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Sustainability and life cycle assessment (LCA) of macroalgae-derived single cell oils

Sophie Parsons[1], Michael J. Allen[2,3], Felix Abeln,[4] Marcelle McManus[1], Christopher J. Chuck[4]

[1]Department of Mechanical Engineering, University of Bath, Claverton Down, Bath, BA2 7AY

[2]Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH

[3]College of Life and Environmental Sciences, University of Exeter, Exeter, Devon, UK, EX4 4QD

[4]Department of Chemical Engineering, University of Bath, Claverton Down, Bath, BA2 7AY

Abstract

Marine macroalgae (seaweed) has many advantages over terrestrial crops as a source of renewable biomass but is severely underutilised at present, especially within Europe. In particular, macroalgae has elevated poly- and monosaccharide content, making it an ideal feedstock as a heterotrophic fermentation sugar source for the production of higher value chemicals. Recent reports have detailed the suitability of seaweeds as a feedstock for the production of single-cell oils (SCOs) which have application in food, oleochemicals and fuels. It is proposed that a biorefinery system based on the production of SCOs alongside other secondary metabolites, has the potential to provide a sustainable replacement to terrestrial oils such as palm oil.

This work therefore evaluates, for the first time, the environmental and economic sustainability of a production process for SCOs from seaweed *Saccharina latissima* using the oleaginous yeast *Metschnikowia pulcherrima*. Two alternative fermentation systems were considered, and uncertainties associated with the seasonal variation in seaweed carbohydrate yield and fermentation performance were integrated into the analysis. From an environmental perspective, the work indicates that seaweed derived SCO lipids and fats can be comparable to a terrestrial oil mix, with a potential climate change impact ranging between 2.5 - 9.9 kg CO_2 eq. kg^{-1} refined SCO. Interestingly and of particular significance, environmental impacts are mainly dominated by energy demand within fermentation and upstream processing steps. From an economic perspective, a break-even selling price for the oil was determined as between €5,300-€31,000 $tonne^{-1}$ refined SCO, which was highly dependent on cost of the seaweed feedstock.

Overall, we demonstrate that key uncertainties relating to seaweed cultivation costs and hydrolysate fermentation at scale result in a large range in values for environmental impact and economic return on investment. Yet even within the constraints and limitations of current knowhow, seaweed already offers a viable proposition for the competitive production of exotic oils similar to cocoa or shea butter in price and nature.

Keywords

Saccharina latissima, oleaginous yeast, single cell oils, life cycle assessment, economic analysis

Highlights

- Life cycle assessment (LCA) and economic analysis of single cell oils (SCOs) derived from seaweed *Saccharina latissima* performed for the first time

- Climate change impact for process determined to be between 2.5 – 9.9 kg CO_2 eq. kg^{-1} refined SCO

- Break-even selling price for the oil calculated as between €5,300-€31,000 $tonne^{-1}$ refined SCO

- Climate change impacts within the range of what has previously been quoted for microalgae and terrestrial oil mixes

- Lower-end break-even pricing is closer to that of exotic oils and fats than terrestrial oils like palm oil

1. Introduction

Macroalgae has wide-ranging use in food, materials, chemicals and health applications. For over 14,000 years seaweeds have played an important role in diet and health provision (Dillehay, Ramírez et al. 2008, Kim, Yarish et al. 2017), and today the global industry is worth more than USD 6 billion (FAO 2018). Aside from food applications (which accounts for 83-90% of the seaweed market) seaweeds are also farmed to produce hydrocolloids such as alginate, agars and carrageenan (40% of the total global hydrocolloid market) (FAO 2018).

There is increasing interest in the use of seaweeds in industrial processes as an alternative to terrestrial biomass. Their fast growth and high photosynthetic efficiency lead to increased production yields per unit area compared with terrestrial lignocellulosics (Subhadra and Edwards 2010, Wei, Quarterman et al. 2013), and a higher rate of carbon dioxide fixation means that they have greater potential for carbon dioxide remediation (Gao and Mckinley 1994, Wei, Quarterman et al. 2013), and the effect of cultivation on bioremediation of contaminated waters can add additional social and ecosystem value (van den Burg, van Duijn et al. 2016). Additionally, seaweeds do not compete for land with other crops, and do not require freshwater for cultivation. From a processing perspective, little or no recalcitrant lignin and cellulose in its crystalline form means that depolymerisation can occur more easily compared with plant biomass. Ecologically, other potential benefits of macroalgae cultivation include the provision of nursery grounds for young commercial fish and crustaceans, the removal of excess nutrients which could cause eutrophication, and the protection of the seabed where otherwise scouring through bottom-trawling could occur (Cottier-Cook, Nagabhatla et al. 2016). However, compared to well defined terrestrial biomass cultivation boundaries; marine boundaries are literally fluidic in nature, are three dimensional and encompass uncontrollable benthic and planktonic components in addition to fixed infrastructures, making production within a designated area more difficult to contain. Accordingly, there is greater variability and functional connectivity of ecosystems within the marine environment making the benefits and risks of large-scale seaweed cultivation both harder to define and measure (Roberts and Upham 2012).

Macroalgae can be categorised into green (Chlorophyceae), brown (Phaeophyceae) and red (Rhodophyceae) varieties (Chen, Zhou et al. 2015). Polysaccharides found within macroalgae include:

cellulose; starch; laminarin (the main storage polysaccharide within brown seaweed); fucoidan (sulphated fucose-rich polysaccharide found in brown seaweed); carageenan (a sulphated polysaccharide found in red seaweed); alginate (a structural polysaccharide found in brown seaweed); and agar (a mixture of two polysaccharides, agarose and agaropectin, found in red seaweed) (Wei, Quarterman et al. 2013). These are extracted from seaweed via a similar process to that of terrestrial lignocellulosic biomass: mechanical milling/chopping to increase surface area followed by dilute acid pretreatment and/or enzymatic hydrolysis. The resulting hydrolysate can be used to produce biofuels and other biochemical through yeast or bacterial fermentation (Kraan 2013). Other fuel product routes from seaweed include use of the whole biomass for hydrothermal liquefaction (Raikova, Le et al. 2017), anaerobic digestion (Vanegas and Bartlett 2013), and conversion to a syngas via pyrolysis or gasification (Milledge, Smith et al. 2014).

From a life cycle assessment (LCA) perspective, few studies have chosen to concentrate solely on cultivation, instead including cultivation within the context of a biorefinery system. To date, this has for biofuels production (Langlois, Sassi et al. 2012, Alvarado-Morales, Boldrin et al. 2013, Aitken, Bulboa et al. 2014, Seghetta, Hou et al. 2016), with several studies also addressing sustainability in the context of high-value compounds and bioplastics (Pérez-López, Jeffryes et al. 2016), (Murray, Moane et al. 2013, Charoensiddhi, Lorbeer et al. 2018, Helmes, López-Contreras et al. 2018). Recently it has been proposed that single cell oils (SCOs) could be produced from seaweed sugars through yeast fermentation as part of a biorefinery concept (Abeln, Fan et al. 2018). SCOs can be used for food, biochemicals, and biodiesel, replacing existing terrestrial oils or higher value oils and fats such as coconut oil or cocoa butter depending on the molecular composition (Kyle and Ratledge 1992). This could have a substantial effect on the sustainability of the oils and fats market, stemming increased demand for oils which otherwise could lead to further deforestation and biodiversity impacts. Yeasts have a high specific growth rate (compared with moulds and microalgae), and are able to accumulate large percentages of intracellular lipids (>40 %w/w) making them suitable for industrial SCO production (Papanikolaou and Aggelis 2011). Much of the literature evaluated to date addressing SCO sustainability (from heterotrophic organisms) has been limited to the use within biofuels (Koutinas, Chatzifragkou et al. 2014, Chang, Rye et al. 2015, Orfield, Levine et al. 2015, Karlsson, Ahlgren et al. 2016). Given this, their wide-ranging potential within foods and other products means analysis across a range of sectors is needed (Parsons, Chuck et al. 2017). Feedstock use and fermentation productivity have been shown to be key factors determining environmental impact (Parsons, Abeln et al. 2019).

Brown kelp species *Laminaria digitata* and *Saccharina latissima* are commonly found in Northern Europe and have attracted attention as a carbohydrate rich feedstock for the production of bioenergy and biochemicals. The industrial cultivation of *Laminaria digitata* currently involves reproduction and culture development under laboratory conditions, before deployment at sea over 6-7 months and subsequent harvesting (Edwards and Watson 2011). This yields approx. 7-8 kg dry weight m^{-1}. Using a 30m x 30m grid system with 6 grids per hectare, this leads to an overall yield of 18.9 tonnes of seaweed per hectare (Edwards and Watson 2011). An alternative ring design for offshore cultivation is described by (Buck and Buchholz 2004). For this design offshore cultivation cost per tonne (dry weight) equates to US$3,450 (Buck and Buchholz 2004). This can be contrasted with a previous Dutch study which estimates cost per tonne (dry weight) at between $155 and $564 (Reith, Deurwaarder et al. 2005). A more recent Irish study put the breakeven cost of production at between €1120 and €2150, with the lower value based on co-production within a scallop hatchery and mussel farm (Edwards and Watson 2011). Van den Burg et al. (2016) reviewed economic feasibility of seaweed cultivation within the North Sea. The authors anticipate large-scale farming to be based on long-line systems, similar to that used by mussel farmers, which could be incorporated into existing off-shore wind infrastructure. With a production yield of 20 tonnes per hectare, a

4,000-hectare scale production facility was envisaged. Economic modelling of this scenario resulted in a break-even price of $1,747 tonne^{-1} dry weight, and a break-even productivity of 63 tonnes hectare^{-1} (dry weight). Despite this, the average price attainable from North Sea seaweed was found to be only US$555 tonne^{-1} dry weight (van den Burg, van Duijn et al. 2016). Given these significant cost ranges, this uncertainty over large-scale cost of production currently inhibits further use of this feedstock across the UK and Europe.

Emerging technologies, such as those utilising seaweed within a biorefinery context, are often challenging to assess given that technology often still at the laboratory scale, and markets are not established for the particular feedstock application. Despite this, it is crucial to understand the environmental and sustainability implications of new and emerging technology at the early stages of commercialisation. This work evaluates the environmental and economic sustainability of a production process for SCOs from seaweed *Saccharina latissima* using the oleaginous yeast *Metschnikowia pulcherrima*. The evaluation of the environmental life cycle impacts associated with heterotrophic fermentation of seaweed sugars has not been carried out before, with SCO production based on a semi-continuous fermentation at the 2L laboratory-scale. The process also yields fragrance chemical 2-phenylethanol and a proteinous yeast extract as part of a biorefinery system. The process could be used to produce a replacement to terrestrial oils such as palm oil, and therefore has clear implications for sustainable consumption and resource use. Given high uncertainties associated with system performance at scale as well as seasonal variability in seaweed carbohydrate content, ranges in fermentation productivity and fermentable sugar yield are integrated into the assessment. Sensitivity of environmental and cost impact to fermentation method is also addressed. Overall, the work explores the potential for seaweed to be used as a feedstock for SCO production integrating uncertainty into the assessment process for seaweed sugars valorisation.

2. Methodology

2.1 Life cycle assessment

The suitability of converting macroalgal sugars into SCOs has been established (Abeln, Fan et al. 2018), however, the sustainability of microbial oil production via heterotrophic fermentation using seaweed as a biomass feedstock has not previously been assessed.

Life cycle assessment (LCA) was used to evaluate potential environmental impacts associated with using *S. latissima* as a feedstock for SCO production. Energy and resource consumption associated with the system was included in this assessment. The following outlines 1. Goal and Scope definition, 2. Life Cycle Inventory (LCI), and 3. Life Cycle Impact Assessment (LCIA), along with assumptions and limitations of the study. The LCA is carried out in accordance with ISO 14040. A consequential approach is taken, applying systems expansion to coproducts: protein production and 2-phenylethanol.

2.1.1 Goal and scope definition

The LCA aimed to understand where environmental hotspots are when using *S. latissima* as a feedstock for SCOs. It also aimed to evaluate the range in environmental impact values under uncertainty, assessing two different fermentation systems: a stirred-tank reactor and a raceway pond. The functional unit was defined as *one tonne of refined SCO produced*.

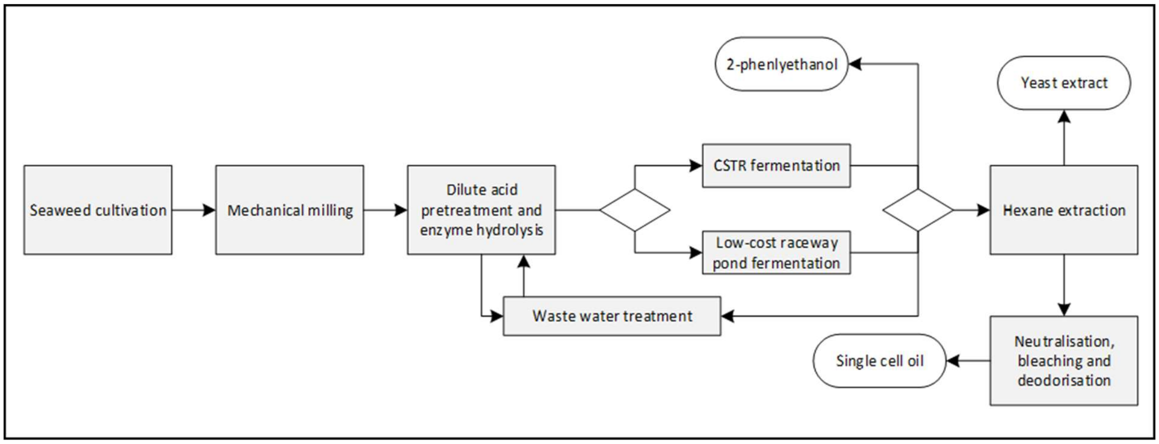

Figure 1. Process flow of single cell oil (SCO) production from seaweed as part of a biorefinery concept

The scope covered energy and raw materials inputs into seaweed cultivation, mechanical milling, dilute acid pre-treatment and enzymatic hydrolysis, fermentation, waste water treatment, extraction using hexane, and further processing via a neutralisation, bleaching and deodorisation step (figure 1). Alongside the SCO produced via fermentation, the process also yields fragrance chemical 2-phenylethanol and a proteinous yeast extract. 2-phenylethanol is extracted directly from the fermentation broth, and the extracted yeast biomass removed following hexane extraction. Production was based on a process which yields 10,000 tonnes of unrefined SCO per year.

2.1.2 Life cycle inventory

Process performance, and raw material and energy inputs were modelled using a combination of experimental data, literature values and the Econivent 3.4 database (Wernet, Bauer et al. 2016). LCI modelling was carried out using Brightway LCA software in Python (Mutel 2017).

Initial hatchery cultivation of *S. latissima* used data for *S. latissima* plantlet production under laboratory conditions (Langlois, Sassi et al. 2012), followed by *L. digitata* off-shore cultivation on ropes as described in Alvarado-Morales, Boldrin et al. (2013). It is assumed that industrial off-shore cultivation of the two species would be the same. Cultivation area required is roughly 7,000 ha. -. Spores are collected from the wild, where plantlets are then cultivated in ponds under laboratory conditions. To facilitate growth, mineral fertilisers, florescent lamps, spargers, and circulation pumps are used (table 1). Total energy demand for laboratory conditioning is 342 kWh per tonne of dry seaweed. The majority of this electricity relates to lamps and sparger use for bubbling. The culture is then deployed on long-line systems out at sea. For deployment 5 L petrol per tonne of dry seaweed is used. Following cultivation (over 4-6 months) the seaweed is collected using a further 25 L petrol per tonne of dry seaweed (Alvarado-Morales, Boldrin et al. 2013). The seaweed was dried and transported 50 km to the biorefinery facility. Sugars are released via acid pretreatment and an enzymatic hydrolysis. Processes for milling and hydrolysis were based on the NREL bioethanol from corn stover model (Humbird, Davis et al. 2011). Total carbohydrates were assumed to be 60% based on Nielsen, Manns et al. (2016). The theoretical yield of fermentable sugars was calculated based on the efficiency of cellulose, hemicellulose and lignin breakdown from corn stover (Humbird, Davis et al. 2011). Electricity consumption for milling is 9.8 kWh per tonne of dry seaweed. Electricity consumption during pre-treatment and enzymatic hydrolysis 119 kWh per tonne of fermentable material produced. During the hydrolysis step 581 MJ of steam was estimated to be consumed per tonne of fermentable material.

Fermentation was modelled using 12 x 250 m^3 stirred-tank reactors, with a maximum working volume of 85%. The yeast used for fermentation was *M. pulcherrima*, with a biomass yield of 0.35 g g^{-1} hydrolysate (sugar) and culture density of 120 g L^{-1}, yielding 1.3 g L^{-1} h^{-1} yeast biomass which corresponds to 0.52 g L^{-1} h^{-1} lipid production (table 1). This is based on experimental data for the continuous fermentation of *M. pulcherrima* on glucose. Two types of reactor system were modelled using the assumptions made in Braunwald, French et al. (2016). A continuously stirred-tank reactor (CSTR) is a commonly used reactor design. This is a simple reactor with a continually rotating shaft with mounted impellers and/or propellers of different types. Because of need for mechanical mixing, CSTR fermentation can be relatively energy intensive. Energy demand for CSTR fermentation was 3050 kWh per tonne of yeast biomass produced based on Koutinas, Chatzifragkou et al. (2014) using data for heterotrophic fermentation at scale. An alternative design is a raceway pond fermenter. 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 (Brennan and Owende 2010). *M. pulcherrima* has previously been grown under non-sterile conditions in a 500 L, open air reactor (Santomauro, Whiffin et al. 2014). There is a 12% reduction in biomass productivity and a decreased lipid content of 35%, caused by the poor mixing and temperature fluctuations within the raceway pond. This leads to an overall reduction in lipid productivity of 23%, but also reduction in electricity demand to 1860 kWh t^{-1} yeast biomass (Braunwald, French et al. (2016)) (table 1). Following fermentation, the product stream was modelled to pass through an adsorption column which removed 2-phenylethanol (Chantasuban, Santomauro et al. 2018). A tonne of yeast biomass produced 9.5 kg of 2-phenylethanol. It was assumed that this displaces the production of benzene from fossil fuels.

Lipid extraction was carried out via a wet extraction with hexane. Modelling for this process is based on data from Davis, Kinchin et al. (2014). This means prior homogenisation and drying is not required. Energy demand for extraction is 330 kWh/t unrefined lipid produced. The yeast biomass contains 40% lipid, with a further 40% removed as a proteinous yeast extract for animal feed. Per tonne of unrefined oil produced this displaces 1 tonne of protein feed (based on global market for protein feed (Wernet, Bauer et al. 2016)). Following this, the oil was refined and upgraded. To this end, the lipid product was mixed with 0.19 wt% phosphoric acid and an additional 10 wt% wash water, which was then centrifuged. This removes any polar phospholipids present. The phosphoric acid was neutralised using sodium hydroxide (2.5 wt%), which removed any free fatty acids from the product stream. The stream was then bleached using clay (0.2 wt%) which removed any other impurities. The efficiency of the purification step was estimated at 95% (Davis, Kinchin et al. 2014). The refined lipid is analogous to the lipid profile of palm oil. All electricity inputs are modelled using the electricity mix for the UK derived from the Digest of UK Energy Statistics 2016 (BEIS 2016).

Table 1. Life Cycle Inventory (LCI) for the production of microbial derived oil from seaweed

Input	Value	Source
Cultivation		
Nursery		(Langlois, Sassi et al. 2012)
Ammonium nitrate	0.08 t^{-1} dry seaweed	
Sodium phosphate	0.03 t^{-1} dry seaweed	

Iron (III) chloride	0.003 t^{-1} dry seaweed	
Anhydrous boric acid	0.02 t^{-1} dry seaweed	
Mineral solution (EDTA)	0.02 t^{-1} dry seaweed	
Electricity (water pumping, lamps, sparger)	342 kWh t^{-1} dry seaweed	
Water	4600 L t^{-1} dry seaweed	
Diesel	30 L t^{-1} dry seaweed	Alvarado-Morales, Boldrin et al. (2013), Edwards and Watson (2011)
Petrol	30 L t^{-1} dry seaweed	
Transport	100 tkm	
Pre-treatment and hydrolysis (incl enzyme production)		Humbird, Davis et al. (2011)
Electricity (milling)	9.83 kWh t^{-1} milled dry seaweed	
Water	2.44 m^3 t^{-1} fermentable hydrolysate	
Ammonia	17 kg t^{-1} fermentable hydrolysate	
Sulphuric acid	33 kg t^{-1} fermentable hydrolysate	
Sodium hydroxide	54 kg t^{-1} fermentable hydrolysate	
Quicklime	21 kg t^{-1} fermentable hydrolysate	
Sulphur dioxide	0.3 kg t^{-1} fermentable hydrolysate	
Sugar	40 kg t^{-1} fermentable hydrolysate	
Heat (Steam)	1314 MJ t^{-1} fermentable hydrolysate	
Electricity	119 kWh t^{-1} fermentable hydrolysate	
Fermentation (CSTR)		Experimental data for *M. pulcherrima*, Koutinas (2014), Braunwald (2016)
Nutrients	0.22 kg t^{-1} yeast biomass	
Electricity	3050 kWh t^{-1} yeast biomass	
Biomass productivity	1.3 g L^{-1} h^{-1}	
Lipid productivity	0.52 g L^{-1} h^{-1}	
Fermentation (raceway pond)		
Nutrients	0.28 kg t^{-1} yeast biomass	
Electricity	1860 kWh t^{-1} yeast biomass	
Biomass productivity	1.14 g L^{-1} h^{-1}	
Lipid productivity	0.4 g L^{-1} h^{-1}	
Lipid extraction and refining		Davis et al. (2014)
Hexane	66 kg t^{-1} unrefined lipid	
Electricity	500 kWh t^{-1} unrefined lipid	
Water	740 kg t^{-1} lipid	
Phosphoric acid	0.3 kg t^{-1} lipid	
Sodium hydroxide	3 kg t^{-1} lipid	
Clay	5 kg t^{-1} lipid	
Heat (steam)	350 MJ t^{-1} lipid	
Electricity (fractionation)	13 kWh t^{-1} lipid	
Water (fractionation)	100 kg t^{-1} lipid	

2.1.3 Life cycle impact assessment

Based on the inputs and outputs of the system (determined in the LCI step), the potential environmental impacts were measured within the life-cycle impact assessment (LCIA) phase. The modelling was carried out within Brightway (Mutel 2017). The ReCiPe method was adopted for conducting the LCIA using the midpoint hierarchist model. The following impact categories were assessed: climate change (kg CO_2 eq), freshwater ecotoxicity (kg 1,4-DB eq), freshwater eutrophication (kg P eq), human toxicity (kg 1,4-DB eq), marine ecotoxicity (kg 1,4-DB eq), marine eutrophication (kg N eq), terrestrial ecotoxicity (kg 1,4-DB eq), terrestrial acidification (kg SO_2 eq), and water depletion (m^3).

These environmental impacts are reported in terms of their relative contributions to total impact per functional unit. Monte Carlo simulations were run within Brightway (Mutel, 2017) (across 10,000 iterations), evaluating uncertainty distributions of the following foreground parameters: distance from seaweed cultivation site to biorefinery, carbohydrate content of the seaweed, and fermentation productivity (Table 2).

Table 2. Distributions assigned to exogenous variables for Monte Carlo analysis

Exogenous variable	Minimum	Maximum	Distribution shape	Source
Seaweed carbohydrate composition (w/w)	0.40	0.70	Triangular	Nielsen, Manns et al. (2016)
Transport distance (tkm)	0	500	Triangular	
Lipid productivity (yeast) (g L^{-1} h^{-1})	0.32	0.56	Triangular	Jin, Slininger et al. (2015)

2.1.4 Assumptions and limitations

To date, very few LCA studies have addressed seaweed cultivation and use within a biorefinery concept. None have addressed the use of seaweed feedstocks for a microbial oil production process using heterotrophic organisms. Data for heterotrophic fermentation is scarce and given the early-stage nature of this process there are a number of limitations to this work which are listed below:

- The scale of seaweed cultivation required for a 10,000 tonne yr^{-1} scale microbial oil production facility is >120,000 tonnes yr^{-1}. This is almost half of the entire European wild harvest, and far more than what is currently produced via formal aquaculture. Material and energy inputs are therefore based on what is known about production at a much smaller scale in Europe.
- Seasonal variability of carbohydrate content is very high which affects the yield of fermentable hydrolysate. Variation of between 40% and 70% (table 2) is built into impact distribution calculations using Monte Carlo analysis.
- Saccharification of lignocellulosic feedstocks typically requires prior hydrothermal or physiochemical treatment in order to solubilise and disrupt lignin and break down the crystalline structure of cellulose. Given the absence of lignin in seaweed such harsh pre-treatment methods may not be needed. For example, previous work has shown non-milled seaweed material to still release glucose and mannitol following enzymatic treatment (Manns, Andersen et al. 2016). There is still uncertainty as to the optimal conditions for sugar release, and therefore a worst-case corn stover process is used, assuming milling, dilute acid pretreatment, followed by enzymatic hydrolysis.
- Experimental performance data was based on a 2 L bioreactor run semi-continuously for 28 days on glucose. There are a number of complex factors affecting scale-up performance, and reliance on laboratory scale data leads to high uncertainty relating to both environmental and economic aspects. Variation in yeast biomass yield is expressed within Monte Carlo analysis (table 2) using data for growth of various oleaginous microbes on lignocellulosic hydrolysate.
- There is limited data in the literature for industrial lipid extraction from yeast. Data for extraction is based on the wet extraction of lipid from microalgae. There is uncertainty on

the ability to extract 95% lipid from yeast biomass using hexane at this scale, and the energy inputs required to adequately disrupt and break apart the cells and then remove water and hexane following extraction.

2.2 Economic analysis

Economic analysis was carried out assuming production of unrefined SCO at a 10,000 tonne year^{-1} scale. Two methods of cost analysis were used: a non-discounted Cost of manufacture (COM) based on Turton, Baille et al. (2009), and a discounted cash flow analysis used to determine a break-even selling price for the microbial oil. The analysis does not include the costs associated with seaweed cultivation, assuming a baseline purchase price of €469 tonne^{-1} dry matter (DM) (van den Burg, van Duijn et al. 2016).

Installed equipment cost was based on milling and hydrolysis from Humbird, Davis et al. (2011), fermentation data in Koutinas, Chatzifragkou et al. (2014) and Braunwald, French et al. (2016), and downstream processing in Davis, Kinchin et al. (2014). These were adjusted using the six-tenths rule for equipment sizing and then converted to the reference year (2017) using the Chemical Engineering Plant Cost Index (CEPCI). Cost data was converted from GBP to Euros (1 GBP = 1.141317 EUR (2017)).

COM calculations per tonne of refined SCO were represented as a probability distribution in order to incorporate uncertainty into calculations. This was carried out in Matlab®, with each distribution sampled 10,000 times.

COM was calculated using equation 1, using assumed relationships between the individual elements given in Turton, Baille et al. (2009). 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. FCI relates to fixed capital investment. Discount rate was excluded from this calculation.

$$COM = 0.180FCI \times 2.73C_{OL} \times 1.23(C_{UT} \times C_{WT} \times C_{RM}) \qquad (1)$$

Break-even selling price per tonne of SCO was determined based on a calculation of net present value (equation 2). This was 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.

$$NPV = \sum_{t=1}^{n} \frac{CF_t}{(1+r)^t} - TCI \qquad (2)$$

For discounted cash flow 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. 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. 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.

3. Results and Discussion

3.1 Life cycle assessment

Life cycle impact assessment (LCIA) was carried out using ReCiPe (H) midpoint impact assessment method in Brightway (Mutel, 2017). An analysis of environmental hotspots, and a comparative environmental impact of each fermentation scenario under uncertainty was evaluated.

Fermentation and acid pretreatment and enzymatic hydrolysis contributed most strongly to environmental impact across the majority of impact categories assessed. This is due to electrical energy demand during fermentation, as well as electricity and heat (steam) provision during hydrolysis. Seaweed cultivation is the third most dominant environmental impact, accounting for 39% of total potential climate change impact. This relates to electricity use during the nursery stage. Percentage impact scores are skewed by the avoided production of protein from terrestrial crop sources which occurs during the lipid extraction and the remaining yeast biomass is used for animal feed. Marine eutrophication, terrestrial ecotoxicity, and water depletion scores were dominated by this avoided production of protein (based on avoided 'market for protein feed, 100% crude' within Ecoinvent 3.4). The heatmap shows that by comparison downstream processing plays a much smaller part in environmental impact than upstream biomass hydrolysis and fermentation (figure 2).

Given the influence of fermentation energy demand on overall environmental impact, a low energy raceway pond design was also investigated. This reduces energy demand per tonne of yeast biomass produced by 40%, but also reduces productivity. The comparison between using a CSTR and a raceway pond for fermentation integrates uncertainty in terms of the range in carbohydrate yield reported from harvested *S. latissima*, transport distance from farm to biorefinery location, and total biomass yield ($g\ g^{-1}$) based on the range of yields reported for yeast biomass from lignocellulosic hydrolysate (Jin, Slininger et al. 2015). Results for cumulative energy demand (MJ) and climate change potential (kg CO_2e) per tonne of refined SCO produced show that despite the reduction in energy use during fermentation, impact is similar when taking uncertainty into account between the two fermentation methods (figure 3). This is due to the reduced productivity of the fermentation process meaning more feedstock is required and hence further upstream processing and hydrolysis. A breakdown of the 9 ReCiPe (H) Midpoint impact assessment methods assessed along with their uncertainty distributions is given in table 3. All Monte Carlo simulations were run using Brightway in Python, sampling 10,000 times.

Compared to direct microalgae oil production (7.12 kg CO_2 eq. kg^{-1} product (Draaisma, Wijffels et al. 2013)) climate change impact for this process using yeast *M. pulcherrima* is lower. Where land use change is included this is comparable to conventional oil crops (4.85 kg CO_2 eq. kg^{-1} product,European market demand: 21.0% palm, 21.1% rapeseed, 9.7% soy, 25.1% sunflower and 23.1% other oils) (Draaisma, Wijffels et al. 2013) at the lower end of the uncertainty distribution (table 3). -

This is particularly important given that this study assumes mechanical milling and pre-treatment steps that are the same as terrestrial biomass (corn stover). The absence of lignin in seaweed means that such harsh treatment conditions is likely not needed. Hence, there is clear potential for environmental impacts to be reduced further. For fermentation, a biomass productivity of 1.3 $g\ L^{-1}\ h^{-1}$ (resulting in a lipid productivity of 0.52 $g\ L^{-1}\ h^{-1}$) is close to the top end of what has been previously reported for oleaginous yeasts across all fermentation modes (batch, fed-batch, semi-continuous) (Papanikolaou and Aggelis 2011). For example, higher lipid productivities from a fed-batch culture (over 50 h) have been achieved using *Cutaneotrichosporon oleaginosus* at 0.59 $g\ L^{-1}\ h^{-1}$ grown on pure glycerol (Meesters, Huijberts et al. 1996). It was with the same yeast, where lipid productivities close to 1.0 g L^{-1} h^{-1} have been reported when cultured continuously on whey permeate (Ykema, Verbree et al. 1988). However, to achieve such high productivities and beyond, for example through culturing at high cell densities (Pan, Kwak et al. 1986) and genetic modification (Xu, Qiao et al. 2017), oxygen typically becomes the limiting factor (Pan, Kwak et al. 1986, Qiao, Wasylenko et al. 2017). Despite the challenges associated with dramatically increasing biomass and lipid productivity further, as shown from the positive environmental impact values during extraction, the production of proteins and other further compounds during fermentation could also substantially reduce impact.

The SCO production process analysed here has the potential to replace terrestrial oils like palm oil within food, chemicals and fuels markets based on its LCA credentials. However, in reality there are other environmental considerations beyond the scope of LCA which dictate the fate of further seaweed cultivation in Europe. Whilst LCA is key to identifying materials and energy hotspots in the value chain, complex site-specific challenges to do with marine ecosystems are outside its scope, and it is the environmental uncertainties relating to cumulative ecosystem effects which (alongside many other factors) influence investment decisions for cultivation and government support. This means that within future work in this area an integrated assessment approach is needed in order to capture all relevant environmental benefits and drawbacks.

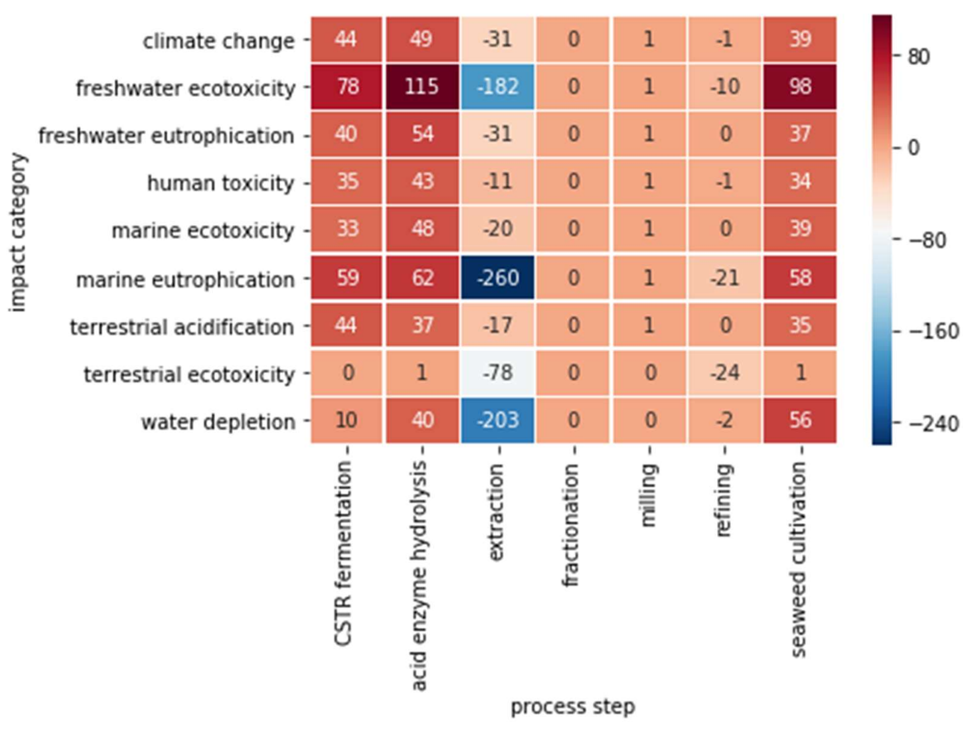

Figure 2. Heatmap of environmental impact across ReCiPe (H) midpoint impact categories for the production of 1 tonne of refined microbial oil

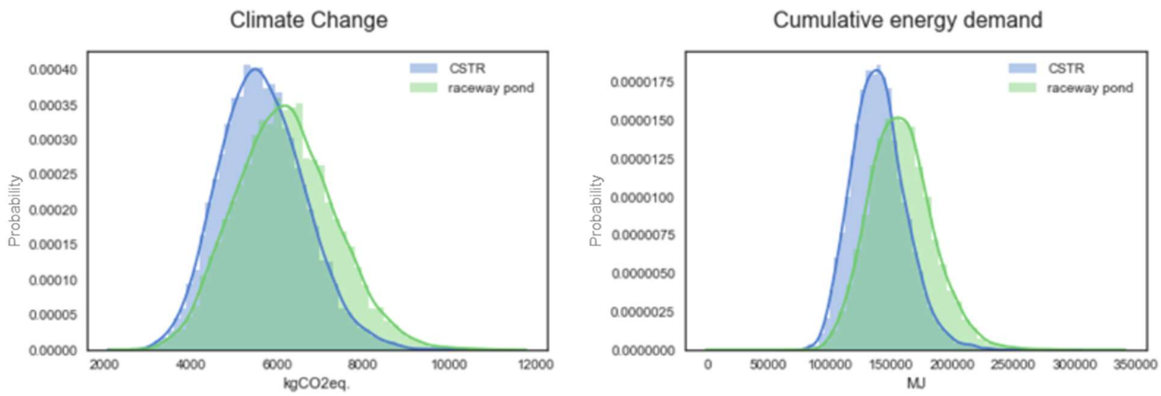

Figure 3. Distribution of Cumulative Energy Demand (CED) (MJ) and Climate Change impact (kg CO_2e) for a stirred-tank reactor system and raceway pond fermentation

Table 3. Probability distributions for ReCiPe (H) midpoint impacts per tonne of refined oil

Impact category	mean	std	min	25%	75%	Max
CSTR						
Climate change (kg CO_2 eq.)	5663	988	2563	4960	6307	9941
Freshwater ecotoxicity (kg 1,4-DB eq.)	52	40	1	32	61	1606
Freshwater eutrophication (kg P eq.)	2	1	0	1	2	29
Human toxicity (kg 1,4-DB eq.)	3826	2739	1066	2558	4203	109197
Marine ecotoxicity (kg 1,4-DB eq.)	75	33	22	57	84	829
Marine eutrophication (kg N eq.)	-3	2	-10	-4	-2	75
Terrestrial ecotoxicity (kg 1,4-DB eq.)	-32	4	-51	-34	-28	-18
Terrestrial acidification (kg SO2 eq.)	49	13	19	39	55	172
Water depletion (m^3)	-134	45	-298	-161	-109	1276
Raceway pond						
Climate change (kg CO_2 eq.)	6188	1146	2670	5391	6940	11204
Freshwater ecotoxicity (kg 1,4-DB eq.)	62	65	5	40	72	5068
Freshwater eutrophication (kg P eq.)	2	2	0	1	2	61
Human toxicity (kg 1,4-DB eq.)	4270	3143	1149	2846	4745	138727
Marine ecotoxicity (kg 1,4-DB eq.)	85	47	30	65	96	2667

Marine eutrophication (kg N eq.)	-2	2	-8	-3	-1	51
Terrestrial ecotoxicity (kg 1,4-DB eq.)	-31	4	-52	-34	-28	-19
Terrestrial acidification (kg SO2 eq.)	51	13	20	42	58	160
Water depletion (m^3)	-101	50	-381	-132	-74	532

3.2 Economic Analysis

Economic analysis investigated both the non-discounted cost of manufacture and profitability based on discounted cash flow analysis. Cost of Manufacture (COM) integrating uncertainty was based on a linear distribution of fixed capital costs (+/- 40%), and a bootstrapped distribution of utilities, waste water treatment (included in water costs), and labour costs across historical cost data for the UK over the past 10 years (ONS 2017). This was performed using Matlab® (across 10,000 iterations).

CSTR fermentation led to a median COM of €16,000 per tonne refined SCO. Using a lower cost raceway pond fermentation (where capital cost is reduced by 90% but productivity is also reduced) this increased manufacturing cost to a median COM of €19,000 per tonne. This means that the lower productivity of the raceway pond cancels out any gains made by reducing initial capital investment. This is due to the significant costs associated with the seaweed feedstock. For this seaweed biorefinery model this indicates that the operational costs (predominately relating to total feedstock cost) had a greater impact on overall manufacturing costs than fixed capital investment (figure 4).

Profitability calculations determined a break-even price for the SCO taking into account sales of co-products. The baseline price used for cost analysis was €469 tonne DM^{-1} which is based on the achievable market price for North Sea seaweed determined by van den Burg, van Duijn et al. (2016). Sensitivity of break-even price to seaweed cost is presented in figure 5. Overall, total annual sales from coproducts 2-phenylethanol and yeast extract was €7,400,000. This was assuming pricing of 2-phenylethanol at €5700 per tonne, and yeast extract and fatty acids at €570 per tonne. This led to a break-even selling price of the refined lipid to be €9700 for the system using a CSTR, and €10,700 for the system using a raceway pond. CSTR break-even price increased to €9800 when assuming lower, bulk chemical pricing for 2-phenylethanol at ~€1000 per tonne. As with the cost of manufacture calculation, savings in initial capital investment and fermentation energy demand did not make up for the lower fermentation productivity which meant more seaweed feedstock was required per tonne of SCO leading to a higher break-even value for the raceway pond compared with the CSTR.

Further seaweed cost information was taken from Edwards and Watson (2011) and Reith, Deurwaarder et al. (2005) and van den Burg, van Duijn et al. (2016) . Using these costs break-even price ranged between €5,300 per tonne, to €31,000 per tonne (figure 5). This demonstrates the influence seaweed cost has on the break-even price of MO, where even at the lowest seaweed cost price per tonne, break-even price is still far higher than the comparative price of terrestrial oil crops such as palm (€750 per tonne (5-year average) (Indexmundi 2018); soy (€880 per tonne (5-year average) (Indexmundi 2018); or even coconut oil (€1,100 per tonne). The break-even price for a seaweed derived MO is closest to those found in the exotic fat market, such as cocoa butter which retails for ~$5000-8000 per tonne (Papanikolaou and Aggelis 2011, Sterk 2018). Cocoa butter is predominately composed of saturated fatty acids, with a higher fraction of stearic acid than palm or soybean, therefore to access this market the SCO (which has a fatty acid profile similar to that of palm oil) would need to contain a higher proportion of saturated C18 fatty acids. One strategy for improving saturated fatty acid content is to use desaturase inhibitors which prevent the desaturation of acylated groups (Papanikolaou and Aggelis 2011). Moreton (1985) showed that an addition of 2 mL L^{-1} of Sterculia oil into the fermentation broth was able to increase C18:0 content in *Rhodosporidium toruloides* from 3.6 to 40.9 % w/w and in *Candida sp. 107* from 5.2 to 44 % w/w. Alternatively, the direct genetic manipulation of fatty acid biosynthesis in *M. pulcherrima* could offer even greater control.

Compared with economic analysis of SCO production from microalgae this can range from $380 – 6900 for biodiesel production (Quinn and Davis 2015) and is highly dependent on the productivity of the algae cultivation system used. For heterotrophic algae and yeast studies found economic cost to range from $1,700-8,000 depending on the type of feedstock used (Koutinas, Chatzifragkou et al. 2014, Parsons, Abeln et al. 2019).

Generic step-change profit sensitivity to yield and seaweed price were also addressed (figures 6 and 7). Yield was increased and decreased by 500 tonnes per year around the 9500 tonne per year needed for the break-even price of €9700 per tonne. An increase of 500 tonnes production per year would increase profitability over the 30-year plant lifetime by €33 million. This corresponds to increasing biomass productivity to 1.37 g L^{-1} h^{-1} and lipid productivity of 0.55 g L^{-1} h^{-1}. It needs to be emphasized that whilst such productivities have been achieved on simpler carbon sources, this level of productivity is far greater than what has ever been reported previously for lignocellulosic feedstocks (Papanikolaou and Aggelis 2011, Jin, Slininger et al. 2015). Similarly, profitability is highly sensitive to a drop in productivity, with a 5% decrease in annual output leading to a €35 million loss over the 30-year plant lifetime. Profitability is also highly affected by seaweed price. This means that volatility and price uncertainty for potential future seaweed markets in Europe has a significant impact on the economic viability of downstream biorefinery systems utilising it as a feedstock.

The economic costs associated with SCO production from seaweed can be compared with the assessment of other seaweed bioprocessing routes to chemicals and fuels. Based on a *S. latissima* feedstock costs of €1.757 kg^{-1}, Marinho et al. (2016) found that a break-even price of 4.77 € kg^{-1} could be achieved for succinic acid production from *Actinobacillus succinogenes* fermentation when obtaining additional value from the solid residue (after hydrolysis) for fertiliser, and extraction of polyphenols prior to hydrolysis. At a feedstock price of €0.55 kg^{-1}, the break-even price for succinic acid can be reduced further to 3.1€ kg^{-1} (Marinho, Alvarado-Morales et al. 2016). Konda et al. (2015) used a *S. latissima* feedstock price of $100/MT for the coproduction of ethanol and alginate. Their minimum ethanol selling price is between $3.6-8.5 gal^{-1}, which is dependent on yield, solids loading and enzyme loading. Based on work by the Pacific Northwest National Laboratory which determined that the minimum allowable feedstock price for seaweed could be $26/MT (dry) for ethanol production to be economically feasible, Konda, Singh et al. (2015) determine a minimum ethanol price of $2.5 gal^{-1}.

Economic analysis showed that despite an improved environmental profile to terrestrial oils, SCO produced from seaweed via a heterotrophic fermentation was not cost comparative to terrestrial oils under current market conditions. At a feedstock price of $155 $tonne^{-1}$ DM minimum selling price is are comparative to the market price of exotic butters such as cocoa butter. This confirms earlier work by Roesijadi, Copping et al. (2008) that short-medium term target markets for seaweed fermentation products would be mid-high value chemicals, with lower value fuels or bulk chemicals very much in the long-term future. In order to reduce the costs further from a biorefinery perspective, improved methods for valorisation of high-value products from seaweed separated out upstream are crucial.

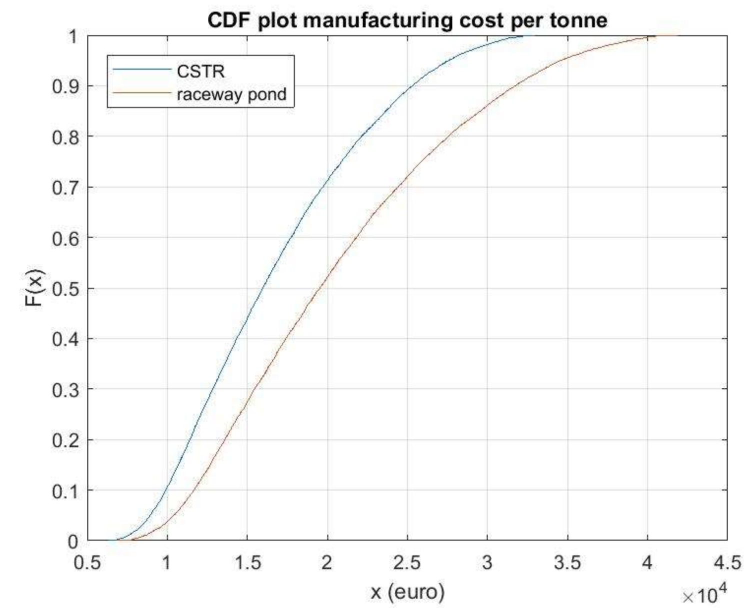

Figure 4. Cost of Manufacture per tonne of refined microbial oil calculated as a probability distribution function (PDF) for CSTR and raceway pond fermentation

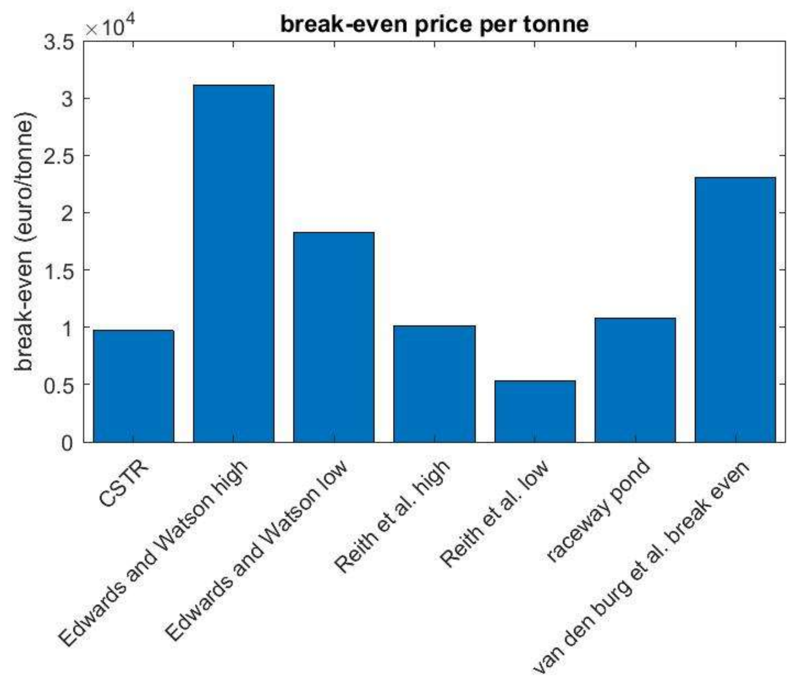

Figure 5. Break-even price of refined microbial oil per tonne based on different seaweed cost prices (Edwards and Watson (2011), Reith, Deurwaarder et al. (2005), and van den burg, van Duijn et al. (2016))

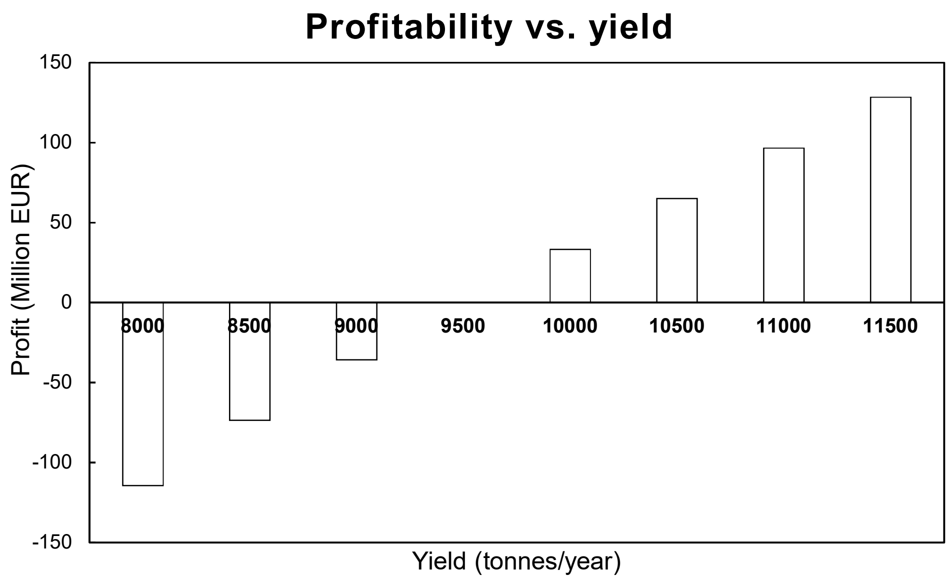

Figure 6. Sensitivity of profitability (Million EUR) to fermentation yield (tonnes/year)

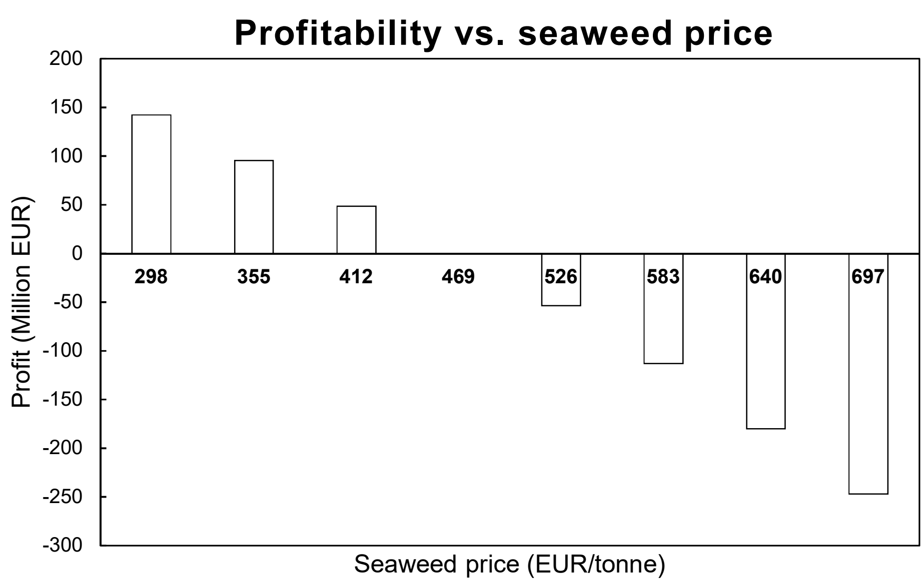

Figure 7. Sensitivity of Profitability (Million EUR) to seaweed price (EUR/tonne)

5.0 Conclusions

For the first time, this LCA and economic analysis evaluates the heterotrophic fermentation of seaweed sugars using yeast to produce a single cell oil. This analysis yields a climate change impact between 2.5 - 9.9 kg CO_2eq. kg^{-1} where variation in seaweed carbohydrate composition and fermentation productivity are taken into account. At the higher end, this is comparable to other single cell oil production processes and at the lower end comparable with terrestrial oil production.

Low-energy raceway pond fermentation did not reduce environmental impact due to the drop in productivity, with an increase in the amount of hydrolysate required. Upstream processing and fermentation steps dominated environmental impact. Overall, economic analysis yields a breakeven selling price of €5,300-€31,000 tonne^{-1} refined SCO depending on seaweed price. At the lower end, this leads to an SCO price roughly comparable to that of exotic butters such as cocoa or shea butter. Where sensitivity analysis was performed we showthat the system has potential for technological improvements that dramatically improve economic viability.

In a rapidly changing geopolitical landscape, where the future value and worth of sustainable, environmentally approaches to industrial biotechnology continues to face huge uncertainty, it is worthy of note that seaweed already offer a viable economic proposition in the higher value oils market. Even ignoring the obvious environmental benefits; the increased pressure on production capability and capacity on the terrestrial environment from a growing population in tandem with the inherent fluctuations in conditions associated with climate change will no doubt create a greater reliance on the marine environment and the relative stability and scale it represents for biomass generation. Our future colonisation and exploitation of the relatively untapped open seas as supplementary cultivation space will undoubtedly lead to improved knowledge, knowhow and understanding of the fundamentals of macroalgae growth and harvesting, opening up new and additional market opportunities along the way.

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