reach $150/barrel by 2040.

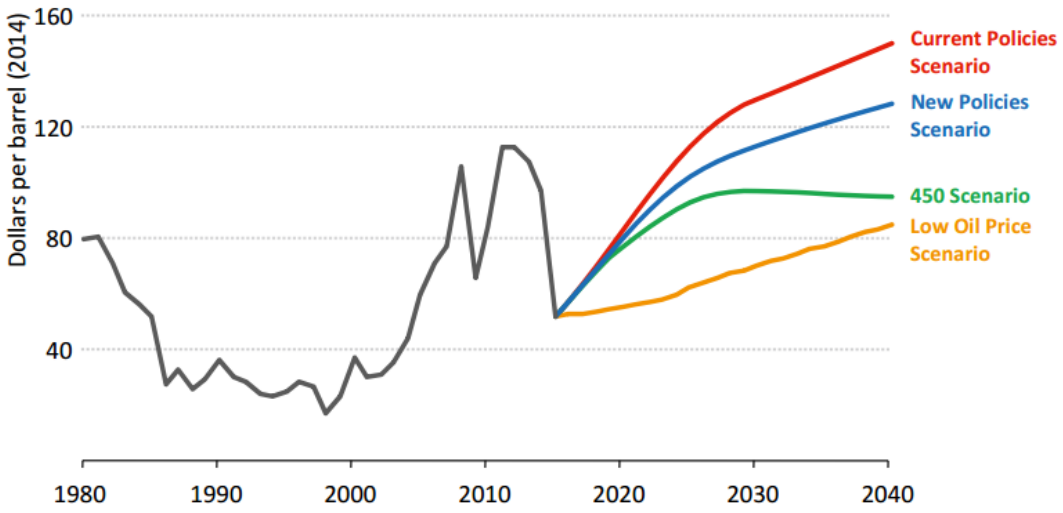

Figure 2 – IEA´s oil price evolution, from 1980 to 2040.

High levels of GHG emissions are also a reason for concern. The transportation sector alone presented the highest rates of GHG emissions growth in any sector over the last ten years, with a predicted 80% increase in energy use and carbon emissions by 2030. Furthermore, energy demand for transportation is expected to increase up to 60% by 2030 (with USA, Europe and emerging economies like China and India set to be the key drivers for this growth) [9].

The source of oil is another problem with no visible solution. Europe, for example, is dependent on imported oil supplies and 80% of its total imported oil share comes from countries such as Russia, Libya and Iraq, with the latter two representing two of the most geopolitically unstable countries in the world, accounting to around 50 million tonnes of oil imports per year (Figure 3) [10].

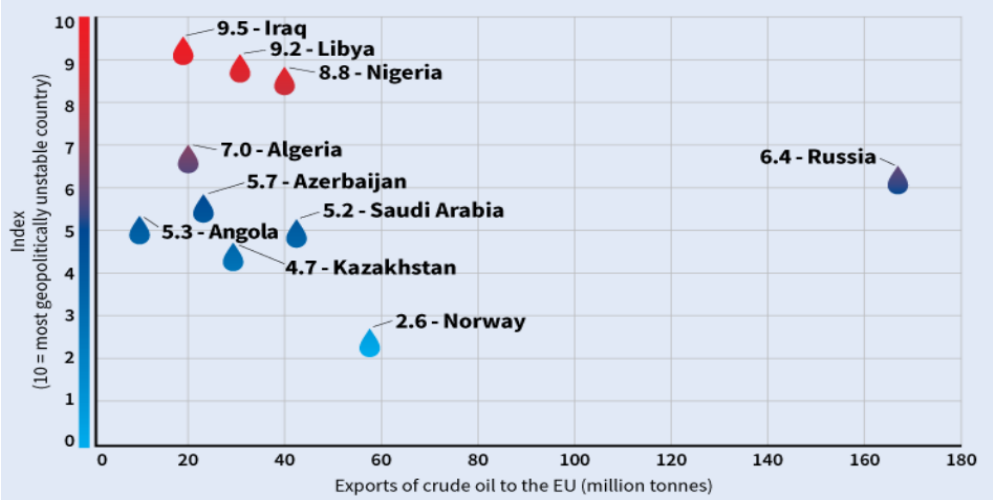

Figure 3 - Index of geopolitical unstable countries [10].

With two thirds of final oil demand being for transportation, a cost competitive and sustainable alternative is vital. As such, biofuels have generated an increasing interest over the last few decades and can be seen as one of the most promising alternatives to replace fossil based fuels in the short to medium term [11, 9].

2.1.2 Biofuels production routes

Several alternative biofuel production technologies are still being developed, while others are already implemented with different degrees of maturity.

A distinction can be made between first and second generation biofuels. First generation ones are typically produced from food crops like oilseeds (rapeseed, palm oil and others) and starch or sugar crops [12]. However, the increasing use of edible crops for biofuels production has raised sustainability concerns regarding food prices and landmass availability for crops

growth. Non-edible energy crops (rich in lignocellulosic biomass) are now being cultivated on a large scale in several countries for biofuels production [13, 9, 14].

Lignocellulosic biomass is mainly composed of three polymers: cellulose, hemicellulose and lignin and it requires a step of saccharification to convert these carbohydrates into sugars (hexoses and pentoses). Both pre-treatment and fermentation processes need to be improved in order to make lignocellulosic processing more economically and energy efficient [15].

Second generation production processes (Figure 4) can use not only lignocellulosic biomass, but also woody crops, agricultural residues or waste [14].

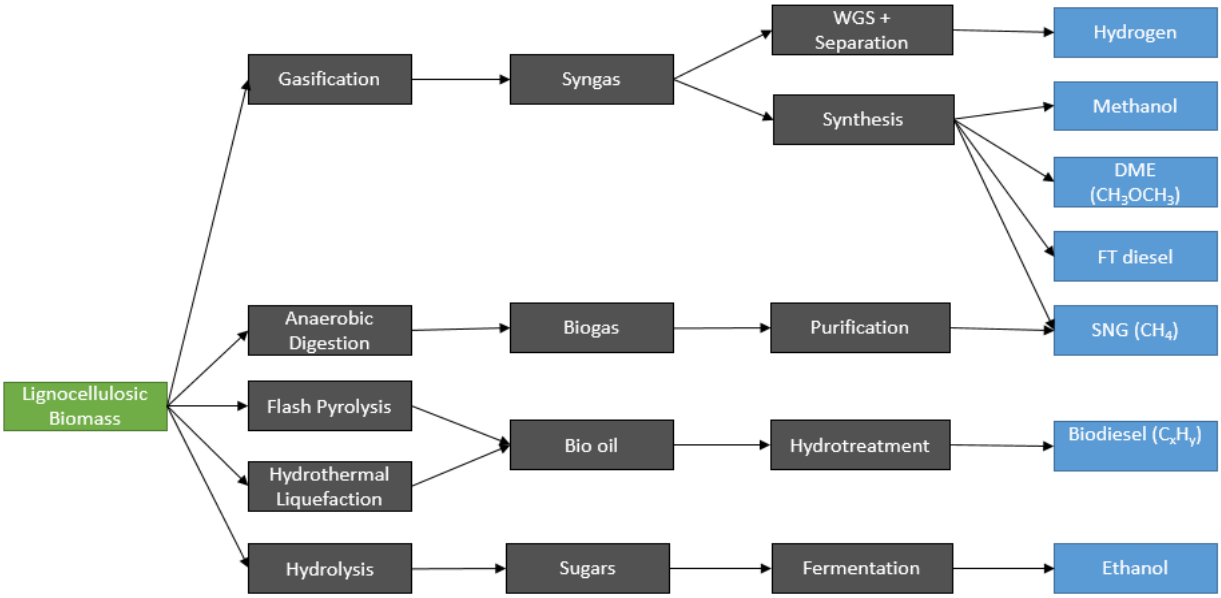

Figure 4 - Simplified second generation biofuel production routes (adapted from [14]).

2.1.2.1 Bioethanol

Bioethanol, along with biodiesel, is currently the largest and most commercially available liquid transportation renewable fuel. Due to its high octane number, low cetane number and high heat of vaporization, it can be seen as a long term replacement for gasoline and is already used as an additive in gasoline blends. While different blends exist, E85 or flex-fuel is the most commonly used [17].

Nowadays, most bioethanol produced comes from first-generation production processes, that use mainly wheat, corn and sugar cane, while second generation processes use a wider range of feedstock, such as grasses, wood and straw. Ethanol can also be produced from lignocellulosic wastes. For instance, cellulosic ethanol can be produced from agricultural residues, woody raw materials or energy crops. Cellulosic ethanol is a promising alternative which is currently in a demonstration stage, due to the more complex production process, involving cellulose hydrolysis [13,16].

Bioethanol can also be produced directly from algae, that can grow in sunlight and produce ethanol directly, which is then extracted without killing the algae. However, such alternative is not economically feasible, due to the low productivity and high cultivation areas required.

While Brazil presents the most mature market in terms of bioethanol usage, significant research is being developed both in Europe and the USA, namely in the wider production of cellulosic ethanol [16].

2.1.2.2 Biodiesel FAME

Plants produce vegetable oils with fatty acids and free carboxylic acids of carbon chain lengths ranging from 12 to 20. Untreated vegetable oils are mainly composed of stearic, palmitic, oleic linoleic and linolenic acid. They possess large hydrocarbon chains (16 to 18 carbons in length) resulting in high viscosity and poor volatility and are unsuitable for direct fuel usage [18].

Biodiesel is usually produced from the transesterification of any type of vegetable oil into fatty acid (m)ethyl esters (methyl ester, FAME; or ethyl ester, FAEE) derived from any type of vegetable oil.

In a transesterification reaction, a triglyceride molecule reacts with an alcohol yielding glycerol, a valuable by-product and an ester (FAME molecule) with one-third of the original molecular weight and a reasonable viscosity and volatility for land engines, whilst carboxylic acids present in the oil will be transesterified into similar (m)ethyl esters (reaction conditions will be discussed in further detail in section 2.2). Regarding reaction stoichiometry, 3 moles of alcohol are required per mole of triglyceride to produce 3 moles of FAME (Figure 5). An excess of alcohol is employed to drive the reaction in the forward direction. An alcohol/oil ratio of 6:1 is normally used in industrial processes to obtain high yields, where higher ratios interferes with the separation of glycerol [19].

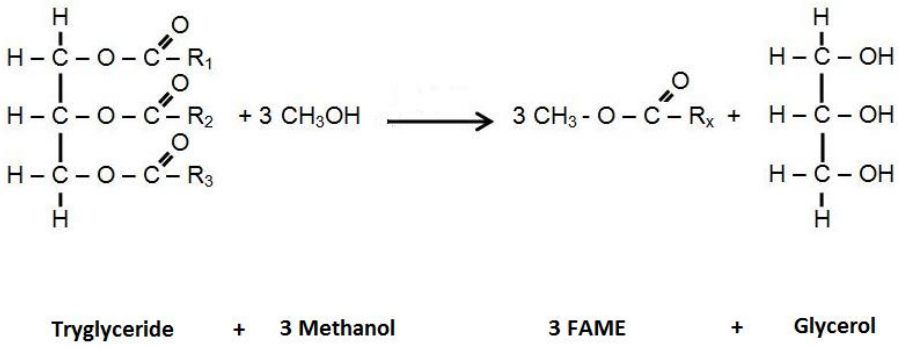

Figure 5 - Schematic representation of a typical transesterification reaction to produce FAME (adapted from [19])

The fatty acid esters obtained not only have similar physical properties to fossil based diesel but can also be used in blends without the need for new infrastructure. Furthermore, its high cetane number is desirable for ignition quality, generating less unburnt hydrocarbons than conventional diesel fuel [19].

First generation biodiesel is typically produced from oil crops (rape, palm or soy), with a later shift to new energy crops (like jatropha or camelina) [20].

As show in Figure 4, biodiesel can also be obtained by a number of alternative routes, including: (i) gasification of lignocellulosic biomass to produce syngas (a mixture of carbon monoxide and hydrogen), which is then converted to liquid fuel via Fischer-Tropsch (FT) (resulting in a BtL (biomass to Liquid) process) and (ii) hydrogenation/hydrotreating to obtain hydroprocessed vegetable oils (HVO).

2.1.2.3 Fisher-Tropsch fuels

In Fischer-Tropsch synthesis, syngas produced by the gasification of biomass to liquid, is catalytically converted to straight chain liquid hydrocarbons. Syngas can be produced from many sources, including natural gas, coal, biomass, or any other hydrocarbon feedstock [21].

Metal based catalysts, such as iron, cobalt or ruthenium are used. Several factors influence product composition, including: syngas composition, catalyst, temperature and pressure. Process temperature is generally in the 150–300°C range. Higher temperatures usually lead to faster reactions and higher conversion rates, but also tend to favour methane production. Increasing the pressure leads to higher conversion rates and also favours formation of long-chain alkanes, both of which are desirable. Typical pressures range from 1 to 20 atmospheres.

A trade-off between reaction conditions and process cost exists. While high pressure may reduce the reaction temperature, it also increases equipment cost and may promote catalyst deactivation [21,22].

Fischer-Tropsch synthesis provides an alternative route for the production of liquid fuels from several sources, such as gasification of coal or biomass, these processes are designated “xTL”, in which (x) stands for C (coal), B (biomass) or G (gas) [23].

The synthesis product must be further processed to improve fuel like qualities. This processing includes chain cracking and isomerization to provide the desired properties. Naphtha, kerosene, and diesel are obtained and can then be distilled into final products.

While Fischer-Tropsch synthesis is already a mature technology it is a high energy intensive process and does not provide enough environmental benefits [21, 24].

2.1.2.4 HVO/HEFA

Hydrotreating is an alternative process to produce diesel from biomass. Hydrotreated/hydroprocessed vegetable oils (HVO or HEFA) are mixtures of paraffinic hydrocarbons free of oxygen, sulphur and aromatics.

HVO´s can be produced from many kind of vegetable oil, including jatropha, camelina, algae or bio oil and already possess good cold properties that can be further adjusted to meet desired requirements by adjusting process intensity or additional catalytic processing. Furthermore, cetane number is very high, and other properties are very similar to the gas-to-liquid (GtL) and FT diesel, produced from biomass to-liquid (BtL) processes [21, 25].

Interest in HVO has been growing, and since the first plant in 2007 by Neste Oil in Finland, several other companies have implemented HVO production [25].

2.1.3 Speciality fuels

2.1.3.1 Winter diesel fuel

A great number of biodiesel blends exist, but the most common include: B20 (6% to 20% biodiesel blended with petroleum diesel), B5 and B2 (5% and 2% biodiesel blended with petroleum diesel respectively) [26, 27].Diesel fuel is susceptible to waxing when in cold climates. At temperatures below the cloud point (Figure 6) the fuel begins to develop solid wax particles. The presence of solidified waxes thickens the oil and clogs fuel filters and injectors in engines. The increasing crystal build up in the fuel filters continues until no more fuel reaches the engine, causing it to stop [28].

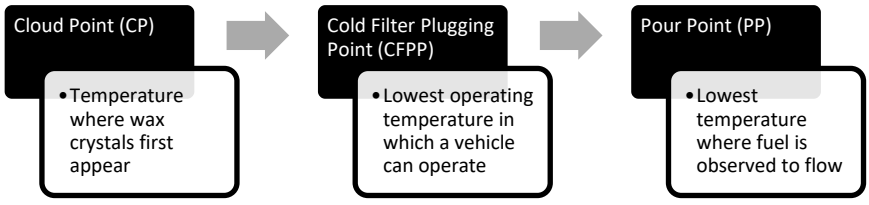

Figure 6 - Cold weather parameters that define diesel and biodiesel operability (adapted from [26]).

Cold flow characteristics of biodiesel fuels are dependent on the feedstock from which they are produced and are highly dependent on the level of saturated fat in the source oil, with low levels of saturated fat promoting cold flow performance (Figure 7).

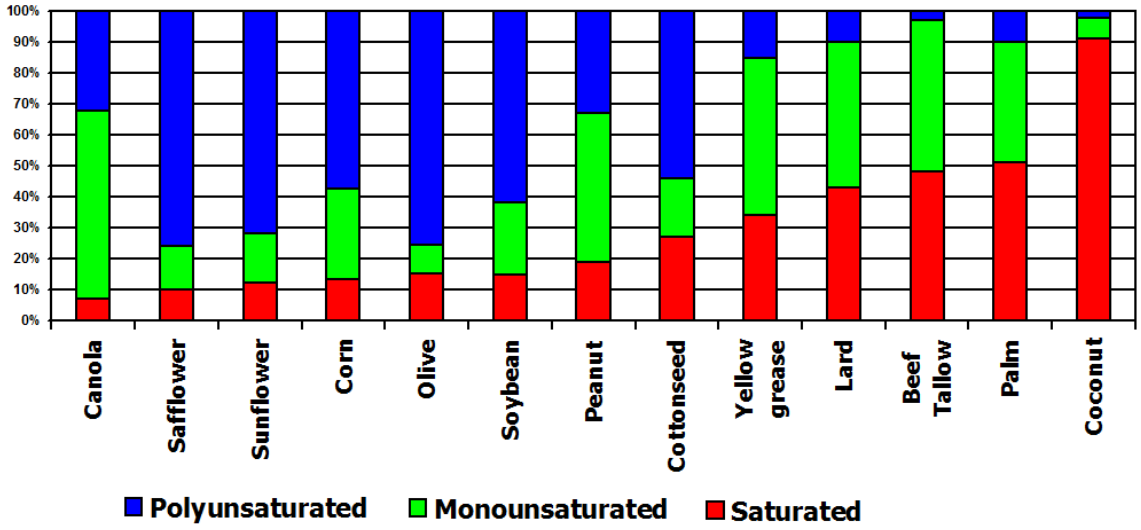

Figure 7 – Cold flow performance by vegetable oil source, where cold flow properties improve from right to left (adapted from [26]).

Anti-freezing strategies have been developed and generally result in the use of additives in conventional biodiesel, ensuring a better cold weather performance and reducing petroleum based products.

Another example includes companies like BioFuel Systems Group Ltd that commercializes compounds, namely the WintronTM range of additives, that are able to lower fuel viscosity. These additives reduce the tendency to increase viscosity as the fuel is cooled which alters cold temperature crystallisation, reducing the freezing point (FP), pour point (PP) and cold filter plugging point (CFPP) [27].

However, while effective in improving cold whether performance, they are not good long-term solutions since they do not provide security of supply.

2.1.3.2 Biojetfuel

Jet fuel is a specific type of fuel, composed by a mixture of several hydrocarbons with a chain length dependent on the type of fuel. Kerosene type jet fuel (including Jet A and Jet A-1) has 8 to 16 carbon atoms, while naphtha-type jet fuel (including Jet B) has around 5 to 15. [13] Aviation fuel has many specific performance and safety requirements. The fuel needs to provide enough energy not only to propel the aircraft from the ground, but also to keep it airborne (Specific energy > 42.3 MJ/kg). Additionally, it needs to have a low enough freezing point (-47ºC) to remain liquid when flying at high altitudes and comply with the necessary safety requirements. These and other important properties (Table 1) must be certified through specific standards.

Table 1 - Aviation fuel requirements (adapted from [30]).

Properties	Density	Flash point (°C)	Freezing point (°C)	Specific energy (MJ/kg)	Boiling point (°C)	Viscosity (mm^2/s)
Requirements	0.78 - 0.85	> 60	< -40	> 42.3	200-300	<8 (at -20°C)

This certification standard, the ASTM D4054 consists of two steps: (i) the certification that the chemical-physical properties complies with the requirements for high altitude operation (such as fuel energy density) and flow characteristics (such as freezing and viscosity), followed by (ii) further testing at low temperatures, materials test and full engine tests.

As such, production of hydrocarbon chains with the above-mentioned length is the aim in the experimental work presented in section 4.3. While a great number of biofuels exists for road transportation, jetfuel stringent requirements make more challenge the production of a biofuel for aviation, with no feasible production developed until date.

A comparison between typical jetfuel properties of same biofuel candidates for aviation is presented in Table 2.

Table 2 - Properties of jet fuel alternatives (adapted from [13]).

	Specific energy (MJ/kg)	Density (15°C)	Energy density (MJ/L)
Jet-A	43.2	0.809	34.9
Liquid hydrogen	120.0	0.041	8.4
Liquid methane	50.0	0.424	21.2
Methanol	19.9	0.796	15.9
Ethanol	27.2	0.794	21.6
Biodiesel	38.9	0.870	33.9
FT synfuel	44.2	0.760	33.6
HVO	45.2	0.780	35.1

Hydrogen can be generated from a variety of feedstocks like municipal and sewage residues, cellulose forest waste materials and crops, biomass gasification, pyrolysis oil by catalytic SMR (Steam Methane Reforming), thermochemical cycles or even using *algae* and bacteria. Biomethane is produced by anaerobic digestion of a large number of renewable feedstocks like waste materials, crops and biomass [21]. However, liquid hydrogen and methane are not adequate for air transportation due to their low density. Methanol and ethanol are far from possessing high enough specific energy to power the airplane engine.

Fisher-Tropsch fuel appears to be in a better position to be a viable jetfuel alternative, since it satisfies the energy requirement parameters. In August 2009, Generic FT Synthetic Paraffinic Kerosene (SPK) was approved for use in blends, at up to 50% volume, with Jet A-1 in ASTM

D7566 [31]. The product from FT synthesis originates a clean burn product, free of sulphur and aromatics present in conventional jet fuel. However, since the fuel is free of aromatic compounds, some fuel leakage problems arise due to shrinkage of the engine. While blending or the use of additives minimizes this issue, it may also worsen environmental performance [21, 24].

Despite this, FT synthesis, while a mature technology, does not provide environmental benefits due to coal and gas requirements and long term viability and implementation is questionable.

HEFA appears to be the technology better placed to be a short term alternative. However, HEFA cannot be applied directly as an aviation fuel, since they are generally produced from C16 and C18 based vegetable oils. An increased chain length increases the freezing point, making it unsuitable for aviation, meaning that an extra energy intensive step of cracking or isomerization is required to obtain the target shorter chain length molecules [13, 21].

HEFA is already being used for testing purposes in commercial passenger flights and received approval for use as an aviation fuel under ASTM D7566-14. In July 1st, 2011 a revised standard was released, allowing up to 50% bioderived synthetic blending components (HEFA) to be added to conventional jet fuel.

Other possible approaches have been studied. One such example is synthetic biology that allows for the design and construction of biological functional systems not yet found in nature. These include the engineering of microbial biosynthetic pathways that aim to produce a range of advanced biofuel alternatives. The majority of metabolism improvements have been reached by slight changes within the production pathway. However, a continuous fuel production at high yields and stable production through building adequate synthetic pathway is still far. While this approach shows promise it is currently not a feasible alternative and it is far from meeting certification standards [32, 33].

2.2. Transesterification Technology

2.2.1 Reaction conditions

As shown in section 2.1.3.2, transesterification consists in the reaction of an ester with an acyl acceptor that can be either an alcohol, another ester or an acid to generate a new ester molecule.

Methanol and ethanol have emerged as the preferred alcohols for these types of reactions. The first has a low cost and good chemical properties, namely polarity and small molecular size. Ethanol has the advantage of being a biobased product. However, commercial grade ethanol contains a small percentage of water, not favorable for the reaction. Higher chain alcohols are not generally used on an industrial scale [34, 19, 35].

Mixing is also needed to reduce mass transfer resistance since alcohol and triglyceride form a biphasic mixture. Initially the reaction rate is slow and the mixing effect is most significant. Later on, when the two phases are almost merged mixing becomes insignificant, with the reaction rate being controlled by temperature. Microwave irradiation and ultrasonic mixing are alternative ways to minimize reaction time [34].

2.2.2 Catalyst Choice

Transesterification can be done under homogeneous and heterogeneous catalysis. Conventional homogenous catalysts are most commonly done with alkali or acid catalysts.

Acid catalysis is mainly used with sulfuric acid (H_2SO_4) when the oil has a higher concentration of free fatty acids. A molar ratio of alcohol:oil of 30-150:1 is usually used, which results in large amounts of alcohol being consumed. Additionally, acid catalyzed transesterification of triglycerides is generally a slow process even with reflux, leading to long reaction times (48 to 96 hours) [36, 19].

Basic catalysis is mainly done with sodium or potassium hydroxide as well as metal alkoxides (such as sodium methoxide). It is also significantly faster than acid catalysis and does not require large quantities of alcohol, often using 6 equivalents of alcohol to 1 of oil [37].

However, some process limitations still exist. Oils with high levels of free fatty acid may promote the occurrence of saponification reactions of triglycerides, leading to unproductive base consumption and complicating downstream processing of the esters. Another disadvantage of this method is the possible saponification that can occur due to the water generated during the reaction of hydroxide ion with the alcohol used, even when water-free reagents are used in the process [37,19].

Purification of the final product is also an important issue, as homogeneous catalysts usage requires elaborate downstream treatment involving neutralization, washing and drying.

Biocatalysts, typically lipases, can also be employed. However, enzyme cost and the presence of alcohols that can deactivate and even denature enzymes remain obstacles for industrial implementation. Additionally, enzyme activity is also influenced by glycerol due to its low solubility in biodiesel, creating a deposit on the catalyst and reducing the enzyme activity [38, 36].

Furthermore, transesterification in super-critical conditions has also been presented, representing the only catalyst-free method. However, high equipment cost and demanding reaction conditions (around 500-600 K and 35-60 bar) have so far limited this alternative to laboratory scale only. [36, 19]

Recently, the use of ionic liquids has also been reported and appears to be a promising alternative in transesterification technology. Ionic liquids can be used to treat byproducts from biodiesel production and can also be used as support for both acidic or alkaline catalysts or supported enzymes [34, 39].

There is nevertheless a clear predominance of acid and alkaline catalyzed transesterification for the production of biodiesel. As such, experimental work presented in section 4.2 will focus on this approach.

2.3. Hydrotreatment Technology

2.3.1 Reaction conditions

Originally, hydrotreating (or hydroprocessing) was the main industrial process to remove SO_X (hydrodesulfurization) and NO_X (hydrodenitrification) compounds from either gasoline, natural gas, diesel or jetfuel, as most countries have strict limits for these pollutants in fuel composition [40].

Later, hydrotreating was also applied to vegetable oils or animal fats as an alternative process to produce bio-based diesel fuels.

In this type of fuel, the straight paraffin components are characterized by a high ratio of H/C and produce high heat per unit mass compared to the other hydrocarbons and are therefore required in large quantities in the aviation fuels. Cyclic paraffins are important for guaranteeing suitable density and reduced freezing points. To achieve these properties and satisfy jet fuel requirements, vegetable oils must undergo appropriate conversion processes [40, 4].

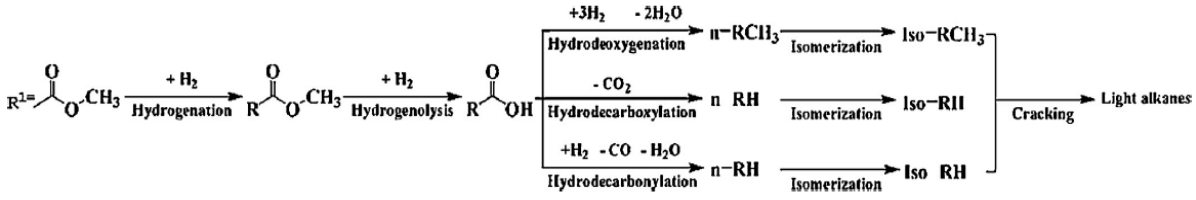

Figure 8 - HDO, HCO and DCO reaction pathway (adapted from [4]).

Figure 8 shows the possible reaction pathway from the vegetable oil source to the liquid alkane fuel product. The reaction starts with the hydrogenation of the C=C bond in the unsaturated vegetable oil, followed by three different reaction pathways: (i) hydrodeoxygenation (HDO), where oxygen is removed in the form of water; (ii) hydrodecarboxylation (HDC), with CO_2 being formed and (iii) hydrodecarbonylation (DCO), where oxygen is released in the form of CO and water.

Reaction pathway (i) is an exothermic reaction that leads to n-alkanes with the same carbon number as the corresponding fatty acid, while pathways (ii) and (iii) are endothermic and lead to n-alkanes with one carbon atom less when compared to the original fatty acid [41,42].

A final step of cracking and/or isomerization is then required to reduce chain length and produce lighter alkanes.

The effects of hydrotreating parameters have been explored in various literatures with temperature, hydrogen pressure, WHSP/LHSV (weight hourly space velocity, WHSP (mass flow/ catalyst mass ratio); liquid hourly space velocity, LHSV (reactant liquid flow rate/reactor volume ratio) and hydrogen/oil ratio being the most important reaction parameters, capable of influencing reaction pathway [41].

While reaction conditions vary with the type of vegetable oil tested, temperature between 250-400ºC have been tested, along with pressures up to 60 bar [40, 4, 41].

2.3.2 Catalyst selection

There are two main types of catalyst mostly used in hydrotreating reaction: (i) metal based catalysts, such as Ni, Pd, Pt or Ru and (ii) bifunctional catalysts, namely NiMo, CoMo, Pd, Pt, Ru supported with Al_2O_3, TiO_2 or zeolite based supports (Pd/SiO_2, $Ni/Sn/Al_2O_3$, Pt/SO_4^{2-}, ZrO_2, $Ni/W/SiO_2/Al_2O_3$, Ni / SAPO-11), among others [4, 41, 42].

Metal based catalysts will usually require a pre-sulfiding step with a sulfiding agent such as Dimethyl disulfide (DMDS) prior to the reaction [40].

Catalyst choice also influences reaction pathway. Metal catalysts are favourable to HDC and DCO, while HDO is favoured by bimetallic sulfide catalysts, like $NiMoS_2$ and $CoMoS_2$. Furthermore, some metal catalysts such as Ni, Pd, and Pt strongly promote methanation reaction, resulting in large hydrogen consumptions [4].

$NiMoS_2$ and $CoMoS_2$ have emerged as the preferred catalyst for hydrotreatment. However, its usage requires a further purification process, since sulfur containing reagents are necessary to maintain catalytic activity and stability not only during the catalysts pre-treatment but also during reaction processing, which inevitably causes sulfur contamination of the final product.

An already available Ni/SAPO-11 catalyst, reported to promote HDO reaction pathway and yielding liquid alkanes was prepared for MEL hydrotreating as discussed in section 4.3 [5].

2.4. Mannosylerythritol Lipids (MEL)

2.4.1 Chemical and biological properties

MEL were first described in 1956 and represent a group of biosurfactants that contain 4-O-β-D-mannopyranosyl-meso-erythritol as the glycosidic and hydrophilic moiety, bonded to one or two short free fatty acids chains (usually C6–C12 length) and acetyl groups as the hydrophobic moiety [43, 2].

Depending on the degree of acetylation in C-4 and C-6 of the sugar moiety (Figure 9), as well as their elution order on thin-layer chromatography (TLC), MEL can be classified as MEL-A, MEL-B, MEL-C or MEL–D.

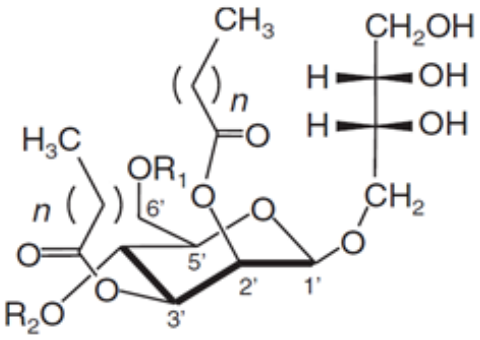

Figure 9 - MEL molecular structure (MEL-A: R1=R2=Ac; MEL-B: R1=Ac, R2=H; MEL-C: R1=H, R2=Ac; n=8-12) (adapted from [44]).

MEL-A represents the diacetylated compound, while MEL-B and MEL-C possess an acetyl group at C-6 and C-4 of the mannosyl moiety respectively. MEL-D possesses a dIacetylated structure. The overall composition of the type of MEL molecule in the mixture will depend on the producer strain. These variants arise due to (i) the number of acylation in mannose and (ii) the fatty acid chain length and their saturation. [45]

2.4.2 Producers and cultivation conditions

MEL are mainly produced by the *Moesziomyces* genus, namely *Moesziomyces antarcticus*, *Moesziomyces aphidis*, *Moesziomyces rugulosus* and *Moesziomyces parantarcticus*, which mostly produce diacetylated MEL-A with smaller amounts of MEL-B and MEL-C. Additionally, they can also be produced by fungal strains like *Ustilago* sp. 9, 10 and 12 or *Pseudozyma* sp. 9, 1 [46,43].

They can be produced from a variety of substrates, preferably vegetable oils or sugars. While soybean oil is the preferred substrate for MEL production due to its high yields and titres,

several substrates have been used for MEL production, including soybean oil, alkanes, glycerol, glucose and xylose [2, 46]. Soybean oil represents the preferred carbon source for MEL production due to leading to higher titres. However, MEL recovery from oil containing broths results in a more complex downstream processing. Furthermore, the use of edible vegetable oils raises questions regarding process sustainability due to the increasing prices of these substrates [2].

2.4.3 Isolation and applications

Due to their diverse range of application, MEL are considered multifunctional molecules and have applications in the pharmaceutical, cosmetic and food industries.

For instance, MEL-A possesses exceptional surface-active properties, being shown to reduce the surface tension of water from 72.8 mN/m (at 20ºC) to less than 30 mN/m [45].

MEL present diverse biochemical functions, namely antitumor and cell-differentiation induction activities against human leukaemia, rat pheochromocytoma, and mouse melanoma cells. MEL-A and MEL-B also show strong antimicrobial activity against gram positive bacteria and weak activity against gram negative bacteria [46, 45]. Furthermore, MEL-A also shows promise as a new ligand, namely due to its high binding affinity towards immunoglobulins, lectins and other glycoproteins. They can also be used as a vehicle for gene or drug delivery due to their ability to form thermodynamically stable vesicles that are able to fuse with the membrane [45].

MEL also have applications in the cosmetic industry, where MEL–A is used in a diverse range of products including skin creams, shaving creams and lotions, enhancing foaming properties, being a moisturizing agent and improving healing properties of the skin. They can also be used to remove soil contaminants, including heavy metals, oils and other toxins [45, 47].

2.4.4 Potential as a biofuel precursor

As stated above, the lipid moiety of MEL molecules possesses alkyl chains with up to two acyl groups, with an ideal chain length (C8-C12) to provide adequate jetfuel and winter diesel. To achieve this conversion, breaking down the ester bond between the acyl groups and the mannosyl moiety is essential.

This separation is what is intended in the experimental work developed in this thesis and can be accomplished by two processes: (i) transesterification to (m)ethyl esters fatty acids adequate to be used as winter diesel and (ii) hydrotreatment to obtain short chain alkanes adequate to be used in jetfuel formulations.

Approach (i) will be discussed in section 4.2 and approach (ii) will be the focus of section 4.3.

3. Materials and Methods

3.1. MEL production

3.1.1 Cultivation conditions

MEL was produced from *Moesziomyces antarcticus* PYCC, JCM 10317T, obtained from the Portuguese Yeast Culture Collection (PYCC), CREM, FCT/UNL, Portugal. Yeasts were cultivated for 3 days at 25°C on Yeast Malt (YM) medium (yeast extract (3 g/L); malt extract (3 g/L); peptone (5 g/L) and glucose (10 g/L)). Dense cultures were then plated on YM agar (yeast malt agar) and incubated for 48 hours at 28 ºC. Colonies having the characteristic morphological appearance of *M. antarcticus* were isolated to prepare stock cultures.

Stock yeast cultures were prepared by propagation of yeast cells in liquid medium (yeast extract (3 g/L); malt extract (3 g/L); peptone (5 g/L); glucose (10 g/L) and agar (20 g/L) and stored for later use.

Inoculum was prepared by incubation of cultures of *M. antarcticus* in liquid medium containing $MgSO_4$ (0.3 g/L), yeast extract (1 g/L), $NaNO_3$ (3 g/L), KH_2PO_4 (0.3 g/L) and glucose (40 g/L). Inoculum was placed in an incubator for 48 h at 28°C and 250 rpm.

Batch cultivations for MEL production were performed in 1000 mL Erlenmeyer flasks containing 1/5 working volume of mineral medium ($MgSO_4$ (0.3 g/L), yeast extract (1 g/L), $NaNO_3$ (3 g/L), KH_2PO_4 (0.3 g/L) and glucose (40 g/L), followed by incubation at 28ºC and 250 rpm for 14 days.

To obtain higher product titres, a fed-batch strategy was implemented, with a pulse of carbon source (40 g/L) at day 4.

Samples were taken at days 0, 2, 4, 7, 10 and 14 to quantify cell dry weight (biomass), sugar consumption and MEL lipidic chain quantification.

3.1.2 Extraction

Liquid-Liquid extraction with ethyl acetate was performed for MEL isolation (Figure 10). An equal volume of fermentation broth and ethyl acetate were added to a separation funnel. After vigorous shaking, the separation funnel was placed in a 4ºC chamber for 24h to promote phase separation. The top organic phase (rich in ethyl acetate) was collected. The bottom aqueous layer remained in the funnel and an extra volume of solvent was added for a second extraction. The

combined organic phases were placed in a rotary evaporator in vacuum conditions (400 mbar in a 45ºC bath) for solvent recovery.

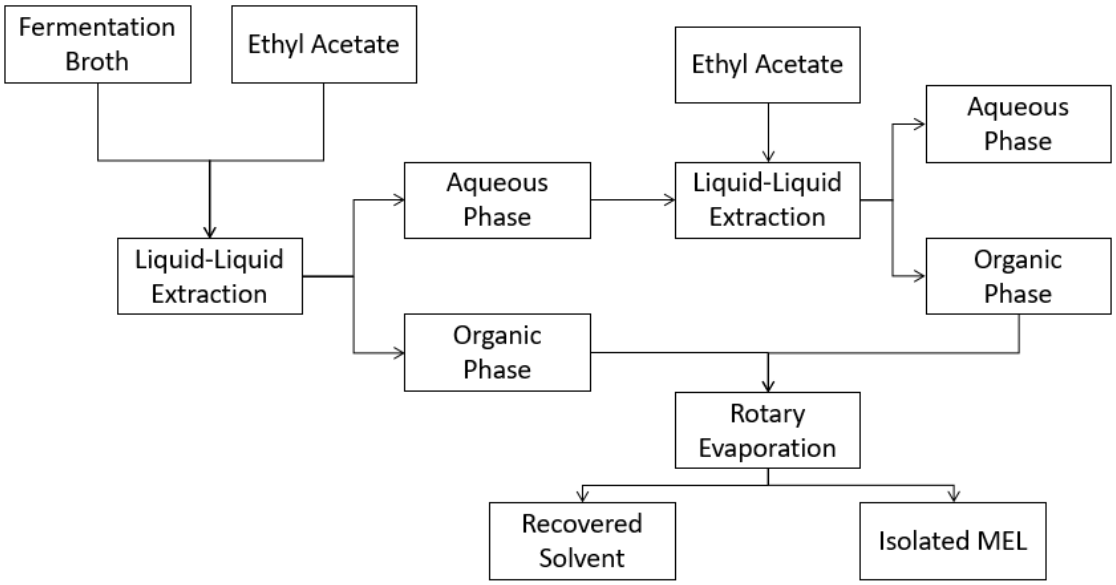

Figure 10 – Operation sequence for MEL isolation**.**

3.1.3 MEL and metabolites analysis

Biomass

Cell growth was followed by measuring cell dry weight. CDW was determined from 2 mL culture broth by centrifugation at 13,000 rpm for 10 min, followed by cell pellet washing with deionized water (twice). The cell pellet was dried in an oven at 100°C for 24 h and weighted.

Sugar consumption

Culture broth samples (3 mL) were centrifuged at 13,000 rpm for 1 minute. The top organic phase was collected and a 1/20 dilution in H_2SO_4 was performed. The organic phase was analysed by HPLC system (Merck Hitachi, Darmstadt, Germany) equipped with a refractive index detector (L--2490, Merck Hitachi, Darmstadt, Germany) and an Rezex ROA--organic acid H+ column (300 mm ~ 7.8 mm, Phenomenex), at 40°C. Sulfuric acid (0.005 M) was used as mobile phase at 0.5 mL/min.

Fatty acid composition

MEL cannot be directly analysed by GC. A procedure for fatty acid analyses based on a transesterification reaction with a mixture of methanol/acetyl chloride, followed by extraction with hexane and water was implemented. The quantification of lipidic chains based on a specific moiety was previously described [3].

Methanol (20 mL) was cooled down to 0°C under nitrogen atmosphere and 1 mL of acetyl chloride was carefully added under stirring, which generated a water-free HCl/methanol solution. Culture broth samples (3 mL) were freeze-dried, weighted and mixed with 2 mL of the HCl/Methanol solution and reacted for 1 h at 80°C for methyl esters production. Heptanoic acid was used as internal standard. The resulting product was extracted with hexane and water (1 mL of each) and 1 µL of the organic phase was injected in a GC system (Hewlett-Packard, HP5890), equipped with a FID detector and a SUPELCOWAX® 10 capillary column (L × I.D. 60 m × 0.32 mm, df 0.25 µm). The oven was programmed from 90°C (held for 3 min) to 200°C at 15°C/min. Nitrogen was used at a flow rate of 50 mL/h.

MEL was quantified through the amount of C8, C10 and C12 length fatty acids considering a molecular weight between 574 and 676 g/mol depending on the length (C8 to C12). of the two acyl chains.

3.2. Transesterification

3.2.1 Experimental Setup

Transesterification reactions were performed in reaction tubes. A specific amount of MEL (generally around 30-40 mg) was placed in reaction tubes, followed by the addition of methanol. The mixture was then vortexed for about 1 minute until all the MEL was completely dissolved in the alcohol. The catalyst was then added and the reaction tube was placed in a pre-heated oven at the desired temperature.

3.2.2 Methyl esters quantification by Gas Chromatography (GC) analysis

Following the reaction, methyl esters extraction from the resulting product was performed as described above. A 1 mL solution of hexane (with 0.4% of methyl heptanoate) plus 1 mL of water were added. Methyl heptanoate was used as internal standard due to being a C7 chain length ester, smaller than the possible C8-C18 chain length esters expected to be obtained from MEL transesterification.

The reaction tube was vortexed for 1 minute. Following phase separation, the upper organic phase was collected and filtered through cotton and magnesium sulphate (a water scavenger) to remove any traces of suspended particles and water.

Finally, 1 µL of the organic phase was injected in a GC system, in the conditions described above.

The reaction yield was calculated as the ratio of moles of methyl esters formed per mole of lipidic chains present in MEL (reaction 1). A yield of 100% corresponds to a reaction in which the substrate is completely converted.

$$Yield\ (\%) = \frac{moles\ of\ lipidic\ chain\ (C8 - C12)}{moles\ of\ MEL \times 2} \quad (1)$$

A combined reaction yield, taking into account all the lipidic chains (C8-C12) was also determined (reaction 2).

$$Combined\ Yield\ (\%) = \frac{\frac{moles\ of\ lipidic\ chain\ (C8 - C12)}{moles\ of\ MEL \times 2} + \frac{moles\ of\ lipidic\ chain\ (C14 - C18)}{moles\ of\ MEL \times 2}}{2} \quad (2)$$

3.3. Hydrotreatment

3.3.1 Catalyst preparation

A bi-functional Ni/SAPO-11 catalyst with 7% metal loading was prepared by incipient impregnation.

An already available SAPO-11 catalyst was impregnated with an aqueous solution of $Ni(NO_3)_2 \cdot 6H_2O$ of 7% by weight. The suspension was stirred for 18h at 25ºC, followed by evaporation of excess water at 80ºC. The solid was then calcinated at 550ºC for 5h. A total of 500 mg of catalyst were prepared.

In the day prior to the catalyst usage, reduction was performed. A pre-treatment was done with Argon at 120ºC (with a heating rate of 5ºC/minute for 2h) to desorb water and other impurities that may still remain in the catalyst. After a period of cooling down to 25ºC, reduction was

performed under a 5% Hydrogen/Argon atmosphere at 120ºC for 2h, with a temperature increase of 5ºC/minute (Figure 12).

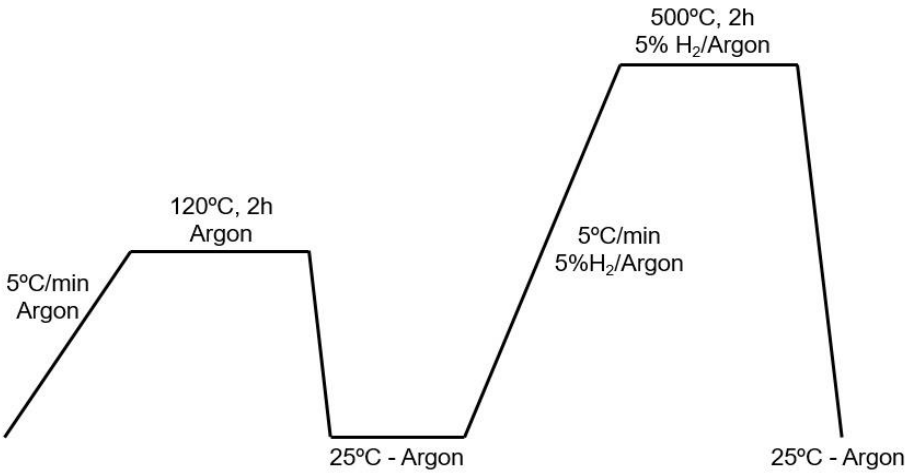

Figure 11 – Catalyst reduction conditions.

Catalyst reduction was performed twice, in the day before usage to minimize the effect of catalyst surface oxidation. A new amount of catalyst was used in each reaction to avoid deactivation effects. No catalyst characterization tests were performed.

3.3.2 Experimental Setup and reaction conditions

Hydrogenation reactions were performed in a pilot scale 75x45 mm batch reactor. An experimental setup was assembled as represented Figure 12 with a monometer for pressure control in position 3, a gas inlet in position 3 and a gas outlet in position 1. The reactor was immersed in a silicon oil bath (position 4) to promote heat transfer and placed in a hotplate (position 5) with a probe for temperature control.

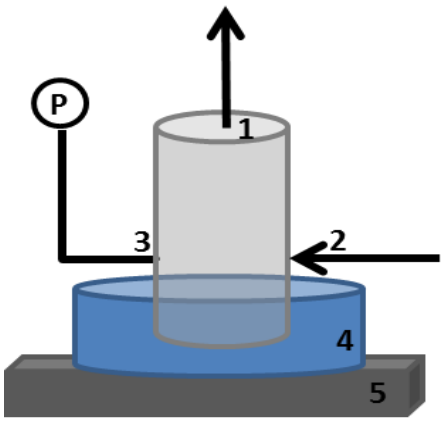

Figure 12 – Hydrotreatment reactor set-up.

The reactor was loaded with around 100 mg of MEL (previously heated to about 60ºC to decrease viscosity) and 20% catalyst by weight. Catalyst loading was done as quickly as possible to minimize surface oxidation. After equipment assembling and loading, security tests were performed, with the reactor being pressurized with nitrogen up to the required reaction pressure (around 60 bar) to verify the existence of leaks in the system. The installation was deemed acceptable when pressure drop did not overcome 3 bar over the course of 4h.

Following the safety tests, the equipment was flushed with nitrogen for 30 min, to ensure that no nitrogen remained in the system. The reactor was then slowly pressurized with hydrogen and heated to around 275ºC with stirring. The reaction was performed for 5h, with occasional hydrogen pulses to compensate the small pressure drop. Several reaction cycles were done in which the reactor was depressurized after a period of 5h and repressurized with hydrogen for a new 5h cycle. After this period the heating was turned off and the reactor was slowly depressurized.

After cooling, the equipment was washed with dichloromethane (DCM) for product and catalyst removal (a volume of around 20-30 mL was used). The resulting solution was centrifuged at 2500 rpm for a few seconds to promote catalyst deposition and filtered to remove any catalyst traces. The recovered catalyst was collected.

Finally, 1 µL of the remaining solution was injected in a GC system, in the conditions described in section 3.2.3.

4. Results and Discussion

4.1. MEL production

4.1.1 Experiments Overview

Figure 13 represents the overall operation sequence followed for experimental work. Inoculum was prepared in a shake flask. Several fermentations with glucose fed-batch were performed for production of MEL. Following extraction and solvent recovery, the isolated MEL was employed as substrate in two types of reactions: (i) transesterification (under both acidic and alkaline conditions) and (ii) hydrotreating.

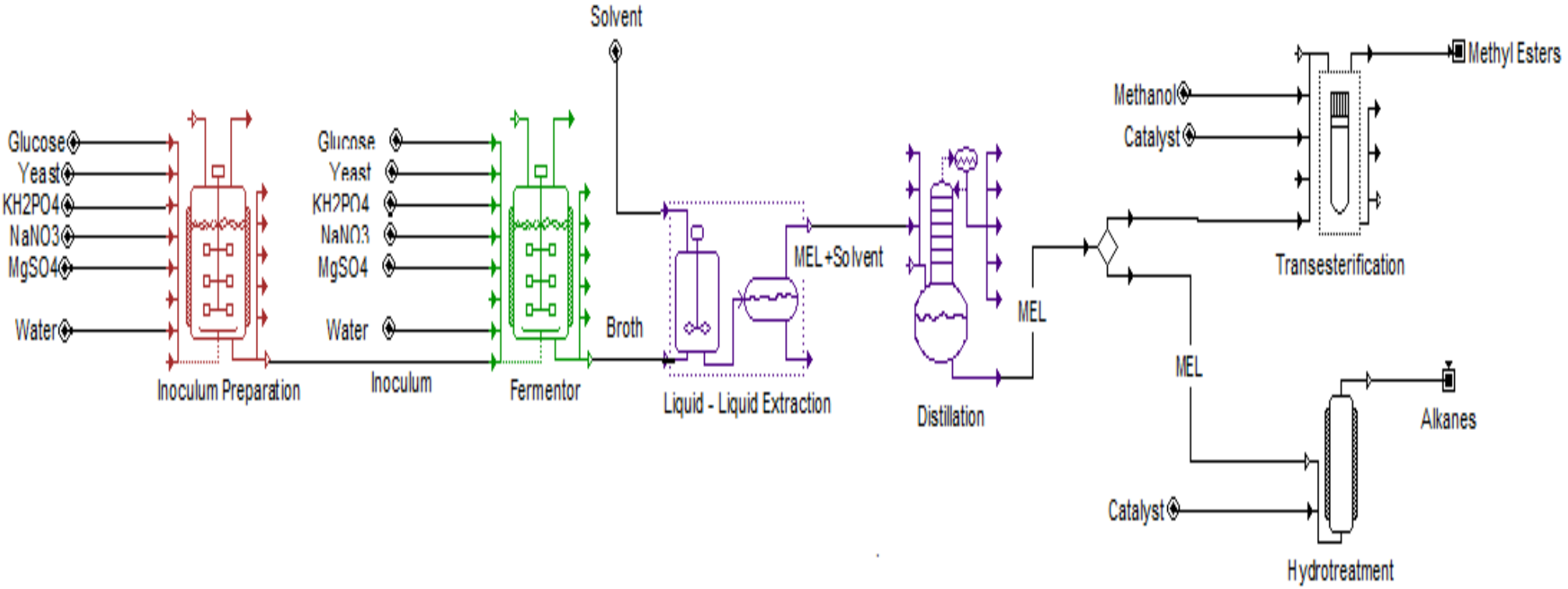

Figure 13 – Operation sequence for the experimental work performed**.**

4.1.2 MEL Production

Several fermentations were carried out with the aim of producing MEL via yeast conversion of glucose. The production of a sufficient amount of MEL to enable a series of further transesterification and hydrotreating reactions was critical for the experimental work presented.

A summary of the range of titres obtained in the several fermentations performed is expressed in Figure 14. As stated above, fermentations were performed with and without an extra glucose feed.

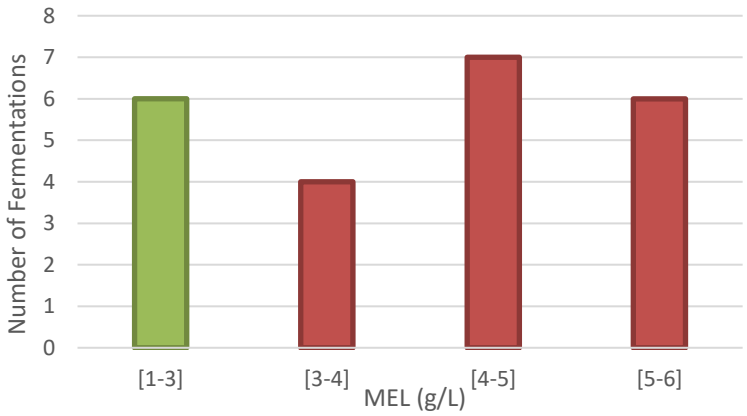

Figure 14 – MEL titres obtained (red represents a fed-batch fermentation at day 4 and green represents a fermentation without further sugar addition).

Initial fermentations resulted in lower MEL titres up to around 3 g/L. Following glucose addition at day 4, higher MEL titres were obtained in the range of 3 to 6. Under the same conditions (*M. antarcticus*, glucose as a carbon source, sodium and nitrate addition and a fed-batch at day 4) a maximum titre of 7.3 g/L was described. [2]

4.1.3 MEL and metabolites quantification

Biomass growth and glucose consumption were also evaluated. Figure 15 represents the biomass and glucose consumption profiles for a no-feed fermentation.

Figure 15 – Biomass growth and glucose consumption curves for a no-feed situation.

As shown by the previous figure both biomass growth and sugar consumption follow the expected profile; glucose is expected to be totally consumed by day 4/5 when an extra carbon source boost is not available. Biomass concentrations increases with time and is similar to the previously value reported for MEL production from *M. antarcticus* grown on D-glucose [2].

It should be noted that the initial glucose concentration is higher than the expected amount (40 g/L). In reality, Figure 15 represents total carbon source consumption, present not only in glucose but also in the yeast extract added.

A similar analysis for a fed-batch fermentation has been previously reported. [2] A similar profile in terms of biomass growth was observed, while glucose was continuously consumed until the end of the fermentation period.

MEL lipidic chain quantification is an essential parameter, especially considering the experimental work developed. A sample of fermentation broth from day 12 was collected and transesterified for methyl esters quantification as described in section 3.2.3. Fatty acid content is quantified in Table 3.

Table 3 – Fatty acid composition of MEL using glucose as substrate.

Fatty acid chain length	**MEL fatty acid content (%)**
C8:n	6.3%
C10:n	55.0%
C12:n	31.7%
Fatty acid chain length	**Long fatty acid content (%)**
C14:n	3.0%
C16:n	2.1%
C18:n	2.0%

The sample analysed comprises 7.1% of long chain esters (C14 to C18), unwanted due to higher chain length, but still in the range of value reported for MEL produced by P. *Antarctica* using glucose as a substrate [2]. The MEL used throughout the experiments reported in the next sections typically has a similar chain distribution to the one presented on Table 3. However, since several fermentations with different time lengths and downstream assays and using different solvent amounts were performed, the chain size distribution varies slightly and the longer fatty acid contain chains is relatively higher in MEL obtained from shorter fermentations (resulting in higher C14-C18 fatty acid chain content).

It should be noted that while this chain distribution was not analysed thorough the fermentation, a build-up in long chains is expected in the initial period of fermentation, which are then used and broken down to generate MEL (shorter length chains). The profile presented in Table 3 already presents a C8-C14 rich composition, since it represents a sample form day 12 of the fermentation period.

4.2. Transesterification

4.2.1 Catalytic ratio effect

Different reaction conditions were tested. Several sets of experiments were performed with the objective of defining ideal reaction conditions, namely (i) catalytic ratio (molar substrate/catalyst ratio), (ii) temperature, (iii) reaction time, and (iv) C (% wt), defined as:

$$C\ (wt\ \%) = \frac{m_{MEL}\ (g)}{m_{MEL}\ (g) + m_{Methanol}\ (g) + m_{Catalyst}\ (g)} \quad (3)$$

MEL transesterification reactions have only been reported for fatty acid chain quantification, under acid catalysis using acetyl chloride/methanol mixture [3]. In order to effectively determine the yield for the reactions performed, a reaction in standard conditions was done using an acetyl chloride/methanol mixture for 1h at 80ºC. This corresponds to a reaction previously validated in which the substrate is completely converted, with a yield of 100%.

In the first set of reactions, different catalytic ratios were tested for increasingly higher concentrations.

Table 4 and Table 5 refer to the experiments performed using alkaline and acid catalysts respectively. All reactions conditions used are expressed (catalyst, concentration, temperature, amount of substrate used, reaction yield and fatty acid content). Methanol was used for all reactions. Fatty acid content (%) represents the molar fraction of C14-C18 esters obtained in the reaction, which are driven for MEL contamination with compounds with longer lipidic chains roduced during the fermentation and also extracted by ethyl acetate. Note that the content of fatty acid varies with fermentation length and extraction conditions used.

Table 4 - Catalytic ratios tested for different concentrations for alkaline catalysis.

Nº	**Catalyst**	**C (% wt)**	**Cat/MEL**	**T (ºC)**	**t (h)**	**MEL (g)**	**Yield (%)**	**Fatty acid (%)**	**Combined Yield (%)**
1	CH_3NaO	5	6	80	4	0.035	56.6%	13.8%	56.5%
2	CH_3NaO	5	8	80	4	0.031	64.6%	16.9%	34.1%
3	CH_3NaO	5	20	80	4	0.031	39.3%	16.2%	43.2%
4	CH_3NaO	10	6	80	4	0.032	54.8%	36.6%	35.1%
5	CH_3NaO	10	8	80	4	0.028	54.3%	8.8%	43.6%
6	CH_3NaO	10	20	80	4	0.035	17.5%	0%	69.4%
7	CH_3NaO	10	35	80	4	0.032	39.3%	0%	62.7%
8	CH_3NaO	20	20	80	4	0.034	10.2%	0%	67.0%
11	AcCl	.	-	80	1	0.031	100.0%	2.7%	100%

Table 5 – Catalytic ratios tested for different concentrations for acid catalysis.

Nº	**Catalyst**	**C (% wt)**	**Cat/MEL**	**T (ºC)**	**t (h)**	**MEL (g)**	**Yield (%)**	**Fatty acid (%)**	**Combined Yield (%)**
1	H_2SO_4	5	8	80	4	0.038	94.3%	4.1%	86.9%
2	H_2SO_4	5	20	80	4	0.035	90.4%	4.2%	83.8%
3	H_2SO_4	5	35	80	4	0.039	88.1%	4.0%	81.4%
4	H_2SO_4	10	8	80	4	0.034	82.4%	4.2%	77.0%
5	H_2SO_4	10	20	80	4	0.034	78.5%	4.1%	73.5%
6	H_2SO_4	10	35	80	4	0.038	88.0%	4.5%	82.3%
7	H_2SO_4	20	8	80	4	0.038	79.7%	3.9%	74.2%
8	H_2SO_4	20	20	80	4	0.033	46.3%	5.8%	48.9%
9	H_2SO_4	20	35	80	4	0.046	83.7%	3.7%	77.3%
10	AcCl	-	-	80	1	0,030	100%	9.2%	100 %

The reaction yields obtained for the different catalytic ratios tested are summarized in Figure 16 (alkaline catalysis scenario) and Figure 17 (acid catalysis scenario).

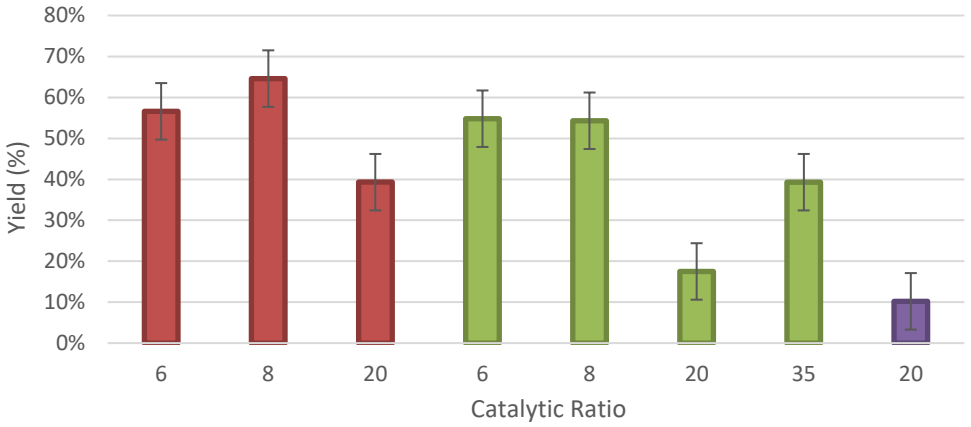

Figure 16 – Alkaline catalysed transesterification yields obtained for different catalytic ratios (red represents catalytic ratios for 5 wt% MEL, green corresponds to 10 wt% MEL and purple refers to 20 wt% MEL).

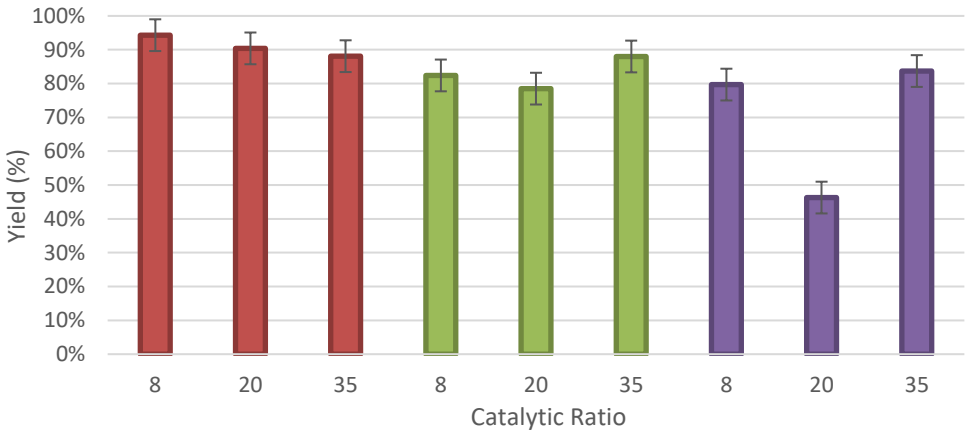

Figure 17 - Acid catalysed transesterification yields obtained for different catalytic ratios (red represents catalytic ratios for 5% wt MEL, green corresponds to 10% wt MEL and purple refers to 20 wt% MEL).

From the experimental data obtained, sulfuric acid catalysed transesterifications result in higher yields for all concentrations and catalytic ratios tested. Lower MEL concentrations seem to favour the reaction in both scenarios, as such conditions correspond to higher ratios of methanol to MEL, which can favour reaction forward and decrease solution viscosity which can also contribute to more efficient reactions.

In the alkaline catalysed-reactions, a catalytic ratio of 8 for 5 wt% MEL contents (reaction 2) originates the higher yield (65%). Lower catalytic ratios of 6 and 8 lead to better yields.

Higher molar catalytic rations than 20 for 20 wt % MEL were also tested, but no phase separation following hexane addition occurred.

In the acid catalysed-reactions, a catalytic ratio of 8 for a 5 wt% MEL (reaction 1) represents the best obtained yield (65%). Lower concentrations and MEL/catalytic ratios resulted in higher yields.

While a direct comparison with literature values is not possible, soybean oil transesterifications with H_2SO_4, have been reported to completely convert the vegetable oil (> 99%). [48]

4.2.2 Temperature effect

The previous reactions were performed at 80 ºC. However, transesterifications reactions are reported to occur in a range of temperatures. For each scenario (acid and alkaline catalysis), one of the previous conditions was chosen and three different temperatures (40, 60 and 80ºC) were tested (Table 6).

Table 6 – Temperature effect for acid and alkaline catalysis.

Nº	Catalyst	C (% wt)	Cat/MEL	T (ºC)	t (h)	MEL (g)	Yield (%)	Fatty acid (%)	Combined Yield (%)
1	CH_3NaO	5	20	40	4	0.031	34.6%	1.3%	20.0%
2	CH_3NaO	5	20	60	4	0.032	58.6%	0.0%	58.6%
3	CH_3NaO	5	20	80	4	0.031	39.3%	16.2%	43.2%
4	H_2SO_4	10	8	40	4	0.032	64.8%	5.3%	63.6%
5	H_2SO_4	10	8	60	4	0.035	95.9%	2.3%	85.1%
6	H_2SO_4	10	8	80	4	0.034	82.4%	8.9%	77.0%
7	AcCl	-	-	80	1	0.030	100%	9.2%	100%

The previous values are presented in Figure 18.

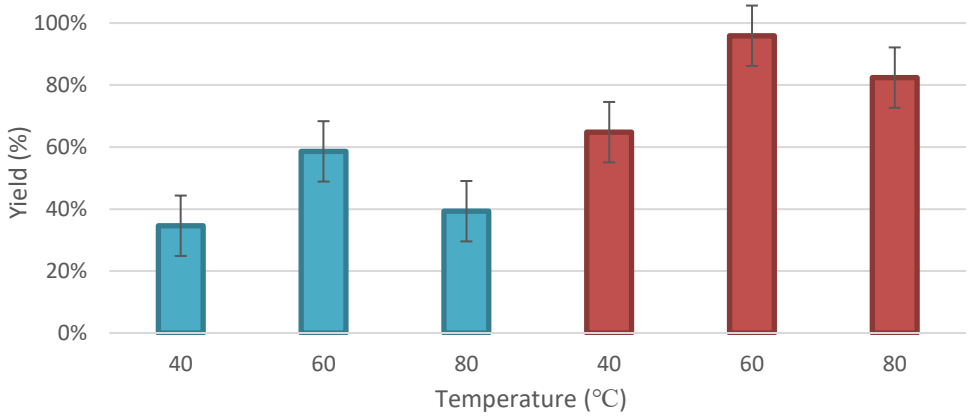

Figure 18 – Reaction temperature effect (blue represents an alkaline catalysed reaction, with a catalytic ratio of 20 and 5 wt% MEL; red corresponds to an acid catalysed reaction with a catalytic ratio of 8 and C= 10 wt%).

In both scenarios, a temperature decrease to 60ºC results in a significant yield increase, while 40ºC results in worse results for both scenarios. Studies reporting sodium methoxide catalysed transesterifications at 60ºC have been reported as the preferable reaction temperature for different vegetable oils sources. [39, 49]. Acid catalysed reactions have also used a temperature of 60ºC for biodiesel production [50].

4.2.3 Reaction time effect

Finally, reaction time was tested for both situations. For the same reaction conditions (concentration and catalytic ratio) presented in section 4.2.2, three different temperatures (40 ºC, 60 ºC and 80ºC) were tested (Table 7 and Table 8).

Table 7 – Reaction time for alkaline catalysis.

Nº	Catalyst	C (% wt)	Cat/MEL	T (ºC)	t (h)	MEL (g)	Yield (%)	Fatty acid (%)	Combined Yield (%)
1	CH_3NaO	5	8	80	4	0.028	54.3%	9%	43.6%
2	CH_3NaO	5	8	80	16	0.029	49.2%	15%	22.1%
3	CH_3NaO	5	8	80	24	0.029	48.8%	14%	19.6%

Table 8 - Reaction time for acid catalysis.

Nº	Catalyst	C (% wt)	Cat/MEL	T (ºC)	t (h)	MEL (g)	Yield (%)	Fatty acid (%)	Combined Yield (%)
1	H_2S0_4	10	8	80	4	0.034	82.4%	4.2%	77.0%
2	H_2S0_4	10	8	80	16	0.032	84.9%	8.7%	74.2%
3	H_2S0_4	10	8	80	24	0.037	95.0%	7.5%	87.4%

The previous results are summarized in Figure 19.

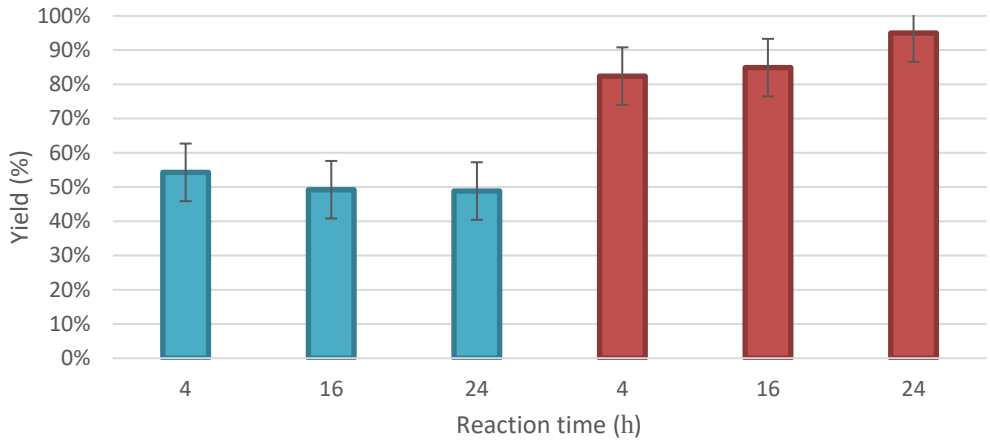

Figure 19 - Reaction time effect (blue represents an alkaline catalysed reaction, with a catalytic ratio of 8 and C= 5 wt% MEL; red corresponds to an acid catalysed reaction with a catalytic ratio of 8 and C= 10 wt%).

For an alkaline catalysed reaction, 4h seems to be a sufficient reaction time, since an increase in reaction time does not result in a higher yield over 54.3%. The same scenario is not verified in Table 8. As expected, acid catalysed reaction requires further reaction time to fully convert the substrate. Increasing reaction time lead to a significant increase in yield from 82.4% to 95% at 4 and 24 hours respectively. Under similar conditions, soybean oil methanolysis, in the presence of H_2SO_4, takes 50 h to reach complete conversion (> 99%) of the vegetable oil. [49]

Considering all the experimental results obtained, the best conditions for both reactions are summarized in Table 9.

Table 9 – Best conditions for MEL alkaline and acid catalysed transesterifications.

Catalyst	**C (% wt)**	**Cat/MEL**	**T (ºC)**	**t (h)**
CH_3NaO	5	8	60	4
H_2SO_4	5	8	60	24

4.3. Hydrotreatment

4.3.1 Product prediction

Two experiments at different temperatures, 170ºC and 238ºC were performed. The experiment at 170ºC included only a single reaction cycle of 5 hours, whereas for the second experiment, a higher temperature of 238ºC included 4 reaction cycles of 5 hours each (Table 10). Both experiments used the same catalyst (7% Ni / SAPO-11), MEL load (around 100 mg), Catalyst ratio (around 20% wt) and H_2 pressure (~60 bar).

Table 10 – Hydrotreating reaction conditions.

Reaction	MEL (mg)	Catalyst (% wt)	T (ºC)	P (bar)	Cycle time (h)	Number of cycles
1	123.4	21%	170	~60	5	1
2	103.8	19%	238	~60	5	4

The resulting reaction product was extracted with about 20 mL of dichloromethane and injected in a GC system, in the conditions described in section 3.1.3. No peaks were visible. The 20 mL solution was then concentrated to around 1 mL and a new injection was performed for GC analysis. The areas and retention times obtained for each experiment are expressed in Table 11.

Table 11 – Hydrotreating product results.

Reaction 1	
R_t (min)	**Area**
1.33	5152543
4.00	1292
4.15	11972
4.76	966
4.91	576
5.46	6527
5.59	1820
5.64	7
5.99	28
6.13	7791
6.18	3536
6.25	8694

Reaction 2	
R_t (min)	**Area**
1.33	5144505
3.26	7060
3.90	10152
4.01	16119
4.09	962
4.16	62073
4.31	6352
4.69	2915
4.76	11827
4.91	17984
4.97	1574
5.04	217
5.35	8240
5.47	32578
5.60	31876
5.65	12006
5.90	245
6.00	13757
6.14	45142
6.19	18946
6.26	41844
6.39	1802
6.49	2932
6.57	368
6.70	130
6.81	3252
6.84	1132
6.96	1545

In an effort to identify some of the peaks presented and identify the reaction product, several possible reaction compounds (alkanes and alcohols) of known chain length were injected in the GC and analysed using the same conditions and column (Table 12).

Table 12 – Retention time in GC.

Compound	Chain length	R_T (min)
Alcohols	C8	2.04
	C10	2.86
	C12	3.81
Alkanes	C8	1.45
	C10	1.81
	C12	2.51
	C16	4.31
	C18	5.05

For ideal jetfuel properties, short range alkanes are required (C8-C14). However, under the reaction conditions tested, no short chain alkanes were identified.

Analyzing Figure 20 it is possible to identify peaks (with retention times of 4.31 and 5.04 min respectively) that may correspond to C16 and C18 length alkanes, matching the retention time presented in Table 12. However, a great number of unidentified compounds with higher boiling points are also present.

Since direct carboxylic acid injection is not possible in the GC column used, an analysis based on the boiling points of possible reactions products was considered. Figure 20 presents the boiling points for C8-C18 length of alkanes, alcohols and carboxylic acids.

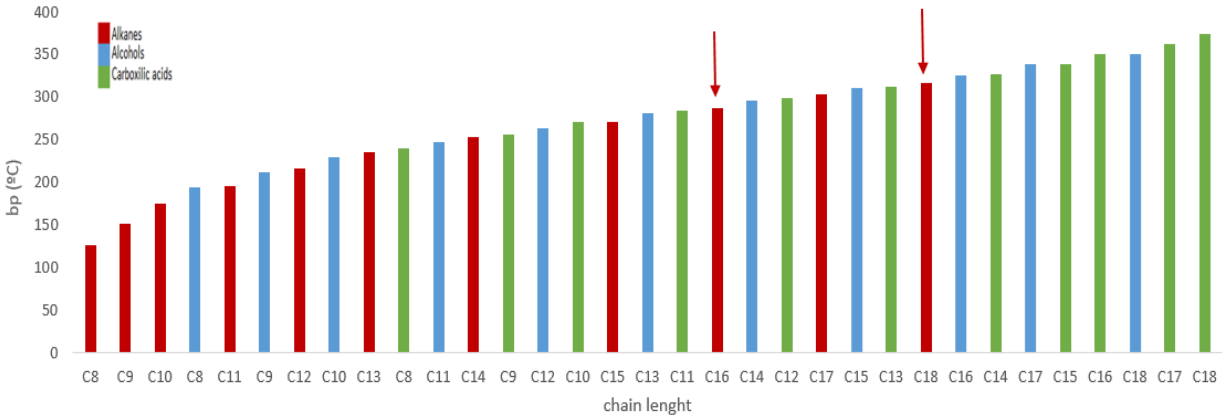

Figure 20 – Boiling points from C8-C18 range alkanes, alcohols and carboxylic acids (red refers to alkanes, blue corresponds to alcohols and green represents carboxylic acids).

Based on the boiling points presented, the reaction product may contain higher weight alcohols (C16 or higher) or carboxylic acids (C14 and higher).

While product identification is not possible without further analytical methods, the primary objective of converting MEL lipidic chains into short chain alkanes was not successful in the experimental conditions tested. However, some additional comments can be made:

(i) The more stringent conditions (temperature and number of cycles) used in reaction 2, definitely resulted in a higher number of products detectable by GC with higher areas

(ii) A 12 mg sample of the post reaction mixture crude obtained after hydrotreating reaction 2 and DCM evaporation was submitted to transesterification and further analysed by GC. Typical C8, C10 and C18 chain length methyl esters peaks were identified. This suggests two situations: (a) some unreacted MEL lipidic chains remained following hydrotreatment and were effectively converted to esters; or (b) the hydrotreating reaction yielded carboxylic acids of varying chain length, that following transesterification were converted to the same methyl esters from situation (a);

(iii) The pressure and temperature intensive reaction conditions (or even the acidic properties of the catalyst) may have may have broken down the glucose moiety and generated higher chain boiling point compounds;

(iv) The experimental set-up was inadequate for the experiment, i.e, insufficient reaction temperature or inefficient heat transfer to the reaction vessel or loss of product and/or catalyst during loading. Note that compounds in the gaseous fraction were not quantified and volatile compounds could be lost in vessel depressurization;

(v) Inadequate catalyst choice. While a 7% Ni/SAPO-11 is a low acidic catalyst, reported to favour HDO and promote oxygen removal in palmitic oil, no extensive research exists for a wider range of bio based substrates. However, under similar reaction conditions, a soybean oil hydrotreatment reaction under a Ni/SAPO-11 with 8 % metal loading, at 340^{o}C resulted in 100% oil conversion with a 99% selectivity of C7 to C14 alkanes [51].

4.4. Process Simulation

4.4.1 Objective and Considerations

A process simulation using SuperPro Designer® was performed to evaluate the feasibility of a biofuel production process from MEL (regarding process production cost and energy requirements). SuperPro Designer® was used to support the decision of which equipment are required and establish mass and component flows along the process considering the different

experimental and assumed yields and production times. The cost and energy analyse was then refined considering different cost and operation scenarios.

The production goal of 1000 units/year was considered as a small demonstration project. To meet this target, a total of 20 fermentations are projected to occur on a yearly basis at a fermentation working volume of 7.5 m^3, considering 14 days full batch fermentation cycle with a titre of 7 g/L of product and plant operations interruption over 1 month a year for cleaning, holidays and equipment maintenance. Glucose and nitrate were assumed to be the only carbon and nitrogen sources for the aerobic fermentation. Upstream steps related with lignocellulose biomass deconstruction, hydrolyses into fermentable sugars and eventual stream detoxification were not considered in this analysis. Downstream separation was assumed to be a liquid-liquid extraction with ethyl acetate of the product from the fermentation followed by solvent exchange.

MEL does not exist in the simulator database and an accurate thermodynamic representation of the compound would require the knowledge of a number of physical and chemical properties (boiling point, density, heat capacity, critical temperature and pressure, among others). As such, for the transesterification steps, soybean oil was used as the source component for MEL properties simulation.

Four different sections were considered: (i) the upstream section that includes reagents storage and sterilization; (ii) fermentation section; (iii) downstream processing, including product isolation and recovery and (iv) reaction section, where a typical soybean oil transesterification reaction for biodiesel production was simulated to mimic MEL transesterification, but corrected with the catalytic conditions used (substrate concentrations, catalyst molar ratios, temperatures used) and yields determined experimentally.

4.4.2 Process Simulation by sections

4.4.2.1 Upstream processing and Fermentation simulation

The upstream section includes both reagents storage and sterilization. Tank T-101 contains water, T-102 stores glucose and T-103 already has a mixture of the mineral medium (yeast extract, KH_2PO_4, $MgSO_4$, $NaNO_3$) required for the fermentation. These three tanks already possess the required concentrations for all solutions and, following sterilization, directly feed the fermenter (Figure 21).

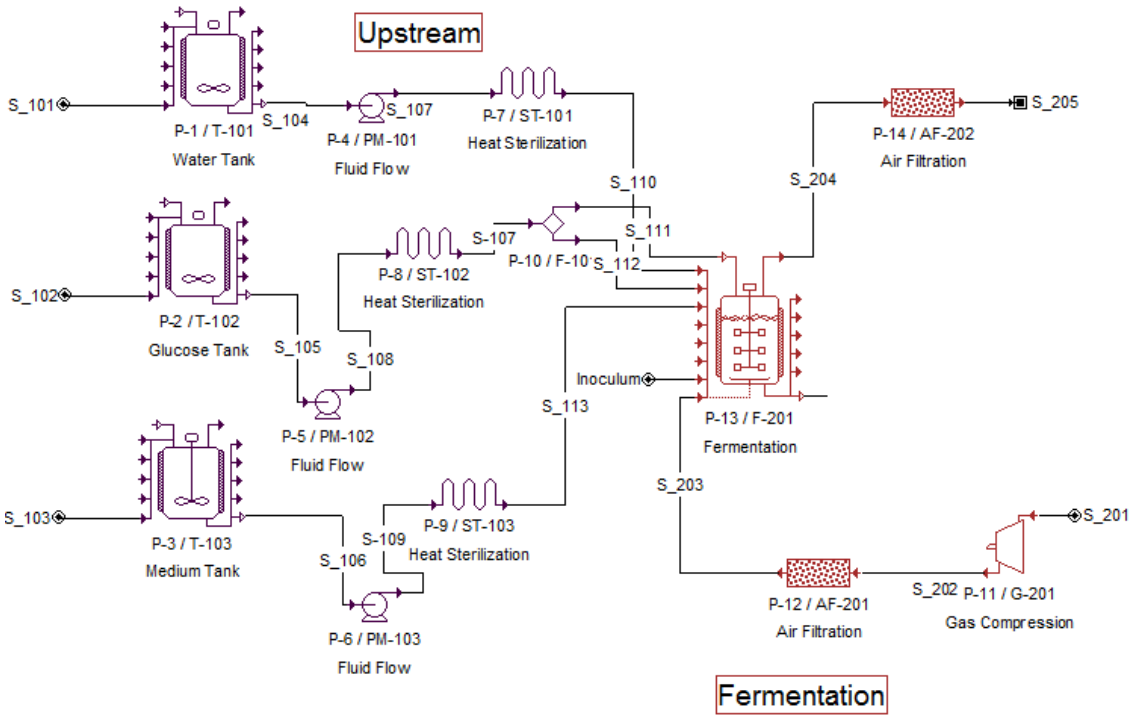

Figure 21 – Process Upstream and Fermentation sections as represented in SuperPro Designer®.

Heat sterilization was done for 5h and 120ºC. An exit temperature of 28ºC was defined (fermentation temperature), to avoid a further heating step in the fermenter. Due to limitations regarding equipment number (a total of 25 units can be used in the version of the software) inoculum preparation was not considered in this simulation, but it was introduced directly in the fermenter.

Air filters were placed upstream and downstream of the fermenter. The fermenter was designed with a working volume of 7.5 m^3 and is expected to produce around 52 units/batch of product, considering a yield of 7 g/L.

A fermentation based on stoichiometry was chosen to model the fermenter. The reaction stoichiometric coefficients were adjusted to allow a yield of 7 g/L of product. A fed-batch strategy was implemented and an extra glucose stream (S_111) was added to mimic the experimental fermentations performed.

Since they do not interfere directly with the reaction stoichiometry introduced, yeast extract, $MgSO_4$ and KH_2SO_4 also exit the fermenter, along with the biomass and the fermentation products, remaining with the product until separation in the final downstream purification stage.

All the carbon dioxide formed in the reaction, along with the remaining air exit the fermenter through a vent stream (S_204), that goes thorough an air filter (AF-201) before being discharged.

4.4.2.2 Downstream process simulation

A downstream processing section was implemented in the simulator (Figure 22). After the fermentation process, a centrifugation step is performed to reduce the amount of biomass present in the fermentation broth by 50% (remaining in the solid rich stream S_304). Following centrifugation, a multi-stage evaporation unit was implemented (P-17) to reduce the water present in the fermentation broth by 75%, in an effort the reduce the amount of solvent required.

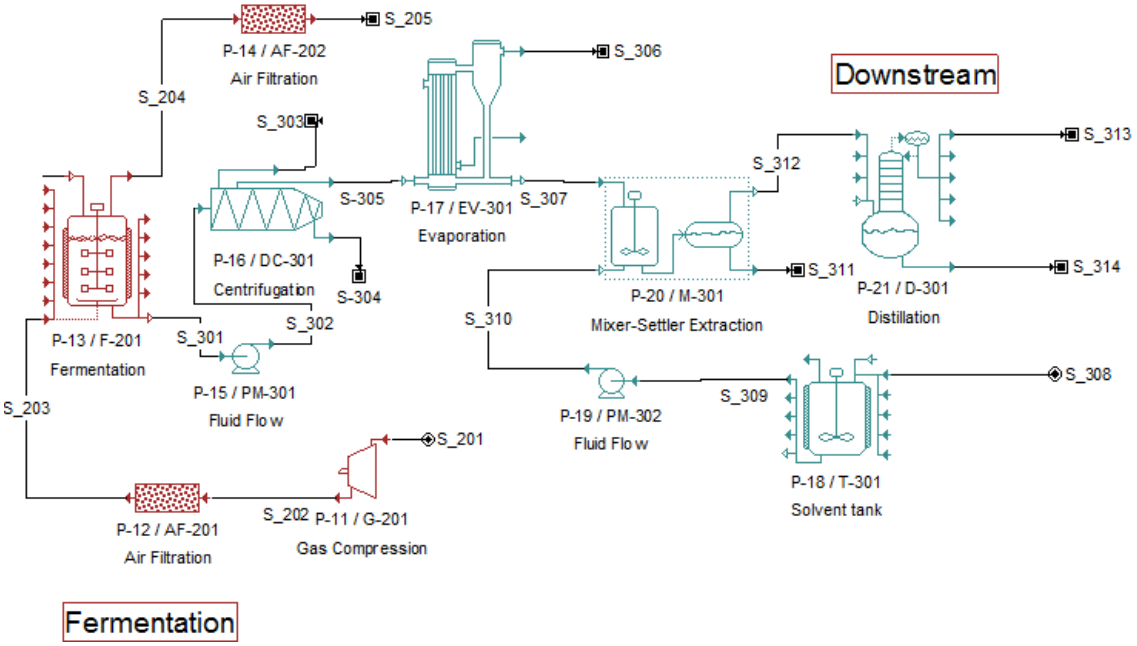

Figure 22 - Process Fermentation and Downstream sections as represented in SuperPro Designer®.

The mixture then goes to a mixer-settler unit where MEL is extracted with ethyl acetate. A single extraction was considered with an amount of solvent equal to twice (volume based) the concentrated aqueous fermentation broth, for process simplification. Finally, a distillation at 80ºC is performed for solvent recovery and MEL isolation. A total of 50 units of isolated product with 98% purity was estimated.

The completed fermentation section is represented in Figure 26.

4.4.2.3 Reaction Section

A typical transesterification reaction for biodiesel production was simulated in a CSTR reactor. An acid catalysed reaction was considered, with a reaction yield of 95% (the maximum experimental yield obtained for MEL transesterification under acid catalysis). A stoichiometric reaction was defined in the simulator and a molar ratio of oil:alcohol of 1:3 (Figure 5) was used to model the reaction.

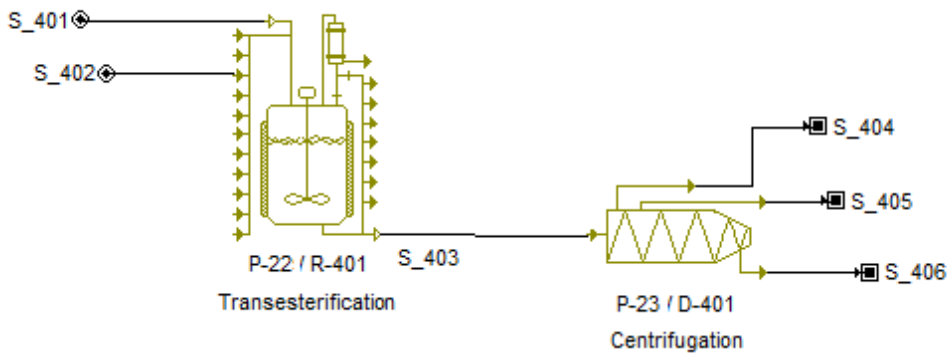

Figure 23 – Process Reaction section as represented in SuperPro Designer®.

A substrate rich stream (S_401) enters the reactor (P-22) along with a stream containing methanol and sulfuric acid. A reaction time of 24h was considered. Following the reaction, a centrifugation step (P-23) was performed to remove glycerol and create a fuel rich stream (S_404), containing 49.1 units of product.

It should be noted that a molar based yield of 95% (determined in section 4.2) was used to model the reactor. As such, a correction to the amount of fuel produced was introduced according to equation 4.

$$mass\ of\ fuel\ produced = mass\ of\ MEL\ produced \times \frac{MW\ (Fuel)}{MW\ (MEL)} \times Yield \qquad (4)$$

Considering a production of 51.5 units of MEL per batch, a total of 21.7 units of fuel are expected to be obtained (equation 5).

$$mass\ of\ fuel\ produced = 51.5\ units \times \frac{296}{667} \times 0.95 = 21.7\ units \qquad (5)$$

4.4.3 Cost and Energy analysis

4.4.3.1 Equipment

A total of 23 equipment were implemented in the process (21 in the MEL production section and 2 in the reaction section). Carbon steel (CS) was defined as the construction material for most equipment. Table 13 (fermentation section) and Table 14 (reaction section) present the purchase cost discriminated by equipment defined by the simulator (purchase cost A). To verify the purchase cost defined by the simulator, a second cost (purchase cost B) was estimated with the help of a cost estimation tool for equipment by Matches. [52].

Table 13 – Equipment list, identification and purchase cost for the fermentation section.

Process	ID	Equipment	Material	Purchase cost A ($)	Purchase cost B ($)
P-1	T-101	Tank	CS	91 000 $	21 110 $
P-2	T-102	Tank	CS	176 000 $	10 700 $
P-3	T-103	Tank	CS	134 000 $	3 300 $
P-4	PM-101	Pump	CS	4 000 $	9 900 $
P-5	PM-102	Pump	CS	4 000 $	9 900 $
P-6	PM-103	Pump	CS	4 000 $	9 900 $
P-7	ST-101	Heat Sterilization	CS	181 000 $	91 300 $
P-8	ST-102	Heat Sterilization	CS	256 000 $	61 110 $
P-9	ST-103	Heat Sterilization	SS316	141 000 $	30 300 $
P-10	F-101	Flow Splitter	SS316	-	-
P-11	G-201	Compressor	SS316	634 000 $	231 000 $
P-12	AF-201	Air Filter	SS316	138 000 $	
P-13	F-201	Fermenter	CS	386 000 $	55 000 $
P-14	AF-202	Air Filter	CS	265 000 $	
P-15	PM-301	Pump	CS	4 000 $	9 900 $
P-16	DC-301	Decanter	CS	288 000 $	120 000 $
P-17	EV-301	Evaporator	CS	121 000 $	130 000 $
P-18	T-301	Tank	SS316	100 000 $	9 900 $
P-19	PM-302	Pump	CS	4 000 $	9 900 $
P-20	M-301	Mixer-Settler	CS	11 000 $	
P-21	D-301	Distillation Tower	CS	302 000 $	80 000 $

Table 14 - Equipment list, identification and purchase cost for the reaction section ($).

Process	ID	Equipment	Material	Purchase cost ($)	Purchase cost ($)
P-24	R-401	Stirred Reactor	CS	180 000 $	5 000 $
P-25	D-401	Decanter	CS	202 000 $	14 300 $

The values obtained from the estimation tool largely differ from the values presented by the software. The estimation tool requires, besides the construction material, the input of some equipment specific parameters that SuperPro Designer® does not specify. Example include type of centrifugal pump and discharge pipe diameter for pumps; type of roof in storage tanks, type of filter, among many others. As such, an accurate equipment estimation cost in not possible. The most expensive case scenario was first simulated and the cost defined by the simulator was chosen for all the calculations performed. Since the software reports values of 2016, no price inflations were considered.

However, to reach a more accurate equipment cost estimation, Lang factors (Table 32) that take into account direct and indirect costs must be introduced and considered individually. The direct-cost items that are incurred in the construction of a plant in addition to the cost of equipment include equipment erection, piping, power, instruments, among others. Indirect costs generally englobe design and engineering costs and contractor's fees [52].

Table 15 – Fixed capital estimation.

Section	**Purchase cost ($)**	**Fixed Capital ($)**	**Depreciation ($/year)**
Fermentation	3 244 000 $	8 580 380 $	858 380 $
Reaction	382 000 $	840 400 $	84 040 $
	Total	9 420 780 $	
	$/year	942 780 $	942 780 $

Considering the Capex required for the production of 1000 units of MEL is about 8.6 M$, considering a depreciation over 10 years, that represents an allocated value of 858 k$. Considering the additional equipment required for the reaction section a total Capex required for the production of 420 units of fuel is about 9.43 M$, representing a depreciated annual value of 943 k$.

4.4.3.2 Labour

The software provides the amount of work hours required per process. A total of 10 workers were considered and a work-force distribution by equipment was defined. A basic salary of 1200 $ was stipulated. The amount of work hours and cost by equipment is expressed in Table 16.

Table 16 – Labour hours and associated cost per batch and per year.

ID	Equipment	Labour (hours/batch)	Operators Required	Cost ($) /batch
T-101	Tank	31.53	0.25	63.06 $
T-102	Tank	21.67	0.25	43.34 $
T-103	Tank	4.64	0.25	9.28 $
PM-101	Pump	0.33	0.25	0.66 $
PM-102	Pump	0.33	0.25	0.66 $
PM-103	Pump	0.33	0.25	0.66 $
ST-101	Heat Sterilization	7.88	0.25	15.76 $
ST-102	Heat Sterilization	3.35	0.25	6.70 $
ST-103	Heat Sterilization	3.33	0.25	6.66 $
F-101	Flow Splitter	-	-	-
G-201	Compressor	0.33	0.5	1.32 $
AF-201	Air Filter	0.02	0.25	0.04 $
F-201	Fermenter	605.16	1	4 841.28 $
AF-202	Air Filter	0.02	0.25	0.04 $
PM-301	Pump	0.33	0.25	0.66 $
DC-301	Decanter	6.67	0.75	40.02 $
EV-301	Evaporator	10	0.75	60.00 $
T-301	Tank	61.87	0.25	123.74 $
PM-302	Pump	0.33	0.25	0.66 $
M-301	Mixer-Settler	10	0.5	40.00 $
D-301	Distillation Tower	21.94	1	175.52 $
R-401	Stirred Reactor	4	1.5	48.00 $
D-401	Decanter	6.67	0.5	26.68 $

4.4.3.3 Raw Materials

The software does not possess an accurate cost of all the materials used. As such, the purchase cost of all materials was taken directly from manufactures (Table 31).

The total amount of raw materials and associated cost considered are summarized in Table 17. In all the following tables the annual cost was determined by multiplying the cost/batch by 20 (the number of projected fermentations).

Table 17 – Raw materials required and associated cost.

Material	Material Cost ($/kg)	kg/batch	Cost/batch ($)
Air	0 $	73504.6	0 $
Biomass	0 $	787.5	0 $
Ethyl Acetate	76.7 $	5418.4	415 556 $
Glucose	10 $	600	5 976 $

KH_2PO_4	113.6 $	2.3	256 $
Magnesium Sulphate	20.6 $	2.3	46 $
$NaNO_3$	29.1 $	22.5	654 $
Water	0.1 $	6187.7	61.88 $
Yeast Extract	193 $	7.5	1 448 $
Methanol	20.7 $	88.96	1 840 $
Sulfuric Acid	166.9 $	0.14	23.4 $

4.4.3.4 Energy and Power

A total of four different utilities are used to heat and cool process streams. Table 18 presents the total amount required.

Table 18 – Amount and cost of utilities.

Utility	**Utility Cost ($/kg)**	**ton/batch**	**Cost/batch ($)**
Steam	12.00	5.1	60 688 $
Cooling Water	0.05	128.9	6 443 $
Chilled Water	0.40	1338.3	535 337 $
NaCl Brine	0.25	7.6	1 897 $

Table 19 discriminates the type of utility and energy requirements by equipment in both cooling and heating situations.

Table 19 – Energy requirements and utilities employed in each equipment.

Process	**ID**	**Equipment**	**Utility**	**Function**	**Q (kcal/h)**	**Process Time (h)**	**Q (Mcal) batch**
P-7	ST-101	Heat Sterilization	Steam	heating	2836	5	7.1
			NaCl Brine	cooling	1988	5	5.0
P-8	ST-102	Heat Sterilization	Steam	heating	8199	5	20.5
			NaCl Brine	cooling	5748	5	14.4
P-9	ST-103	Heat Sterilization	Steam	heating	5177	2	5.2
			NaCl Brine	cooling	4735	2	4.7
P-11	G-201	Compressor	Cooling Water	cooling	160994	2	322.0
			Chilled Water	cooling	3983	4	15.9
P-17	ST-104	Evaporation	Steam	heating	41121	3	61.7
P-15	G-202	Fermenter	Cooling Water	cooling	357696	3	536.5
P-21	D-301	Distillation Tower	Steam	heating	-	6	514.6
	D-301		Cooling Water	cooling	-	6	53.4
						Total:	1561

The cost of the different utilities and the respective temperature range are expressed in Table 33. Power consumption is described in Table 20 (fermentation section) and Table 21 (reaction section). A value of 0.2$/kWh of electricity was considered for cost estimation.

Table 20 – Process power consumption and cost for the fermentation section.

ID	Equipment	Potency/batch (kWh)	Potency/year (kWh)	Cost ($/kWh)	Cost/batch ($)
P-4	Pump	0.1	2.2	0.2	0 $
P-5	Pump	0.1	2.8	0.2	0 $
P-6	Pump	0.01	0.4	0.2	0 $
P-11	Compressor	1864.6	37292.4	0.2	373 $
P-13	Fermenter	7566.3	151326	0.2	1 513 $
P-15	Pump	0.3	5.2	0.2	0 $
P-16	Decanter	65.7	1313.4	0.2	13 $
P-19	Pump	0.2	3.4	0.2	0 $
P-20	Mixer-Settler	3.3	65.2	0.2	1 $
Extra*	-	2375.13	47502.6	0.2	455 $

Note: * represents unregistered equipment and general load.

Table 21 - Process power consumption and cost for the reaction section.

ID	Equipment	Potency/batch (kWh)	Potency/year (kWh)	Cost ($/kWh)	Cost/batch ($)	Cost/year ($)
P-24	Reactor	0.01	0.2	0.2	0.0 $	0.04 $
P-25	Decanter	0.93	18.6	0.2	0.2 $	3.7 $
Extra*	-	0.23	4.6	0.2	0.0 $	0.9 $
	Total	0.94	18.8	-	0.2 $	3.8 $

Note: * represents unregistered equipment and general load.

A summary of the previous values including raw materials, labour, utilities, power and equipment are presented in Table 22.

Table 22 – Contribution /%) of raw materials, labour, utilities, power and equipment for MEL and fuel production cost.

%	MEL	Fuel
Raw material	39.3%	39.2%
Labour	0.5%	0.5%
Utilities	56.0%	55.7%
Power	0.2%	0.2%
Equipment	4.0%	4.3%

Considering the total production cost required per batch it is possible to estimate a production cost for MEL (Table 23).

Table 23 –MEL and fuel production cost.

	Production/batch (unit)	**Cost ($/unit) x 10^3**
MEL	-50	20.95 $
Fuel	-20	49.98 $

4.4.3.5 Scenarios

Analysing Table 22, it is possible to verify that utilities and raw material cost represent the major contributors (85%) for the total production cost (Figure 24).

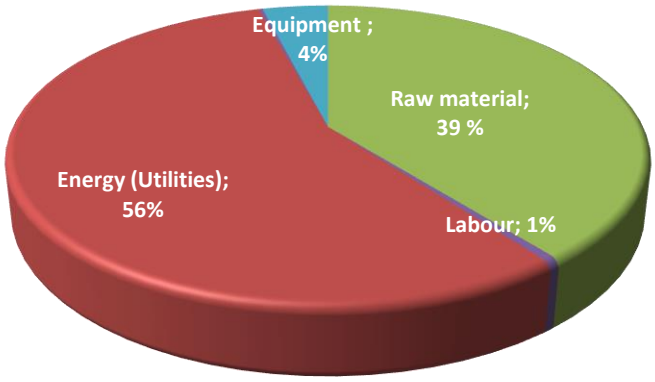

Figure 24 – Contribution for production cost (red represents energy spent on utilities, green represents raw materials, blue represents equipment and purple indicates labour costs).

With the purpose of reducing production costs presented on Table 23, several scenarios can be discussed (Table 24). Scenario 1 describes the production cost and conditions presented in the previous section.

Table 24 - Different scenarios considered.

Scenario 2	Scenario 1 +	2.1 Cold utilities removal
		2.2 Price of Raw materials = 1/3
Scenario 3	Scenario 2 +	3.1.1 Solvent recycle with 5% loss
		3.1.2 Solvent recycle with 10% loss
		3.2 Price of Steam =1/2
Scenario 4	Scenario 3 +	4.1.1 MEL titre =10 g/L
		4.1.2 MEL titre = 14 g/L

Scenario 2 considers the possibility of eliminating cold utilities. Due to the high volume of materials that require sterilization, a large amount of utilities is required per batch. Furthermore, a high temperature reduction is required (from 120ºC to 28ºC) and since cooling water cannot be used due to the target temperature required, costly alternatives must be used. As such, since the simulations refers to a batch production process, a possibility would be to wait until the sterilized materials and air cool up on their own, thus eliminating the need for cold utilities, reducing the production cost significantly.

Furthermore, reactants cost was taken from Sigma-Aldrich. An accurate pricing of the reactants would result in lower prices, especially considering he high quantities required.

Scenario 2 implements these changes resulting in a substantial reduction in the production cost (Table 25).

Table 25 – Production cost reduction for scenario 2.

	Production Cost Reduction (%)
MEL	77 %
Fuel	76 %

Scenario 2 allows a production cost reduction for fuel of 76%. The removal of cold utilities (2.1) contributes with 50% and the reduction in the raw materials price (2.2) with around 26%.

While this scenario already reduces production cost significantly, a third hypothesis was also tested. The extraction process requires the use of large amounts of solvent per batch (around 5.4 m^3). Following distillation for product recovery, the liquid condensate of the column can be recollected and reintroduced directly into the mixer-settler or saved in an extra tank for future usage. Scenario 3 includes two situations, considering both a 5% (scenario 3.1) and 10% (scenario 3.2) of solvent loss in the recycle process.

Even considering the price reduction of scenario 3.1, another hypothesis can be studied. The cost of steam can also be reduced, possibly by reapplying lower pressure steam resulting from nearby production processes. Scenario 3.3 considers the reduction in the price of steam from 12 $/kg (as defined by SuperPro Designer) to 6 $/kg.

Table 26 presents the cost associated with each scenario.

Table 26 – Solvent recovery and steam price influence on production cost.

Production Cost Reduction (%)	**Scenario 3.1**	**Scenario 3.2**	**Scenario 3.3**
MEL	49%	46%	12%
Fuel	48%	45%	12%

Table 27 represents the possible production cost if conditions 3.1 (recycling with 5% solvent loss) and 3.3 (cost of steam reduced by half) are implemented.

Table 27 - Production cost reduction (%) for scenario 3.

	Production Cost Reduction (%)
MEL	61%
Fuel	60%

Scenario 3 results in a production cost reduction of 61% from Scenario 2. Solvent recycle with a 5% solvent loss (3.1) results in a 49% reduction, while reducing steam cost by half (3.2) contributes with a reduction of 12%.

Finally, a fourth situation was studied, in which the future possibility of achieving higher MEL titres of 10 g/L and 14 g/L was considered. The results are summarized in Table 28.

Table 28 – Production cost reduction (%) for scenario 4.

Production Cost Reduction (%)	**4.1 – MEL titre = 10 g/L**	**4.2 - MEL titre = 14 g/L**
MEL	30%	50%
Fuel	30%	50%

Scenario 4.2 introduces a final cost reduction of 50% from scenario 3, with a production cost of 0.96 $.

The production costs estimated for scenario are summarized in Table 29. It should be noted that the values presented for cost reduction in each scenario assume that the previous scenario is already implemented.

Table 29 – Production cost reduction for each individual scenario.

Production Cost Reduction (%)	**Scenario 2**	**Scenario 3**	**Scenario 4**
MEL	77%	61%	50%
Fuel	76%	60%	50%

A significant cost decrease can be achieved if the different scenarios are implemented. The overall production price reduction from scenario 1 is represented in Figure 25.

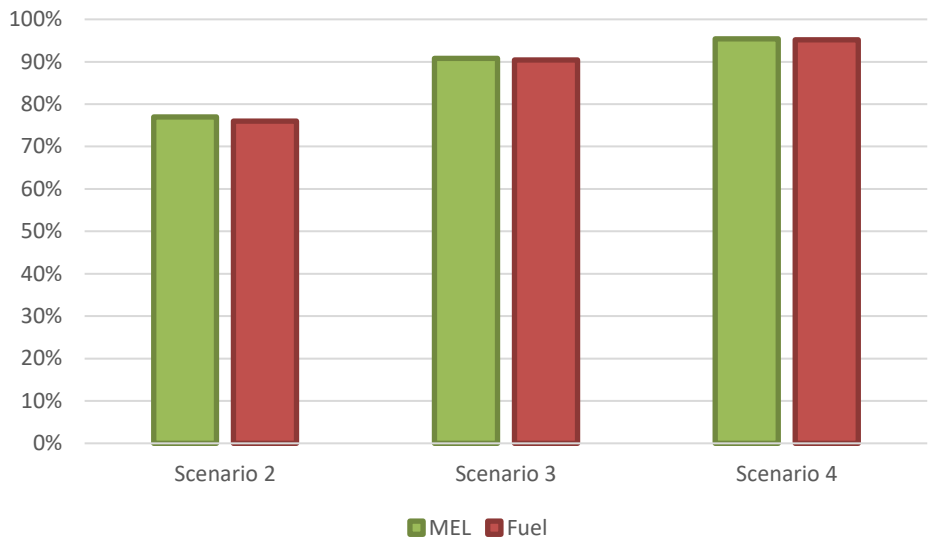

Figure 25 – Production cost reduction (%) for the scenarios hypothesized.

Considering the 20 units of fuel produced by batch, the total energy and power requirements to produce 1 unit of fuel can be determined (Table 30).

Table 30 – Energy and power requirements to produce 1 unit of fuel in each scenario.

Scenario	**1**	**2**	**3**	**4**
Energy (Mcal)	72	28	28	14
Power (kWh)	547	547	547	274

5. Conclusions and Future Work

The possibility of biofuel production through MEL transesterification was confirmed by the experimental work developed. Maximum yields of 95% and 65% were obtained for acid and alkaline catalysed transesterification respectively. The best reaction conditions were determined for both acid (C=0.05 g MEL/g solution, molar catalytic ratio =8, 60ºC and 24h) and alkaline (C=0.05 g MEL/g solution, molar catalytic ratio =8, 60ºC and 4h) catalysed reactions

Sulfuric acid catalysed transesterifications resulted in higher yields for all concentrations and catalytic ratios tested. Lower MEL concentrations seem to favour the reaction in both types of reactions, as such conditions correspond to higher ratios of methanol to MEL, which can favour reaction forward. Transesterification reactions present better results with lower catalytic ratios and lower concentrations, a scenario that may lower material and production costs, as well as facilitating further product purification. Future work could include a process scale up in the same conditions tested, a wider range of catalytic ratios, different alcohols (like ethanol or isopropanol) or different catalysts. A process to determine if mannose erythritol is recoverable from the transesterified MEL, could also be relevant from a biorefinery perspective.

While MEL possesses an ideal chain length for adequate jetfuel properties, conversion of said molecule into short alkanes by hydrotreatment was not verified. While higher molecular weight alkanes and carboxylic acids could be possible reaction products, no short-chained alkanes were identified. This may result from: (i) reaction set-up used, including pressure drop, inefficient heat transfer, loss of substrate/catalyst, loss of volatile compound during nitrogen flushing or depressurization or (ii) inadequate catalyst choice.

Despite the experimental work presented not verifying the production of biojetfuel range alkanes from MEL, only a few conditions were tested and in very limiting conditions. Alternative heterogeneous catalysts can be tested, namely noble metal based catalysts, like Pd/Al_2SO_3 or Rd/Al_2SO_3 also reported to be efficient in vegetable oils. A more suitable method for product analysis like CG-MS could prove helpful in identifying the reaction product. Furthermore, a system that allows the capture of the reaction gas streams would be invaluable to identify reaction products and adjust process intensity.

It should be noted that a very limited amount of experiments was performed due to the time restrictive nature of this project. In order to validate the results obtained all experiments should be repeated in the same conditions used in order to verify the results obtained and evaluate their statistical significance.

A process for biofuel production from MEL was assessed using SuperPro Designer®. Utilities and raw materials prices were found to be the major contributors for the high production cost (around 86%). Different scenarios were implemented in order to reduce the production cost.

The more plausible scenario 3 results in a production cost reduction of 91% and 90% for MEL and fuel respectively.

While scenarios 3 and 4 may present a sufficient cost reduction for a feasible MEL production process, even in the most optimistic and less plausible scenario 4, that assumes a future increase in MEL titre, 1 unit of fuel is still expected to require around 14 Mcal in the conditions simulated. While possible, fuel production from MEL was not found to be a viable alternative regarding both production cost and energy requirements.

It should be noted that the upstream stage was simplified, since refined glucose was directly used and no inoculum preparation was simulated. The reaction zone was also simplified to only include a reaction and a separation stage, with no further fuel purification stage.

Since a simulation based in a series of hypothesis was performed, all the presented values are merely indicative. Production cost could be lower with more mature technology and process up-scale.

Despite the preliminary analysis presented, a more detailed process simulation is required for a better assessment of the production process.

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Appendix

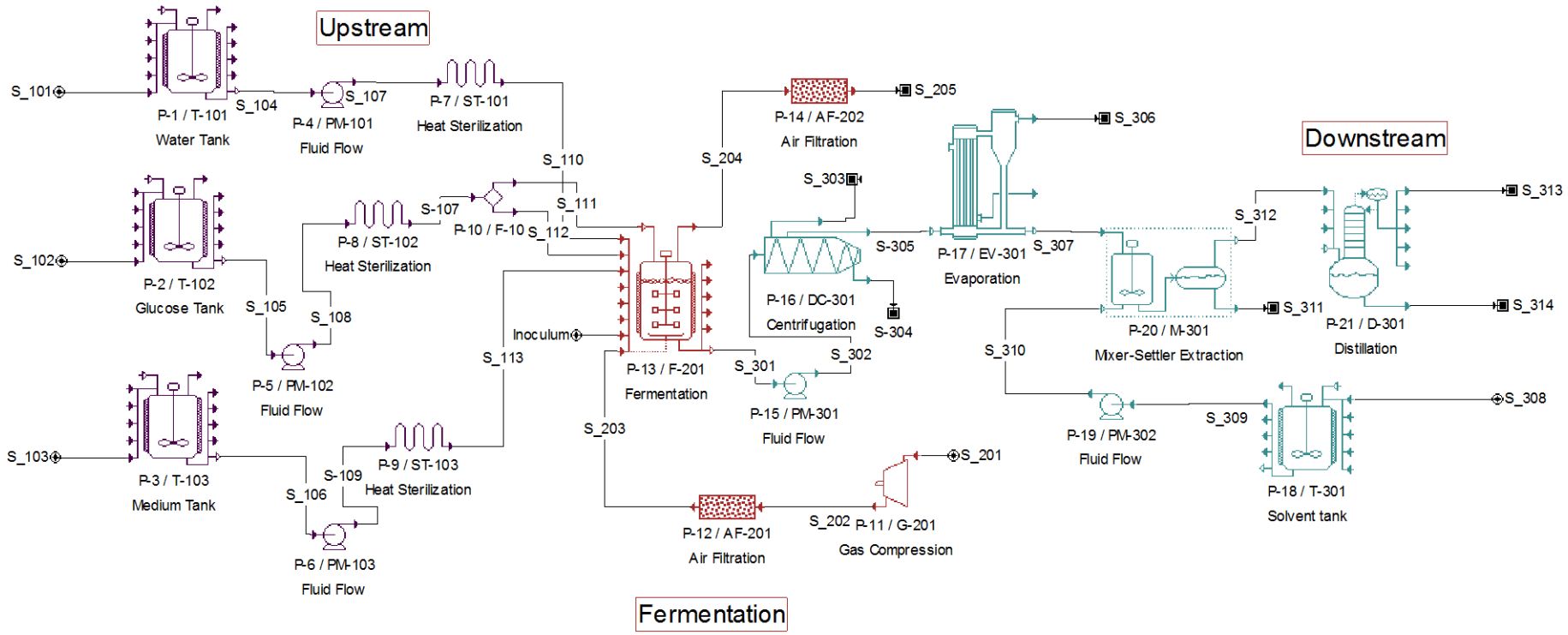

Figure 26 – Full production process as represented in SuperPro Designer®.

Table 31 – Price ($/kg) of raw materials considered.

Raw Material	**Price ($/kg)**
Ethyl Acetate	76.7
Glucose	9.96
KH_2PO_4	113.6
$MgSO_4$	20.55
$NaNO_3$	29.08
Yeast extract	193
Methanol	20.68
Sulfuric Acid	166.89

Table 32 – Typical factors for estimation of project fixed capital cost

	Item	**factor**
f1	Equipment erection	0.40
f2	Piping	0.70
f3	Instrumentation	0.20
f4	Electrical	0.10
f5	Buildings	0.15
f6	Utilities	0.50
f7	Storages	0.15
f8	Site Development	0.05
f9	Ancillary buildings	0.15
f10	Design and Engineering	0.30
f11	Contractor´s fee	0.05
f12	Contingency	0.10

Table 33 - Utility temperature range and cost.

Utility	**T in (ºC)**	**T out (ºC)**	**Price ($/kg)**
Steam	152	152	12.00
Cooling water	25	50	0.05
Chilled water	5	10	0.40
NaCl brine	-10	0	0.25