

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, 8 catalyst to substrate molar ratio and 60°C for 4 h or 24 hours reaction times for acid or alkaline catalysed reactions, respectively. Hydrotreating reactions were performed at 240°C and 55-60 bar, using a 7% Ni/SAPO-11 bifunctional catalyst. 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 unit/year MEL and (ii) chemical conversion of such MEL into the fuel molecules.

Keywords: Mannosylerythritol lipids (MEL), Transesterification, Hydrotreating, Process Simulation

1. Introduction

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 [1, 2].

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 [3, 4].

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 [5]. 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, mainly composed by cellulose, hemicellulose and lignin) are now being cultivated on a large scale in several countries for biofuels production [6, 4, 7].

1.1. Specialty fuels

While a number of alternative methods for biofuels production exist, no feasible alternative exists for winter and aviation biofuels.

Winter diesel fuel refers to diesel with improved cold properties. Diesel fuel is susceptible to waxing when in cold climates. At temperatures below the cloud point 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 [8].

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 [9].

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

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 (-47°C) to remain liquid when flying at high altitudes and comply with the necessary safety requirements.

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.

Fischer-Tropch (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 [6, 10].

Along with FT fuel, HEFA (Hydroprocessed Esters and Fatty Acids) is already being used for testing purposes in commercial passenger flights and received approval for use as an aviation fuel under ASTM D7566-14. Currently, up to 50% bioderived synthetic blending components (HEFA) to be added to conventional jet fuel.

1.2. Mannosylerythritol Lipids

MEL 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 [11, 8].

Depending on the degree of acetylation in C-4 and C-6 of the sugar moiety (Figure 1), 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.

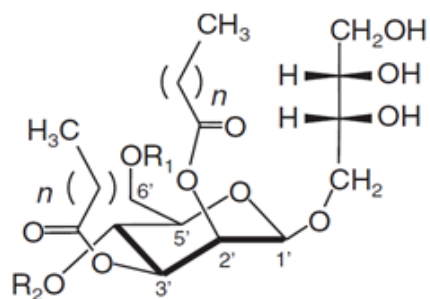


Figure 1 - MEL molecular structure (MEL-A: R1=R2=Ac; MEL-B: R1=Ac, R2=H; MEL-C: R1=H, R2=Ac; n=8-12) (adapted from [8]).

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 [8, 13].

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

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 alkenes adequate to be used in jetfuel formulations.

2. Materials and Methods

2.1. MEL production

2.1.1. Cultivation conditions and extraction

MEL was produced from *Moesziomyces antarcticus*, 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 °C. 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 MgSO₄ (0.3 g/L), yeast extract (1 g/L), NaNO₃ (3 g/L), KH₂PO₄ (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 (MgSO₄ (0.3 g/L), yeast extract (1 g/L), NaNO₃ (3 g/L), KH₂PO₄ (0.3 g/L) and glucose (40 g/L), followed by incubation at 28°C 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.

A Liquid-Liquid extraction with ethyl acetate was performed for MEL isolation.

2.1.2. Fatty acid composition

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 [11].

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.

2.2. Transesterification Reactions

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.

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.

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 μ L of the organic phase was injected in a GC system, in the conditions described above.

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.

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

3. Results and Discussion

3.1. 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.

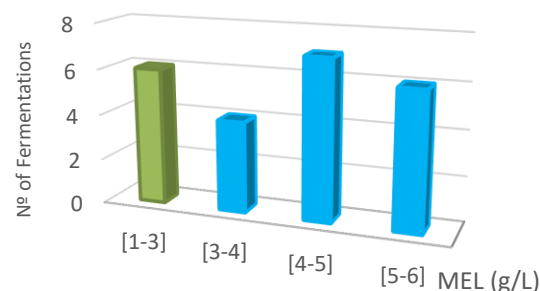


Figure 2 – MEL titres obtained (blue represents a fed-batch fermentation at day 4 and green represents a fermentation without further sugar addition).

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 [8].

3.2. Transesterification

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).

Figure 3 refers to the experiments performed using alkaline and acid catalysts to determine the best conditions regarding molar catalytic ratio for different concentrations.

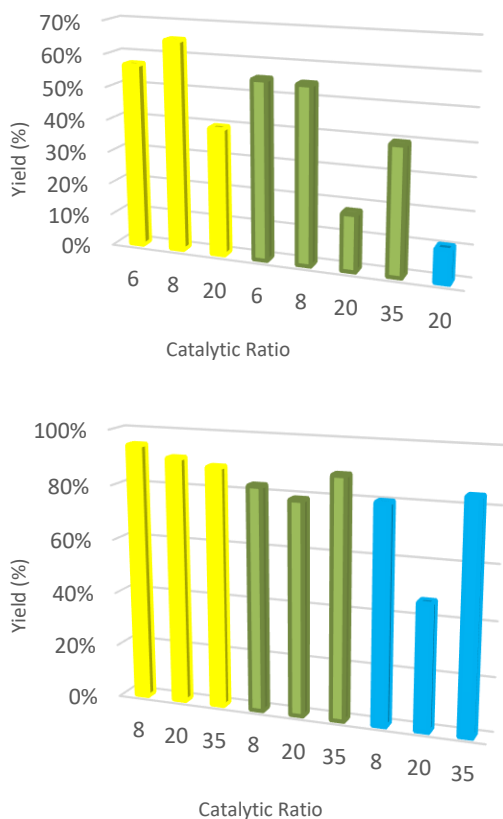


Figure 3 – Alkaline (upper figure) and acid (lower figure) catalysed transesterification yields obtained for different catalytic ratios (yellow represents catalytic ratios for 5 wt% MEL, green corresponds to 10 wt% MEL and blue refers 20 wt% MEL).

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.

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%). [15]

Reaction time and temperature effect were also evaluated (Figure 4).

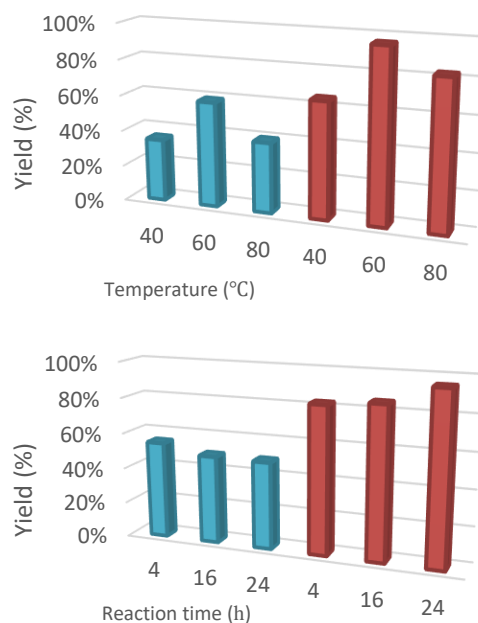


Figure 4 – Temperature and reaction time effect (blue represents an alkaline catalysed reaction, with a catalytic ratio of 8 (upper figure), 10 (lower figure) and 5 wt% MEL; red corresponds to an acid catalysed reaction with a catalytic ratio of 8 and 10 wt% MEL).

In both scenarios, a temperature decrease to 60°C results in a significant yield increase, while 40°C results in worse results for both scenarios. Studies reporting sodium methoxide catalysed transesterifications at 60°C have been reported as the preferable reaction temperature for different vegetable oils sources. [16, 17]. Acid catalysed reactions have also used a temperature of 60°C for biodiesel production [18].

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%. 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.

3.3. Process Simulation

A process using SuperPro Designer® was performed to evaluate the feasibility of a biofuel production process from MEL (regarding process 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 m³, considering a 14 days full batch fermentation cycle with a titre of 7 g/L of product (around 50 kg per batch) 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.

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 correcting for the catalytic conditions used (substrate concentrations, catalyst molar ratios, temperatures used) and yields determined experimentally.

The first three sections are represented in Figure 6. The reaction section is represented in Figure 5 - Reaction simulation in SuperPro Designer®

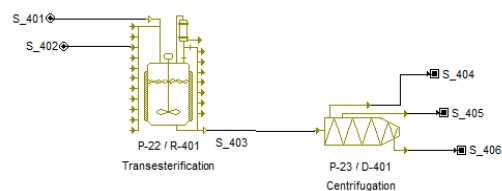


Figure 5 - Reaction simulation in SuperPro Designer®.

Raw materials, labour and equipment cost; utilities and power consumption were determined and the overall productions costs estimated (Table 1).

Table 1 – 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 2).

Table 2 – Production cost of MEL and fuel per batch.

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

MEL production cost is highly dependent (85%) on the cost of utilities and raw materials (Table 1).

In this context, a series of hypothetical scenarios were purposed to reduce production cost (Table 3).

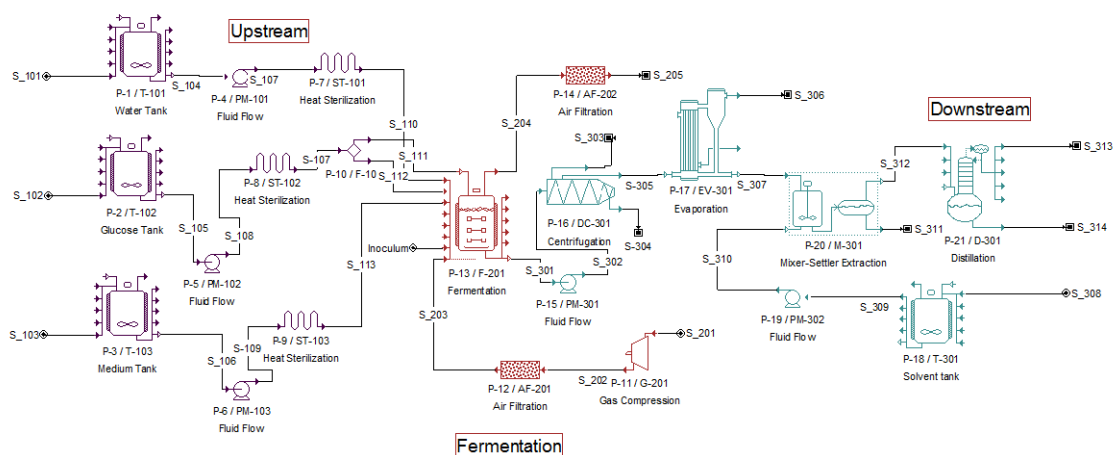


Figure 6 – Fermentation section in SuperPro Designer®.

Table 3 – 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 1 corresponds to the production costs presented in Table 1. 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 120°C to 28°C) 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 significantly 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 the high quantities required.

Scenario 2 implements these changes resulting in a substantial reduction in the production cost (Table 4).

Table 4 – Production cost reduction for scenario 2.

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

Scenario 2 allows a production cost reduction 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 m³). 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 6).

Table 5 – Solvent recovery influence on production cost.

Cost reduction (%)	Scenario 3.1	Scenario 3.2	Scenario 3.3
MEL	49%	46%	12%
Fuel	48%	45%	12%

Table 6 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 6 – 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 7.

Table 7 – Production cost reduction for scenario 4.

Prod. Cost Reduction (%)	titre=10 g/L	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 \$.

It should be noted that the values presented for cost reduction in each scenario assume that the previous scenario is already implemented.

Table 8 – Overall production cost for all scenarios.

Prod. Cost Reduction (%)	Sc. 2	Sc. 3	Sc. 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 7.

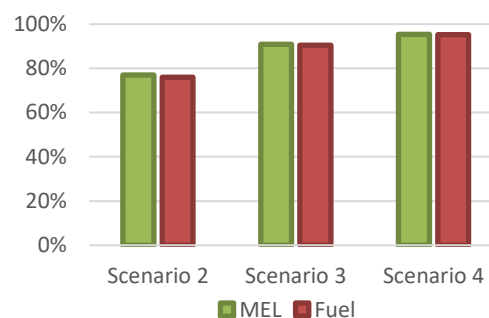


Figure 7 – Production cost reduction (%) from scenario 1 for the scenarios hypothesized.

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 9).

Table 9 – 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

4. 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. Higher yields were obtained 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.

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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6. References

- [1] - Huijben, M. (2016). Nanomaterials for Energy. Retrieved from <https://www.utwente.nl/mesaplus/nme/Introduction>. Consulted on: 12/07/2016.
- [2] - Diefenderfer, J., assumptions Vipin Arora, M., & Singer, L. E. (2040). *International Energy Outlook 2016 Liquid fuels* (Vol. 484).
- [3] - The Nuffield Council on Bioethics. (2011). "Chapter 1 - Why Biofuels? Drivers for Biofuels Production".
- [4] - Luque, R., J. Campelo, and J. Clark. (2011). "Introduction: An Overview of Biofuels and Production Technologies." Woodhead Publishing Limited.
- [5] - Macedo, I. C., & Seabra, J. E. A. (2008). *Mitigation of GHG emissions using sugarcane*

bioethanol. Sugarcane ethanol - Contributions to climate change mitigation and the environment.

[6] - Smith, Maxwell. 2007. "Aviation Fuels." 9891:495.

[7] - Biofuel.org.uk. (2010). Types Of Biofuel Retrieved from <http://biofuel.org.uk/types-of-biofuels.html>. Consulted on: 12/07/2016.

[8] – Biodiesel Cold Flow Basics. National Biodiesel Board. Retrieved from https://www.google.pt/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEWiP4aD8prjQAhWhwVQKHY4YDusQFggiMAA&url=http%3A%2F%2Fbiodiesel.org%2Fdocs%2Fdefault-source%2Fffs-performance_usage%2Fcold-flow-basics---ppt.ppt%3Fsfvrsn%3D6&usq=AFQjCNHR7rLciA-WBVvVeRBaAzre-EDIXw. Consulted on: 08/08/2016.

[9] - Biofuel Systems Group Ltd, Wintron ® - Biodiesel Cold Flow Additives. Retrieved from <https://www.biofuelsystems.com/wintron.htm>. Consulted on: 12/07/2016.

[10] - Kandaramath Hari, Thushara, Zahira Yaakob, and Narayanan N. Binitha. (2015). "Aviation Biofuel from Renewable Resources: Routes, Opportunities and Challenges." *Renewable and Sustainable Energy Reviews* 42:1234–44.

[11] - Yu, Mingda et al. (2015). "Characteristics of Mannosylerythritol Lipids and Their Environmental Potential." *Carbohydrate Research* 407(2015):63–72.

[12] - Faria, Nuno Torres et al. (2014). "Production of Glycolipid Biosurfactants, Mannosylerythritol Lipids, from Pentoses and D-Glucose/d-Xylose Mixtures by Pseudozyma Yeast Strains." *Process Biochemistry* 49(11):1790–99.

[13] - Faria, Nuno et al. (2014). "Conversion of Cellulosic Materials into Glycolipid Biosurfactants, Mannosylerythritol Lipids, by Pseudozyma Spp. under SHF and SSF Processes." *Microbial Cell Factories* 13(1):155.

[14] - Rodríguez-Ruiz, J., Belarbi, E. H., Sánchez, J. L. G., & Alonso, D. L. (1998). "Rapid

simultaneous lipid extraction and transesterification for fatty acid analyses." *Biotechnology Techniques*, 12(9), 689–691.

[15] - Schuchardt, U., Sercheli, R., & Matheus, R. (1998). "Transesterification of Vegetable Oils: a Review General Aspects of Transesterification Transesterification of Vegetable Oils Acid-Catalyzed Processes Base-Catalyzed Processes". *J. Braz. Chem. Soc.*, 9(1), 199–210.

[16] - Fan, Mingming, Jianglei Huang, Jing Yang, and Pingbo Zhang. (2013). "Biodiesel Production by Transesterification Catalyzed by an Efficient Choline Ionic Liquid Catalyst." *Applied Energy* 108:333–39.

[17] - KoohiKamali, S., Tan, C. P., & Ling, T. C. (2012). "Optimization of sunflower oil transesterification process using sodium methoxide." *The Scientific World Journal*, 2012 (2007).

[18] - Canakci, M., & Gerpen, J. Van. (1999). Biodiesel Production Via Acid Catalysis.

