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Techno-economic analysis (TEA) of microbial oil production from waste resources as part of a bio-refinery concept: assessment at multiple scales under uncertainty

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Abstract

BACKGROUND: Microbial oils, often termed single cell oils (SCOs), offer an alternative to terrestrial oil crops across the energy, food, and chemical industries. In addition to oils, a range of secondary metabolites can be produced from the heterotrophic organisms as part of a bio-refinery system. Techno-economic analysis (TEA) is an important tool for evaluating economic viability, and while TEA is subject to high uncertainties where production is still at the laboratory scale, the tool can play a significant role in directing further research to evaluate suitability of scale-up.

RESULTS: SCO production from the oleaginous yeast *Metschnikowia pulcherrima* using sucrose, wheat straw and distillery waste feedstocks was evaluated at two production scales. At a scale of 100 tonnes a^{-1} oil production a minimum estimated selling price (MESP) of €14k per tonne was determined for sucrose. This reduced to €4-8k per tonne on scaling to 10,000 tonne a^{-1}, with sucrose and wheat straw yielding the lowest MESP.

CONCLUSIONS: Feedstock price and lipid yield had the greatest impact on overall economic return, though the valorisation of co-products also had a large effect, and further play between feedstock and system productivity strategies could bring the price down to be competitive with terrestrial oils in the future. The novel approach demonstrated here for the first time integrates uncertainty into economic analysis whilst facilitating decision-support at anearly technology development stage.

Key words
Microbial oil, single cell oil, biorefinery, techno-economic analysis, TEA, uncertainty

1. Introduction

Advanced biorefinery concepts based on the production of microbial or single cell oils (SCOs) offer a solution to environmental challenges posed by use of vegetable oils for production of biofuels, oleochemicals and food products. Use of SCOs creates opportunities for co-product utilisation, offering sustainable routes to a number of different product streams. In order to understand the long-term sustainability of technologies for the production of SCOs, techno-economic analysis (TEA) is required. This is not only helpful in determining economic viability, but also in defining key factors important for successful commercialisation. Despite the importance of TEA to risk minimisation at the early stages of technology development, there are a number of challenges when applying this type of assessment method whilst still at the laboratory scale.

Uncertainty and variability are inherent characteristics of any process system, but play a far greater role in emerging technology systems and those defined at the laboratory scale. For biorefining, these uncertainties can be considered in the following way (1):

(i) Process-inherent uncertainties (product yield, bioprocessing system performance, feedstock variability)

(ii) Modelling uncertainties at unit operation level due to unknown scaling and transformation of laboratory scale processes

(iii) Uncertainties associated with market factors, policies and availability of financing

Systematic accounting for all types of uncertainty is not commonly performed within early stage TEA studies (1). There are a number of deterministic and stochastic methods for uncertainty assessment including sensitivity, and scenario analysis; Monte Carlo, and Global Sensitivity Analysis (GSA); and qualitative determination of quantitative uncertainty values from pedigree matrices obtained via expert elicitation. Van de Spek *et al.* (2015) used pedigree matrices to validate a process model for CO_2 capture with monoethanolamine (2). Others have applied sensitivity analysis (3), and Monte Carlo analysis (4, 5) to TEA evaluation of new technologies.

To date, the majority of techno-economic studies for microbial biorefineries have focused on phototrophic microalgae (6, 7). A smaller number of TEAs have also been performed on yeast and heterotrophic algae (8-10), with all studies assessing the use of SCOs for biodiesel rather than for food or other terrestrial oil product replacement (6). Amongst all TEA studies conducted on SCO routes to bioproducts none have fully accounted for uncertainties in their modelling.

Lipid accumulation in yeast and other microorganisms to form SCOs typically occurs under a nutrient-limited environment where a sufficient excess of extra-cellular carbon during growth phase leads to synthesis of storage lipids and *de novo* lipid accumulation. Different neutral lipids are accumulated by different microorganisms, with oleaginous yeasts predominately accumulating triacylglycerols (TAGs) and sterols (11). For yeast, ideal lipid accumulation occurs when the carbon/nitrogen ratio is between 30-80. Species from the genera *Rhodosporidium, Cryptoccus, Lipomyces,* and *Rhodotorula* can accumulate lipid at up to 70% their dry biomass (12, 13). Of this 60-90% can be neutral acylglycerols. These largely contain α-linolenic (C18:3) linoleic (18:3), oleic (18:1), steric (18:0), palmitoleic (C16:1), and palmitic (C16:0) acids (14, 15).

As heterotrophic organisms, yeasts metabolise carbon from simple sugars or carbon-containing compounds such as glycerol. This means fermentation feedstocks can be monosaccharides such as glucose, or C5 and C6 saccharide-containing hydrolysate derived from the breakdown of lignocellulosic biomass. Cellulose is hydrolysed to form glucose, whereas hydrolysis of hemicellulose yields glucose, xylose, mannose and galactose. However, at high temperatures, xylose degrades to furfural; and mannose, galactose and glucose to 5-hydroxymethyl furfural (HMF). Xylose and HMF also further degrade to form formic acid. The partial breakdown of lignin forms phenolic compounds. Formation of HMF, furfural, formic acid and phenolics has an inhibitory effect on cell growth (16). For this reason, lignocellulosic hydrolysis methods must be substantive enough to breakdown material components but not lead to further degradation and formation of inhibitory compounds.

Conventional routes to biomass hydrolysis include acid pretreatment and enzymatic hydrolysis, ammonia fibre expansion and steam explosion (17). All require acid or high temperatures to obtain a hydrolysate capable of being utilised by yeast or heterotrophic algae during fermentation. A detailed process and cost model for acid pretreatment and enzyme hydrolysis for the production of bioethanol has been previously produced by Humbird *et al.* (18). However, aerobic conversion of hydrolysates at large-scale are poorly documented in the literature, with only a small number of TEA studies evaluating heterotrophic organisms (7-9). Within these, agitation and aeration during fermentation were suggested to be the most significant areas of electricity use within the whole process (8, 9); however, changes in the extraction of lipid and drying gave the greatest impact on mass and energy balance (9).

Cell harvesting and lipid extraction are key elements of downstream processing following fermentation. For lipid extraction, a cell disruption step is required to break open the cell wall, before (typically) a solvent is used to extract the lipid. Industrially, this can be combined in one processing step, where solvent is added and the mixture is homogenised before solvent and lipid recovery using a distillation column and recovery of the solvent (8, 10). Alternative disruption methods include bead milling, ultrasound, microwave and acid or enzymatic hydrolysis. These methods have varying potential for industrial scalability (13). Wet extraction using hexane has been modelled in detail on an industrial scale (19). The model obtains a lipid yield of 99.7% (95% fatty acid lipids), with a hexane recovery rate of 99.4%. Baseline experimental data assumes a 3-step extraction strategy where the lipid is extracted, hexane is recovered before another final extraction step is applied.

Previous estimates of heterotrophic SCO production ranges between \$1.76 - \$6 per kilogram depending on achievable lipid productivity (8, 10). An approximate comparison can be made with phototrophic algal biodiesel costs at \$5- \$150 per litre (7). Based on current literature cost is most sensitive to productivity; however, assessment that includes a range of production scenarios and potential feedstocks is missing. Given the lack of industrial data for parts or all of these processes, and challenges associated with modelling achievable productivities at commercial scale based on laboratory data, this makes realistic cost estimation difficult. Where uncertainty is high, there are a number of different methods for both representing/communicating uncertainty and evaluating the process model in a decision-orientated way. Stochastic approaches such as Monte Carlo analysis or NUSAP (Numeral, Unit, Spread, Assessment and Pedigree) based Pedigree Matrices (20) enable the level of uncertainty in results to be communicated. Methods such as sensitivity analysis and scenario analysis help explore how variation in input data and assumptions made in the model can affect the model outputs. Whilst some of these methods are commonly applied to TEA studies in the literature, full systematic assessment of uncertainty is lacking. This is particularly important for industrial biotechnology given the specific challenges associated with moving to scale.

One promising route to SCO is through oleaginous wine yeast *Metschnikowia pulcherrima.* The yeast can produce up to 40% oil content per cell though catabolism of a wide range of oligosaccharides and monosaccharides (21). Excitingly, the yeast can be cultured in non-sterile conditions due to a combination of culturing at low pH and production of antimicrobials. This has been demonstrated by growing axenically in raceway ponds (22). In addition the yeast can produce co-products such as a proteinous fraction and 2-phenylethanol, an aromatic fragrance (23).

The work presented here contributes an evaluation of a novel route to SCOs as part of a biorefinery system using *M. pulcherrima*, comparing economic viability at two different scales of production. Different feedstocks were evaluated using continuous stirred-tank (CSTR) fermentation and low-cost, low-energy raceway pond fermentation. These feedstocks are wheat straw, distillery wastes (distiller's dried grains with solubles (DDGS) and draff) and sucrose. The range of scenarios evaluated demonstrate how TEA can be applied towards emerging technologies such as SCO biorefineries in a way that incorporates uncertainty for the first time but still supports decision-making. This novel approach has wide ranging application across the economic assessment of emerging technologies.

2. Methodology and process description

In the TEA model, two production scales were evaluated: 100 tonnes and 10,000 tonnes of unrefined SCO per year. The assessment showed economic viability at both demonstration and full commercial scale, factoring in the production of a range of co-products as part of a biorefinery system. Here tonne refers to a metric ton (1000 kg). The 100 tonnes per year (figure 1 A) assumed a sucrose feedstock only. This scale was also used to show cost associated with running a smaller, bespoke lipid production facility. At 10,000 tonnes per year a sucrose feedstock (figure 1 B) was contrasted with lignocellulosic feedstocks wheat straw and distillery waste (DDGS and draff) (figure 1 C) From the lignocellulosic feedstocks additional co-products (yeast protein and 2-phenylethanol) and process steam and electricity are produced alongside the refined, fractionated SCO.

Figure 1. Microbial oil production process at two different scale (a) 100 tonne per year scale using a sucrose feedstock, (b) 10,000 per year scale using a sucrose feedstock, (c) 10,000 tonne per year scale using a lignocellulosic feedstock

Both 100 and 10000 tonne per year facilities were assumed to be running for 8410 hours per year, with a plant life of 30 years. Cost analysis was calculated as a conservative order of magnitude estimate for equipment cost (+/-40%). A breakdown of assumptions used for the analysis is given in table 1. Two cost analysis methods are used - Cost of Manufacture (COM) (based on the method given in (24)) and discounted cash flow analysis. Due to the differences that internal rate of return (IRR) or discount rate have on the estimated minimum selling price (18), COM gives an estimate for annual cost of manufacture which excludes discounting and additional coproduct revenue, whereas, discounted cash flow analysis includes discounting and additional revenue from co-products. The yeast productivity was based on experimental results at the 2 L scale, though similar productivities have been reported for alternative species (25).

The cost year for the analysis was 2017. All calculated costs have been converted from GDP to euros assuming an average exchange rate of 1.141317 (2017). The cost of equipment was scaled based on the six-tenths rule (eq. 1) (where $Cost_A$ refers to known cost and $Cost_B$ refers to approximate cost) and then equipment further levelised using the Chemical Engineering Plant Cost Index (CEPCI) (eq. 2).

$$\frac{Cost_B}{Cost_A} = \left(\frac{Capacity_B}{Capacity_A}\right)^{0.6} \qquad (1)$$

$$Present\ cost = Original\ cost\ \times \left(\frac{index\ at\ present}{index\ when\ cost\ was\ obtained}\right) \qquad (2)$$

Discounted cash flow analysis was used to obtain a minimum estimated selling price (MESP) based on a net present value (NPV) of zero at an IRR equal to the assumed discount rate of 10%. This was used to determine economic viability of SCO production from yeast *M. pulcherrima* compared with low-mid range commodity oil/chemical costs.

Table 1. Breakdown of techno-economic assumptions at 100 tonnes per year and 10,000 tonnes per year scale

2.1 100 tonne per year scale facility

Equipment cost was based on the production of 100 tonnes of unrefined microbial oil per year, yielding 95 metric tonnes of refined microbial oil. Unit processes within the demonstration facility were separated into: fermentation, harvesting, extraction and refining. This included direct purchased cost and cost of installation. The cost estimates for each unit process were calculated as +/-40% as an order of magnitude estimation. Cost was calculated using information from literature for yeast fermentation, and algal and yeast downstream processing.

The process used unpublished experimental data relating to work described by (22) and (23). Fermentation was carried out in semi-continuous mode using the oleaginous yeast *M. pulcherrima*. The yeast is commonly found in wine making, and can be cultured in non-sterile conditions due to the production of a range of antimicrobial compounds, including the fragrance chemical 2-phenylethanol (22). At this scale the feedstock for fermentation was assumed to be sucrose. Cell concentration during fermentation was held at 120 g/L with a lipid content of 40%. Sucrose to microbial mass conversion was 0.35 g/g. The lipid profile was assumed to be analogous to that of palm oil (dominated by triglycerides 2-oleo-dipalmitic, POP and palmitic-oleic-oleic, POO). The stoichiometric equation for the fermentation reaction is given in equation 3.

$$C_{12}H_{22}O_{11} + 0.245(NH_4)_2SO_4 + 4.79O_2 \rightarrow 0.35C_4H_{6.5}O_{1.9}N_{0.7} + 0.245C_3H_7NO_2S + 0.06C_{54}H_{101}O_6 + 0.008C_8H_{10}O + 6.561CO_2 + 6.915H_2O \quad (3)$$

For the mass balance, $C_4H_{6.5}O_{1.9}N_{0.7}$ was used as the molecular equation for the yeast (26). Cysteine (molecular formula $C_3H_7NO_2S$) was used as a proxy for protein production in order to balance nitrogen and sulphur elements. Accumulation of lipid (molecular formula $C_{54}H_{101}O_6$) was based on an average of palmitic and oleic acid containing triglycerides. This reflects the dominance of C18 and C16 fatty acids within the lipid profile of the yeast (22).

Fermentation was modelled as being carried out in a 30 m^3 stirred-tank reactor with a 25 m^3 working volume. Information on energy calculation for different impeller types is given in the supplementary information.

Yeast biomass was produced at a rate of 31.29 kg/hr. 2-phenylethanol was produced at a rate of 0.250 kg/hr. The stream leaving the fermentation vessel passes through an adsorption column which removes 2-phenylethanol from the process stream and was then filtered via continuous rotary vacuum filtration, similar to recent reports on 2-phenylethanol production (23). The wet yeast biomass was mixed in a mixing tank with hexane (25% w/w yeast in hexane). The mixture was homogenised to rupture and break open the cell, solid and liquid phases were separated, with the solid stream containing the extracted yeast biomass which forms the yeast extraction co-product stream. Hexane was recovered via an evaporation step with hexane losses at 0.5%. The process was calculated to yield 100 tonnes of unrefined microbial oil per year.

To purify the lipid further, the stream was then mixed with 0.19 wt% phosphoric and an additional 10 wt% wash water. Following this, the mixture was centrifuged. This is based on an NREL algal purification process for product upgrading (19). This removes any polar lipids (such as phospholipids) present in the unrefined lipid mixture. Phosphoric acid was then neutralised using sodium hydroxide (2.5 wt%), removing free fatty acids from the process stream. The next step was then a bleaching step with clay (0.2 wt%) which removes any other impurities. A slurry is formed and then filtered to remove the clay. The efficiency of the purification step was assumed to be 95%.

Terrestrial oils like palm oil are often sold fractionated – typically into palm olein or palm stearin fractions. Palm stearin contains a higher proportion of saturated fatty acids and TAGs, where palmitic acid content is 49-68% and oleic acid content 24 to 34% (27). Palm olein has a lower proportion of palmitic acid, and higher proportion of oleic acid (18:1) with a lower boiling point than the stearin fraction. With a fatty acid profile similar to that of palm oil, the microbial oil derived from yeast *M. pulcherrima* was assumed to be fractionated in the same way as terrestrial palm oil fractionation, carried out using a distillation column to yield 75% fraction containing majority palm olein, and a 25% fraction containing majority palm stearin. In further economic analysis this fraction was taken together to yield an annual production of 95 tonnes of fractionated refined microbial oil. A further 2.5 tonnes of 2-phenylethanol (at €5,700 per tonne) and 160 tonnes of yeast extract (€340 per tonne) are produced.

2.2 10,000 tonne per year scale facility

Waste lignocellulosic biomass offers a route to lipid production which avoids first generation crop usage, and therefore does not directly compete with food production. Utilisation of nonedible biomass feedstocks or by-products from agriculture and industrial processing helps to maximise per hectare crop productivity and increases industrial circularity as wastes from one process become feedstocks for another. Lignocellulosic biomass components (cellulose, hemicellulose, lignin, volatiles/extractives and ash) can be highly inconsistent, even within the same resource type, due to different strains, harvesting and growth conditions (28). Feedstock variability presents a number of challenges for biochemical processing. Physical properties such as moisture content, particle morphology, density, compressibility, and biomass microstructure influence effectiveness of pre-treatment and hydrolysis pathways. Chemical properties such as higher lignin and volatiles content can lead to increased inhibitor production, impacting fermentation and product yield (28, 29).

Production using lignocellulosic feedstocks was at 10,000 tonnes of unrefined lipid production per year. The process evaluated the use of wheat straw, assuming a composition of 34.6% glucan, 21.2% xylan, 2.3% arabinan, 0.9% galactan, 18% lignin, 2.2% acetate, 5.6% ash and 15.4% extractives (30). Pricing for wheat straw is assumed to be €70 per tonne (31). This was then compared with by-products from the distillery industry, DDGS and draff (unprocessed/spent grains). DGGS and draff are currently sold as animal feed for cows, sheep, goats and horses across the UK. The majority of distillery waste produced in the UK is generated in Scotland as by-products of whisky production. The potential output from Scotland alone is estimated at 466,000 tonnes (2012); however, sources of DDGS from bioethanol production could be far higher than this (estimated at 750,000 tonnes) (32). The price of DDGS per tonne was taken as €228, (32). The DDGS used in the model was sourced from the Vivergo biorefinery plant in Yorkshire, UK. This is composed of neutral detergent fiber (NDF) (31.5%), starch (2.3%), sugar (1.1%), protein (undegradable dietary protein and crude protein) (47%), oil (7%), based on a total solids content of 89%. Composition of distillers' malted barley draff is NDF (62%), starch (1.7%), sugar (2%), protein (undegradable dietary protein and crude protein) (28.7-30.7%), oil (9%), based on a total solids content of 18-24%.

Data on acid and enzyme hydrolysis was taken from the NREL corn stover to bioethanol model (18). This includes capital cost data and operating costs, scaled accordingly. Efficiency at breaking down the lignocellulosic components in wheat straw, DDGS, and draff were calculated based on the efficiency of cellulose, hemicellulose and lignin breakdown from corn stover. Based on the efficiencies outlined in (18), 95% of the theoretical lignin present remains unsolubilized and can therefore be utilised for process heat and electricity. The steam and electricity generated was then fed back into the acid pre-treatment and enzymatic hydrolysis step. Excess electricity was sold back to the grid. Lignin from wheat straw produced 98,670 kg steam and 36,011,235 kWh of electricity per year. Given that lignin content for DDGS and draff is less well defined, a content of 15% for both was assumed based on (33). This yielded 71,571 kg steam and 26,123,603 kWh process electricity per year.

Fermentation was modelled as using 12 x 250 m^3 stirred-tank reactors, with a maximum working volume of 85%. System performance was assumed to be the same as at the demonstration plant scale, with the *M. pulcherrima* culture as productive (0.35g/g hydrolysate) with a culture density of 120 g/l, yielding 1.3 g/l/hr yeast biomass which corresponds to 0.52 g/l/hr lipid production. Yeast biomass was produced at a rate of 3.1 tonnes/hour. To evaluate sensitivity of capital cost to fermentation productivity, a lower cost raceway pond route was also investigated as a potential fermentation scenario. Raceway ponds are typically used for photoautotropic microalgae cultivation as an alternative to a closed photobioreactor systems. The ponds are built in concrete with a closed loop and oval shaped recirculation channels. Their advantages are that they are cheap and easy to maintain, but are limited by poor biomass productivity and ease of contamination. Their lower productivity when used in algae cultivation is attributed to aspects such as poor mixing and temperature fluctuations (34). *M. pulcherrima* has been previously demonstrated to grow well in open, non-sterile conditions (22), owing to its ability to produce a range of antimicrobials and grow at low pH. A total annual productivity reduction of 23% was assumed for the raceway pond system based on the work in (8), given a biomass productivity drop of 12% and a reduction in lipid content to 35%. However, the installed equipment cost for fermentation dropped by 92%.

Following fermentation, the stream leaves the fermentation vessels passing through an adsorption column which removed 2-phenylethanol from the process stream. Hexane extraction was assumed to be via a wet hexane extraction (19). This negated both a prior homogenisation and drying step. The counter-current column yielded a 95% extraction efficiency. The model here assumed a solvent:biomass ratio of 5.8. For this process, a conservative 5% hexane loss was assumed. Electricity usage was also calculated based on (19).

Given the intended use of the lipid product as a replacement for palm oil constituents further refining, upgrading and fractionation of the oil, the model bases this on (19) and (35). The stream was mixed with 0.19 wt% phosphoric and an additional 10 wt% wash water, which was then centrifuged. As outlined at the demonstration scale, this removed any polar lipids (such as phospholipids) present in the unrefined lipid mixture. Phosphoric acid was neutralised using sodium hydroxide (2.5 wt%), removing free fatty acids from the process stream. The next step was a bleaching step with clay (0.2 wt%) which was assumed to remove any other impurities. A slurry was formed and then filtered to remove the clay. The efficiency of the purification step was estimated at 95%. The oil was then fractionated based on (35). This did not include capital costing, for which a distillation column sized using (10). In further economic analysis this fraction was taken together to yield an annual production of

95 tonnes of fractionated refined microbial oil. A further 250 tonnes of 2-phenylethanol (€5,700 per tonne) and 10,000 tonnes of yeast protein for animal feed (€570 per tonne) were produced from all feedstocks. Electricity and steam produced were fed back into the acid-enzyme process. This reduced utilities costs by up to 68%.

2.3 Limitations and uncertainty

To date, TEA studies on SCOs have not fully accounted for uncertainty. Given the early-stage nature of this type of oleaginous yeast to lipid process and the limited data available in literature there are a number of limitations to this work which are listed below:

- Experimental performance data for both the demonstration and pilot plant scale was based on a 2 L bioreactor run semi-continuously for 28 days. There are a number of complex factors affecting scale-up performance, and reliance on laboratory scale data leads to uncertainty in cost analysis results and subsequent evaluation of economic viability. The performances used in the study are indicative of those assumed elsewhere for oleaginous yeast (9, 10).
- There is substantial variability across feedstocks in their ability to be broken down and hydrolysed to form a fermentable hydrolysate. This process bases theoretical breakdown of cellulose, hemicellulose and lignin of the feedstocks assessed on a corn stover acid pretreatment and enzymatic hydrolysis process, given that this models biomass hydrolysis at a large scale. Performance of this process on different feedstocks is likely to vary, with other established methods for biomass breakdown (steam explosion etc.) potentially more suited. There is therefore significant uncertainty related to using laboratory data to model this process on a larger-scale where very little performance data exists.
- For lipid extraction, the majority of literature to date has focused on the extraction of phototrophic algal lipids for biodiesel. There is limited description in the literature of

industrial lipid extraction from yeast. Given this, extraction was based on previous published techno-economic data for yeasts (9, 10) (100 tonne per year), and on larger-scale wet algal extraction (19) (10,000 tonne per year). There is uncertainty on the ability to extract 95% lipid from yeast biomass using hexane at industrial scale, and the energy inputs required to adequately disrupt and break apart the cells and remove water and hexane following the extraction step.

- Literature to date has focused on the use of microbial lipids to produce biodiesel (6). This means that following extraction, the unrefined lipid is transesterified to produce fatty acid methyl esters. The refining, bleaching and deodorisation of lipids needed for lipid applications outside that of biofuels is poorly defined. In the model it was assumed to be similar to the refining required for crude palm oil entering a refinery. Equipment cost data for this step was taken from an algal upgrading process (19). There is uncertainty here on processing steps required, equipment cost, and input quantities needed.

Given the level of uncertainty in modelling the process at this early stage of development, the approach applied to the techno-economic model was to determine a range of feedstock and processing (biomass hydrolysis and fermentation) scenarios in order to understand overall sensitivity to feedstock and process choice at two different technology readiness levels (TRLs). Uncertainty relating to the COM was communicated through the use of Monte Carlo analysis, and scenarios evaluated through both COM and profitability.

3. Results and discussion

3.1 100 tonne per year facility

3.1.1 Capital expenditure

Equipment cost was calculated based on installation costs for equipment outlined in (36). This is given as an order-of-magnitude cost estimate. For modelling at demonstration scale, sucrose was used as the carbon source for fermentation, meaning additional equipment for processing lignocellulosic biomass was not needed. Fermentation took place in a stirred-tank reactor.

Total fixed CAPEX cost ranged between €794,768 and €1,854,446 for the 100 tonne per year facility. Further information on this is found in the supplementary information.

3.1.2 Cost of manufacture

Cost of manufacture (COM) was calculated based on fixed CAPEX cost (FCI) (1.2 ×total cost), labour cost, raw materials cost, utilities cost, and waste management cost. Where uncertainty associated with parameter inputs is high, COM per tonne of oil produced was represented as a cumulative probability distribution profile. This was calculated by: 1. defining uncertainty values for each parameter, 2. determining appropriate distribution profiles (uniform, triangular etc.), 3. randomly sampling each profile in order to then propagate this through the COM calculation to obtain a cumulative probability distribution for COM per tonne. The Monte Carlo calculation was carried out in Matlab®. Each distribution was sampled 10,000 times.

Monte Carlo analysis has previously been applied to other TEA metrics including minimum fuel selling price for thermochemically (37) and biochemically derived (38) fuels. These studies demonstrate the role Monte Carlo can play in defining confidence interval estimates for TEA metrics, particularly where uncertainty is high in assessing new and emerging technology.

COM was calculated using equation 4, using assumed relationships between the individual elements given in (24). Where C_{OL} refers to the cost of operating labour, C_{UT} to utilities cost, C_{WT} to waste treatment, and C_{RM} refers to cost of raw materials. Discount rate was excluded from this calculation.

$$COM = 0.180FCI \times 2.73C_{OL} \times 1.23(C_{UT} \times C_{WT} \times C_{RM}) \qquad (4)$$

Operating labour was calculated based the number of operators required per shift. This was based on the relationship between number of processes handling particulate solids and the number of processing steps involving non-particulate solids (24).

A linear distribution was used for capital investment given the nature of order of magnitude estimation for capital cost. Triangular distributions are typically given to parameters where substantial uncertainty exists, particularly outside that of minimum, most likely, and maximum values (5, 39). Therefore, raw materials inputs were distributed triangularly. Utilities, waste water treatment (included in water costs), and labour costs used a bootstrapped distribution across historical cost data for the UK over the past 10 years (40) .

The median COM was €24,000-€25,000 per tonne. Relative standard deviation was 2% . Based on the median costs for manufacture per tonne, the refined SCO is currently not price competitive with standard terrestrial oils such as palm oil (€400-800 per tonne) or higher value coconut oil (€800-1600 per tonne). A COM of €20,000 per tonne puts the SCO into a pricing bracket for high value speciality chemicals. Under these conditions the SCO would be required to offer additional functionality not found in bulk terrestrial oils. The SCO would therefore be entering the market as a speciality chemical (based on enhanced performance properties for applications such as surfactants) rather than bulk chemical replacement within the terrestrial oils market.

3.1.3 Profitability

A discounted cash flow analysis was used to calculate a minimum estimated selling price (MESP) for the SCO. This is where net present value (NPV) is equal to zero, at a finite rate of return.

NPV is commonly used to assess economic performance over a project's lifetime. It accounts for the fact that returns on capital investment made at the start of the project are not received until later on. This is accounted for by the discount rate which takes into account the decreasing value of future returns made based on initial capital outlay. Thus, this determines the earning power of an investment (36). NPV is calculated based on nominal net cash flow (CF_t) at year t; r is the plant's discount rate; n is the plant's lifetime; and TCI refers to total capital investment (eq. 5).

$$NPV = \sum_{t=1}^{n} \frac{CF_t}{(1+r)^t} - TCI \qquad (5)$$

Internal rate of return (IRR) is defined as any discount rate that results in a NPV of zero. Hence, given the calculation of MESP, discount rate was assumed to be the same as IRR at 10%. For the discounted cash flow rate of return (DCFROR) analysis plant lifetime is assumed to be 30 years, with a 3-year construction period, and 3-month start-up period in the first year. The plant was assumed to be 40% equity financed, with a 10-year loan period at 8% APR. For capital depreciation, a straight-line depreciation was assumed over 10 years. Tax rate was assumed to be 30%. Working capital was 5% of total fixed capital investment. Direct costs for warehousing, piping and site development, along with indirect costs for permitting, construction and other expenses were included in the calculations for total fixed capital investment. Total sales per year from co-products – 2-phenylethanol and yeast extract were estimated to be €66,200.

The MESP for refined oil was calculated to be €14,000 per tonne. The calculation of NPV at the MESP has a 97% relative standard deviation, with 5% and 95% percentiles ranging between -0.571 MM€ and 0.448 MM€. Further analysis of the small-scale facility is available in the supplementary information.

3.2 10,000 tonne per year scale facility

The production of 10,000 tonnes of SCO per year was modelled using either lignocellulosic feedstock or sucrose (as a comparator). The lignocellulosic feedstocks assessed were wheat straw, DDGS and draff obtained as waste from the distillery/bioethanol industry. The results for each feedstock and fermentation scenario were assessed using the same non-discounted and discounted cash flow metrics as for the pilot facility – cost of manufacture (COM) and discounted cash flow analysis-derived minimum estimated selling price (MESP).

3.2.1 Capital expenditure

As assumed in the 100 tonne per year facility, installed equipment expenditure per process step was calculated with an associated range of +/- 40%. Based on capital equipment and installation costs (figure 2) the sucrose raceway pond scenario has the lowest initial capital costs (€35MM), whereas the highest capital costs were associated with DDGS CSTR (€111MM) and draff CSTR (€110MM) scenarios. The initial capital investment required for a plant using sucrose as opposed to a lignocellulosic feedstock (and hence not requiring upfront pretreatment and hydrolysis equipment) was comparable to the raceway pond scenarios for lignocellulsoic biomass. As with the smaller 100 tonnes per year facility, fermentation equipment was the greatest contributor to capital cost at €39MM.

Figure 2. Capital expenditure for each scenario at full commercial scale production (showing 25th and 75th percentiles and median for each processing step and total based on a uniform distribution between maximum and minimum values)

3.2.2 Cost of Manufacture

Cost of manufacture (COM) was calculated for each scenario based on capital cost, labour cost, raw materials cost, utilities cost, and waste management cost. As for the demonstration scale facility, cost was given as a cumulative probability function (CDF) based on uncertainties associated with input values using equation 6.

The uncertainty ranges and distributions used to determine COM as a probabilistic cumulative distribution were calculated based on a linear distribution of FCI values (+/- 40%) calculated from equipment costing given in 3.2.1. Raw materials inputs were distributed triangularly. Utilities, waste water treatment (included in water costs), and labour costs used a bootstrapped distribution across historical cost data for the UK over the past 10 years (40). This was performed using Matlab® (n = 10,000).

Figure 3. Cumulative distribution function showing cost of manufacture (COM) at a commercial scale facility under a range of feedstock scenarios

Table 2. Median Cost of Manufacture (COM) and standard deviation for commercial facility

Median COM per tonne ranges from €4-10k (figure 3, table 2). The lowest costs were associated with the sucrose feedstock scenarios (€4700-5100) and the highest cost to manufacture was associated with DDGS (€8900-10300). DDGS is the highest priced lignocellulosic feedstock at €228, and coupled with its higher protein, lower carbohydrate content this means that more is required leading to higher raw materials costs and therefore cost of manufacture. At this COM the SCO would be entering the market as a mid-high value chemical, requiring enhanced performance properties not currently provided by existing terrestrial oil markets.

A route to reducing cost is to consider additional revenue from co-products. This is not included in the COM calculation, but is included when calculating the MESP. The importance of producing microbe-derived chemicals as part of a biorefinery system in order to be cost-effective is discussed in previous studies (41-43). Biddy *et al.* (2016) showed that through diversion of a C5-rich fraction following lignocellulosic pretreatment to produce succinic acid, they were able to reduce biodiesel minimum fuel selling price from $9.55/GGE to $5.28 (41). The potential for costs to be reduced further by producing fragrance chemical 2-phenylethanol and yeast extract was explored through discounted cash flow analysis. This also takes into account changing value of capital investments over the 30-year plant lifespan.

One-way ANOVA testing based on data samples from each distribution for the different feedstocks and fermentation methods (CSTR or raceway pond), returned very low p-values for comparison across feedstocks; however, within feedstock groups for sugar and DDGS comparing their two fermentation scenarios, p-values exceeded a 0.05 significant level. This indicates that distributions for scenarios within these feedstocks groups are more strongly similar (assuming acceptance of the null hypothesis that their mean values come from the same group) than between CSTR or raceway pond scenarios across feedstock types.

3.2.3 Profitability

Profitability was calculated using the same assumptions as were used for the 100 tonne per year facility. A discounted cash flow analysis was used to calculate MESP for the refined oil. Hence, given the calculation of MESP, discount rate was assumed to be the same as IRR at 10%. For the DCFROR analysis plant lifetime was assumed to be 30 years, with a 3-year construction period, 3-month start-up period in the first year and 40% equity financed. As for the demonstration facility a straight-line capital depreciation was assumed over 10 years. Tax rate was assumed to be 30%. Working capital was 5% of total fixed capital investment. Direct costs for warehousing, piping and site development, along with indirect costs for permitting, construction and other expenses are included in the calculations for total fixed capital investment. NPV at the MESP is calculated using equation 7.

Additional revenue was obtained from the following: animal feed protein produced at €570 per tonne, 2-phenylethanol at €5700 per tonne and fatty acid (obtained from the refining step) at €685 per tonne. Lignin produced process heat and electricity reducing utilities consumption from the pretreatment and hydrolysis step.

MESP was calculated between €3600-7800per tonne (figure 4). The lowest calculated MESP was for the scenario using sucrose as a feedstock at €3600-4200 per tonne (assuming a feedstock cost €230 per tonne); however, the wheat straw scenarios (feedstock cost €70 per tonne) roughly equivalent to this at €4000-4200 per tonne. DDGS and draff scenarios were found to have an MESP at €5700-7800 per tonne (feedstock cost €228 and €40 per tonne respectively). This was due to increased amounts of material required based on carbohydrate content and marginally higher equipment cost based on higher annual throughput. This is particularly true for draff where even though cost per tonne is low, the feedstock is very dilute (18-25wt% solids).

In 2018 wheat straw prices in the UK rose dramatically to between £80-100 per tonne (44). Under these conditions the MESP using wheat straw rises to €4700 per tonne. Similarly, volatility in the cost of sucrose has a dramatic effect on overall MESP values. Evaluating global sugar prices over the period 2017/2018, the highest value reached is €400 per tonne (45). Based on this feedstock price, MESP increases to €5500.

In this analysis co-product 2-phenylethanol was sold at €5700 per tonne. Sensitivity analysis evaluating the effect on MESP if this was sold at a price comparable to that of ethylbenzene (as a bulk commodity chemical rather than a high-value fragrance chemical) shows that this increases MESP by between €100-200, leading to an MESP for the CSTR sucrose scenario of

€4300. Low-cost raceway pond fermentation (which assumes a 23% drop in productivity based on (8)) lowers MESP for sucrose and wheat straw scenarios, but increases MESP for DDGS and draff (figure 4). This is because gains made in CAPEX reduction using a raceway pond are not made back again by the increase in feedstock cost based on the lower productivity. This indicates that where feedstock cost/feedstock processing cost is low, gains can be made by employing a lower cost fermentation method, however, at higher feedstock/feedstock processing costs and with a drop in fermenter productivity, lower-cost fermentation does not provide an economic advantage.

Sensitivity analysis of the sucrose CSTR scenario shows greatest sensitivity to overall lipid yield (figure 5). This is followed by feedstock cost, then variable and fixed operating costs, then, total co-product yield and initial capital investment. Based on this ±20% sensitivity analysis it can be concluded that economic viability is most sensitive to lipid productivity. If revenue stream from co-products were to increase, then this would lead to an increased sensitivity to co-product yield also. These findings confirm those of (8, 10) that productivity has a substantial impact on selling price. However, on evaluating specific market price fluctuations for feedstocks, the cost of the feedstock can vary far beyond ±20%, and therefore this can have as substantial an effect on MESP and overall economic viability as productivity, if not surpassing it.

Figure 4. Minimum estimated selling price (MESP) for SCO at commercial scale under a range of feedstock scenarios

Figure 5. Sensitivity analysis of net present value (NPV) for SCO at commercial scale using a sucrose feedstock

Each biomass feedstock was assigned a price range based on the market rate, in order to reflect their use in the agricultural industry as animal feed or animal bedding, rather than as a waste material (assigning a nominally low value). DDGS and draff are promising feedstocks from a processing perspective, having already been partially processed, they also contain nutrients, nitrogen and other elements used by the yeast during fermentation.

In this analysis sucrose and lignocellulosic biomass all yield the same co-products (with lignin from the biomass also used for process energy generation), with low-cost fermentation achievable due to the ability of *M. pulcherrima* to grow in non-sterile conditions (22). However, from alternative yeast or other lignocellulosics a range of other biochemicals and biomaterials could be obtained as part of the biorefinery system. This includes succinic acid (46), hydroxymethylfurfural (HMF) (47) , and nanocellulose (48). Both HMF and succinic acid are important platform chemicals. More effective utilisation of biomass components during hydrolysis could yield additional co-products which may lead to lignocellulosics matching, if not surpassing, the MESP for sucrose. This also has important environmental implications - moving away from reliance on first generation feedstocks competing directly with food production.

Figure 6. Minimum Estimated Selling Price (MESP) for sucrose STR as a function of productivity (tonne/hour) for a range of feedstock prices

Productivity of refined SCO for the sucrose STR scenario modelled in the TEA analysis is 1.13 tonnes hour^{-1}. Based on a sucrose price of €230/tonne, sensitivity analysis shows that even with an improvement in productivity of 50% this does not take the MESP below €2000/tonne (figure 6).

4. Conclusions

Based on this model for an SCO biorefinery, the impact of feedstock choice and fermentation method are demonstrated. The work shows that at a scale of 10,000 tonnes per year economic viability is highly dependent on feedstock price and fermentation productivity. Sucrose and wheat straw scenarios led to the lowest MESP (€3600-4200 per tonne) compared with distillery by-products which had a far higher MESP at €5700-7800 per tonne. This difference was based on higher feedstock and feedstock processing costs.

Low-cost raceway pond fermentation was shown to significantly lower the MESP of sucrose when compared with CSTR fermentation, but for distillery by-products MESP was increased, as reduced initial capital costs did not overcome the drop in productivity where feedstock and processing costs are higher. This shows that lower-cost fermentation methods (which result in a lower productivity) are only cost-effective where feedstock/feedstock processing costs are low.

Uncertainty relating to optimal process design for emerging SCO technology at scale is high, and insight into the performance of the SCO biorefinery system has been demonstrated under uncertainty for the first time. The MESP determined here for a range of feedstocks shows that the SCO can only be economically viable as a mid to high-value chemical – therefore needing to offer additional functionality and benefit over existing terrestrial oils. It is therefore, even at higher productivities, not comparable to existing oil products, but could become a viable technology in the future through greater valorisation of coproducts, integration with existing processes and waste product streams – reducing feedstock cost, and improved overall fermentation productivity.

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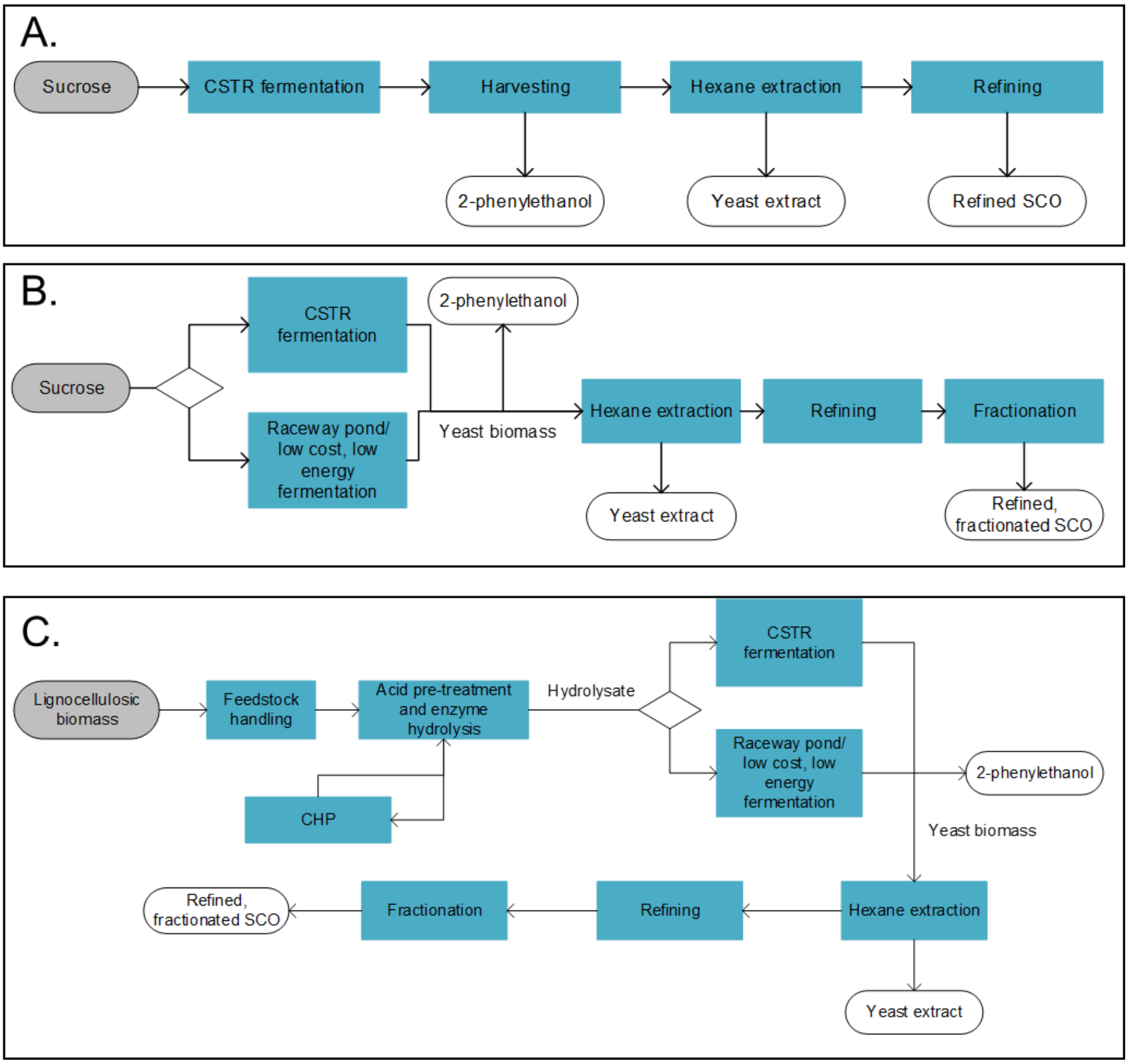

Figure 1. Microbial oil production process at two different scale (a) 100 tonne per year scale using a sucrose feedstock, (b) 10,000 per year scale using a sucrose feedstock, (c) 10,000 tonne per year scale using a lignocellulosic feedstock

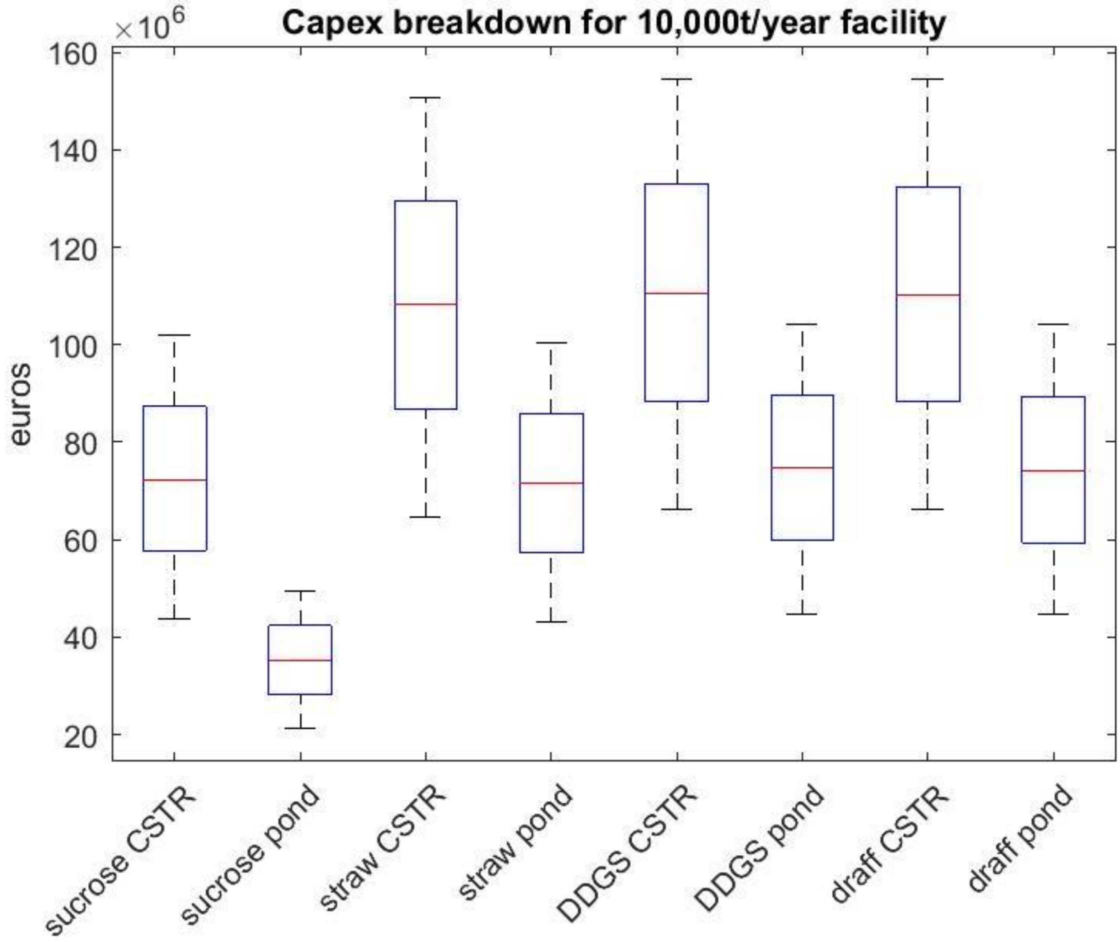

Figure 2. Capital expenditure for each scenario at full commercial scale production (showing 25th and 75th percentiles and median for each processing step and total based on a uniform distribution between maximum and minimum values)

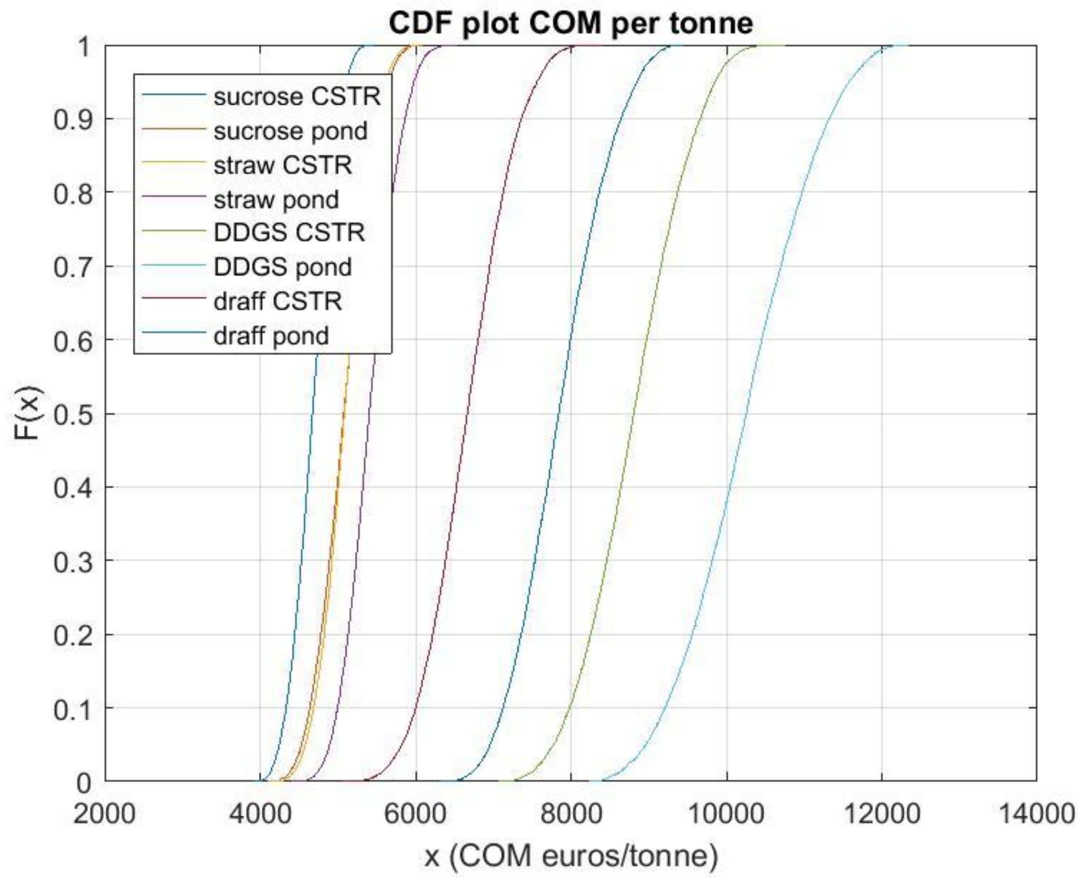

Figure 3. Cumulative distribution function showing cost of manufacture (COM) at a commercial scale facility under a range of feedstock scenarios

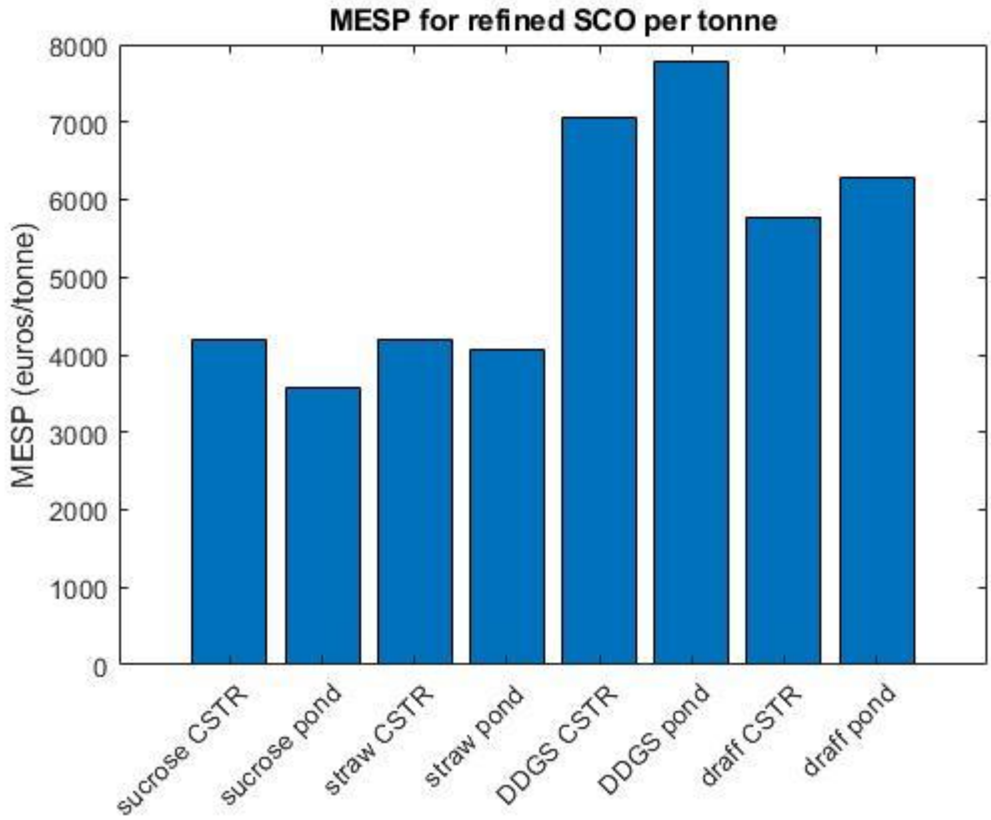

Figure 4. Minimum estimated selling price (MESP) for SCO at commercial scale under a range of feedstock scenarios

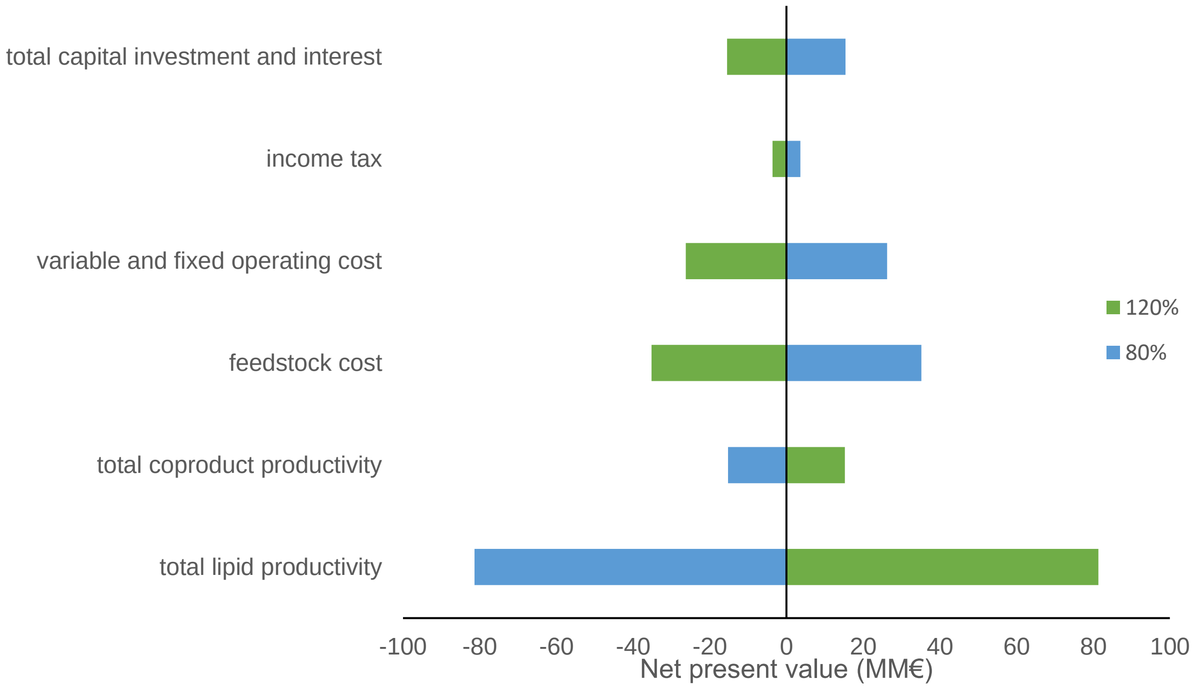

Figure 5. Sensitivity analysis of net present value (NPV) for SCO at commercial scale using a sucrose feedstock

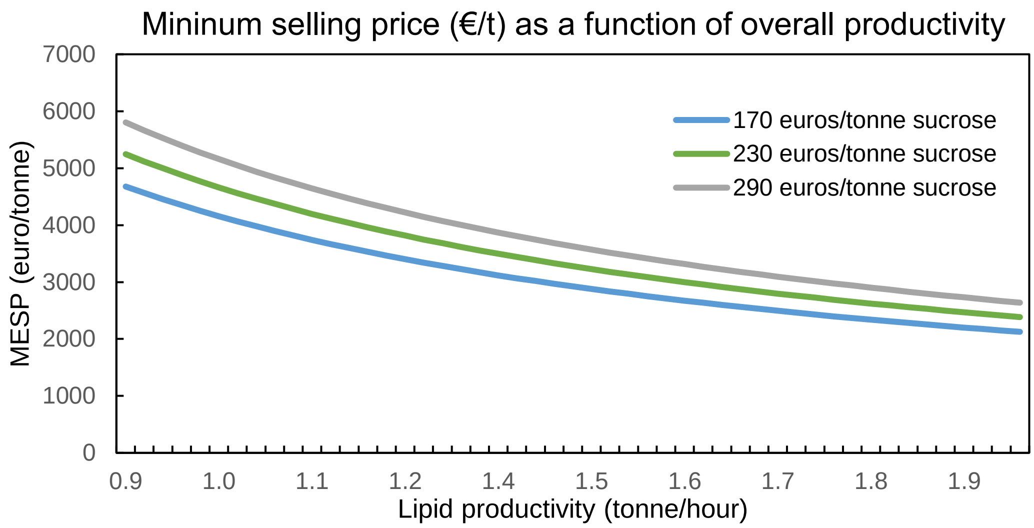

Figure 6. Minimum Estimated Selling Price (MESP) as a function of productivity (tonne/hour) for +/- 25% feedstock price

Table 1. Breakdown of techno-economic assumptions at 100 tonnes per year and 10,000 tonnes per year facility

Techno-economic analysis assumptions for the SCO production facilities			
Plant life span	30 years	Interest rate	8%
Operating hours	8410 per year	Loan term	10 years
Cost year	2017	Depreciation	Straight-line
CEPCI	562.1	Salvage value	0
Discount rate	10%	Construction period	3 years
Income tax rate	30%	Working capital (% of FCI)	5%
Equity percentage of total investment	40%	Yeast productivity	1.3 g l^{-1} hr^{-1}

Table 2. Median Cost of Manufacture (COM) and standard deviation for commercial facility

Scenario	Median COM per tonne (€)	Standard deviation (SD)
Sucrose CSTR	4674	261
Sucrose pond	5077	341
Wheat straw CSTR	5084	308
Wheat straw pond	5404	329
DDGS CSTR	8809	629
DDGS pond	10257	788
Draff CSTR	6672	522
Draff pond	7844	567