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# Climate impact of yeast oil from fast-growing perennial biomass (willow)

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ABSTRACT

Global demand for fats is predicted to increase in coming decades. Production of vegetable oil causes environmental issues, and further agricultural expansion could compromise sensitive and valuable habitats and substantially increase greenhouse gas emissions. Oleaginous yeasts can convert lignocellulosic hydrolysate into an oil that is similar in composition to rapeseed oil and could provide an alternative to agricultural expansion. In this study, an energy and climate assessment was performed for a system where different varieties of willow (*Salix* spp.) were assumed to be grown on fallow land and used as feedstock for yeast oil production. The effects of biomass growth and soil organic carbon sequestration by six different commercial varieties of *Salix* on the climate impact of the system were also assessed. The results showed that production of 1 tonne of yeast oil required 18.1 GJ in fossil primary energy demand and 15.2 GJ in process electricity, while 18.5 GJ of biomethane and 6.5 GJ of excess power were generated simultaneously. Global warming potential of the oil was on average 1.86 kg $CO_2$-eq $kg^{-1}$ and was substantially lower when carbon sequestration in soil by *Salix* was included in the analysis. Similar trends in net climate impact in terms of temperature change were observed. These findings indicate that edible oil can be produced from *Salix* feedstock via the novel conversion pathway of yeast fermentation, with considerable climate benefits. However, varietal differences in Salix, especially regarding soil carbon sequestration potential, can have a strong influence on the net climate impact of the yeast oil, highlighting the importance of including varietal differences when assessing climate impacts from a systems perspective.

## 1. Introduction

Global consumption of vegetable oil is predicted to increase from on average 214 Mt in 2019–2021 to 249 Mt in 2031, mostly due to rising demand in developing countries, where food consumption is the main driver (OECD-FAO, 2022). Palm, soybean, rapeseed, and sunflower oils are the most widely produced vegetable oils worldwide, and their production is associated with climate impact, deforestation (some regions) and loss of biodiversity (Vijay et al., 2016; Alcock et al., 2022). Meanwhile, competition for land and water resources is expected to increase further by 2050 (FAO, 2022). In order to meet the rising demand for vegetable oils while mitigating climate change and avoiding further stress on land and freshwater resources, alternative ways of producing oils for human consumption and other purposes need to be found.

Oils with similar nutritional composition and technical properties to plant oils can be derived from oleaginous yeasts (Brandenburg et al., 2018; Chmielarz et al., 2021). Microbial fat synthesis technology originated in Germany following fat shortages during the first and second world war (Lundin, 1950) and is well-proven, but has not been successfully commercialized due to high feedstock and fermentation costs (Ratledge and Cohen, 2008). The high feedstock costs could be addressed by using lignocellulosic biomass, which with its high abundance and relatively low cost could be a suitable substrate for industrial fermentation (Passoth and Sandgren, 2019; Passoth et al., 2023). Previous systems analyses have examined the fossil energy demand of using wheat straw for production of oleaginous biodiesel (Karlsson et al., 2016) and yeast oil for feed purposes (Sigtryggsson et al., 2023). However, removal of straw from the cropping system can have a substantial impact on soil organic carbon (SOC) content, and thus the climate performance of straw-derived yeast oil (Karlsson et al., 2017).

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Several varieties of plants under the genus *Salix* (commonly known as willow) can be a suitable biomass feedstock for producing a wide range of biobased products (Baker et al., 2022). *Salix* is well-adapted for growing in temperate regions and can thrive on wet and marginal cropland that might not be suitable for other crops (Volk et al., 2006; Karp et al., 2011). It is typically cultivated in short-rotation coppice (SRC) plantations, which are harvested every 3–5 years over 25 years before removal and replanting (Volk et al., 2004; Karp and Shield, 2008), which reduces resource use and soil disturbance compared with annual crops (Boehmel et al., 2008). *Salix,* in particular, has the potential to fix and accumulate carbon in soils and thereby mitigate climate change (Kalita et al., 2021). It has also been shown to provide additional benefits such as phytoremediation, flood risk mitigation, reduced nutrient losses, reduced erosion, and biodiversity improvements (Bressler et al., 2017; Weih et al., 2020). In past decades, Salix varieties with rapid growth, high biomass yield, and resistance to diseases and pests have been developed for commercial biomass applications (Mola-Yudego and González-Olabarria, 2010; Lindegaard et al., 2016). These varieties exhibit distinct differences in their traits and characteristics among them. Accounting for these varietal differences in systems analysis can lead to more accurate results when estimating the climate impacts of using *Salix* in a biorefinery system.

Previous studies on biorefinery systems producing yeast oil or yeast oil-based biodiesel using wheat straw feedstock have shown that fossil energy use in the system is similar to or better than that in the original system based on e.g., vegetable oil or fossil diesel (Karlsson et al., 2016; Sigtryggsson et al., 2023). Replacing fossil diesel with yeast oil-derived biodiesel has also been shown to reduce the climate impact of diesel production substantially (Karlsson et al., 2017). Use of *Salix* as a feedstock for bioenergy production has been investigated previously, but a novel aspect of the present study was to perform a systems analysis of *Salix*-to-oil production that evaluated energy use and climate impact. Another novel aspect was to take varietal differences into consideration when evaluating the climate impacts of producing yeast oil from *Salix* from a systems perspective. Six commercial varieties were included in the analysis, which considered in particular the effects of using a perennial feedstock that contributes to carbon sequestration when producing yeast oil in a commercial-scale biorefinery. The intention was to provide a basis for future work on impacts and implementation of energy crops like *Salix* for biorefinery purposes.

## 2. Methods

### *2.1. System overview*

The production system studied is presented schematically in Fig. 1, with system boundaries represented by a dotted line. It is similar to the system studied by Sigtryggsson et al. (2023), but with wheat straw replaced with *Salix* and sequential changes in processing due to this biomass change. The system started with cultivation of *Salix*, including all necessary inputs for cultivation and fuel for machinery, followed by transport by truck to the biorefinery. In the biorefinery, the *Salix* biomass was assumed to be pretreated by steam and then fermented into an oil-rich yeast biomass. Solids that could not be hydrolyzed were used for internal heat and power production. Yeast oil was extracted and the remaining microbial biomass was sent to a biogas fermenter. Digestate produced as a by-product from biogas production was assumed to be used as a liquid fertilizer on agricultural fields with annual rotation of winter and spring cereals, replacing a fraction of mineral fertilizers. Nitrous oxide ($N_2O$) emissions arising from the Salix cultivation and digestate application on fields were included in the assessment, as were avoided emissions from the replacement of mineral fertilizers. The biogenic carbon flux from soil carbon sequestration and growing Salix plants were included. The system did not include inputs, energy use, or emissions for manufacturing the machinery used in cultivation or building the biorefinery. Emissions from machinery and infrastructure are spread over long lifetimes and contribute only a small fraction to the total lifecycle emissions per unit of yeast oil produced. The system boundaries were chosen to focus on the operational phase of the conversion process and address primary processes, such as feedstock growth, harvesting, and conversion.

Energy use as measured in MJ fossil primary energy demand ($PED_{fossil}$) and climate impact (global warming potential and time-dependent climate impact) were assessed from a life cycle perspective. The flow of resources and energy throughout the yeast oil production system was used for estimating its emissions and climate impact. The time frame was 50 years, long enough to assess the long-term climate effects of growing *Salix*. Possible future changes in the production system or the background system were excluded. The functional unit used was 1 tonne of yeast oil. Yeast oil has comparable technical and nutritional properties to rapeseed oil (Sigtryggsson et al., 2023) and could be used for similar purposes, e.g., biofuels, biochemicals, food, and feed.

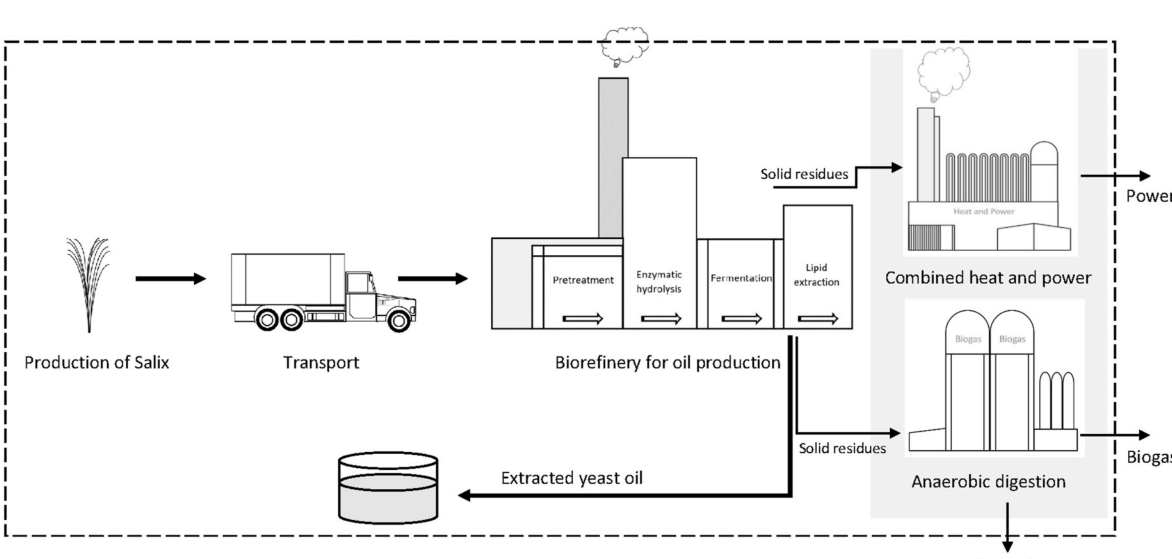


**Fig. 1.** System overview of the main process steps from Salix cultivation to fermentation of yeast oil and energy production.

### 2.2. Salix cultivation and varieties

The six commercial varieties of *Salix* considered in this study were 'Björn' (*Salix schwerinii* E. Wolf. × *S. viminalis* L.), 'Gudrun' (*S. burjatica* Nasarow × *S. dasyclados* Wimm.), 'Jorr' (*S. viminalis*), 'Loden' (*S. dasyclados*), 'Tora' (*S. schwerinii* × *S. viminalis*) and 'Tordis' ((*S. schwerinii* × *S. viminalis*) × *S. viminalis*). Individual *Salix* plantations were assumed to be harvested every third year after establishment, while after 25 years the plantation was assumed to be terminated and re-established with new cuttings. A 50-year cultivation period was considered in impact assessment, comprising two 25-year rotations for individual *Salix* plantations. Technologies and management practices were assumed to remain unchanged during the cultivation period. As *Salix* is harvested every three years, plantation establishment on different plots was assumed to be staggered over a three-year period, so that a harvest could be obtained annually to ensure a steady supply of biomass for the biorefinery.

The *Salix* cultivation chain included all processes from field preparation to harvest, field transport, and production of inputs such as pesticides, fertilizers, and cuttings, as shown in Fig. 2. The rotation started with soil preparation by ploughing and harrowing and weed control, followed by establishment of cuttings. Fertilizer was assumed to be applied from the second year onwards. Every third year, whole-stem harvest, field transport, and road transport to the biorefinery took place. After eight three-year cutting cycles, the old plantation was assumed to be terminated by mechanical uprooting of stumps, followed by establishment of a new rotation. *Salix* is normally harvested during the winter, while the demand for biomass is evenly distributed over the year. Fresh *Salix* chips are difficult to store, so whole-stem harvesting and collection in bundles was assumed. The bundles were assumed to be transported 35 km by truck (one-way) to a biorefinery for storage and chipped on demand (108 MJ electricity per tonne *Salix* DM). Energy data on *Salix* harvest, transport, and chipping were taken from Baky et al. (2009).

The material inputs, energy inputs, and GHG emissions in *Salix* production were adapted from a system analysis performed by Kalita et al. (2021) using data from previous studies on SRC *Salix* in Sweden (Tables S1 and S2 in the Supplementary Material). *Salix* cultivation was compared with a reference land use of green fallow where the *Salix* was assumed to be cultivated on land that was previously fallow which

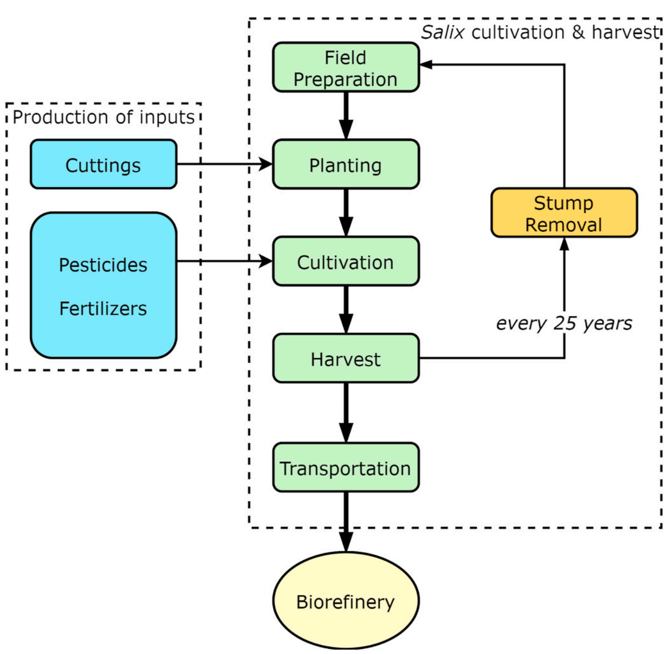


**Fig. 2.** Overview of inputs and activities related to cultivation of Salix.

represents a change in land use. A reference land use scenario was established of green fallow that was cut annually with biomass left on the field. The SOC changes and emissions from fallow cultivation were calculated and subtracted from the Salix cultivation system to account for the land use changes. The data used to calculate energy and emissions related to management of the green fallow can be found in Table S3 in the Supplementary Material.

Initial data on biomass yields, soil conditions, and plant characteristics for the selected *Salix* varieties were based on a field study in Pustnäs, Uppsala, in central Sweden by Weih and Nordh (2005). The soil type at that site is Vertic Cambisol with sandy loam topsoil, mean air temperature during the growing season is 12.5 °C and mean annual precipitation is 841 mm. Fertilization with 100 kg N, 14 kg P, and 47 kg K (NPK 21:3:10) per hectare was performed annually (except in the year of establishment). Biomass yield and plant growth characteristics in our system were allocated the average values from the field data. The first harvest of *Salix* plants typically provides lower yield than subsequent harvests, when the plants have matured. Field data on yield of the six selected varieties of *Salix* are presented in Table 1. Soil carbon data were available for the 0–20 cm soil layer over two time periods, with initial soil carbon stock of 28.8 Mg ha$^{-1}$ and soil bulk density of 1.3 g cm$^{-3}$. See Baum et al. (2020) for full details of the soil sampling procedures, analysis, and fertilization.

### 2.3. Oil production in the biorefinery

In the biorefinery (Fig. 3), the *Salix* chips (size mainly below 45 mm) were assumed to be pretreated with dilute sulfuric acid and steam explosion, to hydrolyze hemicelluloses and increase the accessibility of cellulose to enzymatic hydrolysis. The composition of the six varieties comprised on average: cellulose 50–56%, xylan 9–10%, galactan 2%, arabinan 1%, mannan 2%, hemicellulose 13–15%, and lignin 24–29% (Kalita et al., 2023).

Enzymatic hydrolysis was assumed to be carried out using a crude version of the enzyme mix Cellic® CTec3 HS from Novonesis, with primary energy demand of 13 MJ kg$^{-1}$ and a carbon footprint of 1 kg $CO_2$-eq kg$^{-1}$ (Jesper Kløverpris, Novonesis, personal communication April 19, 2016). The dose was assumed to be 16 kg enzyme tonne$^{-1}$ biomass and sugar recovery was assumed to be 631–687 g kg$^{-1}$ *Salix*. The lignin and other solid fractions were assumed to be separated and used for heat and power production, with net power production of 1.54 MWh per tonne dry matter (DM). Fermentation was assumed to be carried out using the yeast strain *Rhodotorula babjevae* DBVPG 8058 (Brandenburg et al., 2018) for 72 h, with lipid yield of about 0.2 g g$^{-1}$ sugar and 50% lipids per dry cell of biomass. Full details of the fermentation process can be found in Sigtryggsson et al. (2023). After extraction of lipids, the remaining yeast biomass (12–13 kg lipids and 1215–1320 kg tonne$^{-1}$ *Salix* DM) was assumed to be used as substrate for biogas production. The biogas process was modeled based on theoretical yields and with an efficiency of 80 %. The raw biogas was upgraded by water scrubbing to 96 % methane content, comparable to that of natural gas. The electricity use at the biogas plant, for all process steps included, was estimated to 0.3 kWh per kilo upgraded methane. The digestate was assumed to be transported 30 km by truck and applied

**Table 1**
Average biomass yield in tonnes of dry mater (DM) per hectare during the first and subsequent harvests of the six Salix varieties when grown under fertilized conditions in central Sweden.

| Variety | Average 1st harvest (Mg ha$^{-1}$) | Average subsequent harvests (Mg ha$^{-1}$) |
|---|---|---|
| Björn | 15.5 | 42.7 |
| Gudrun | 11.6 | 20.6 |
| Jorr | 16.9 | 36.9 |
| Loden | 10.4 | 18.3 |
| Tora | 16.6 | 38.3 |
| Tordis | 19.8 | 48.5 |

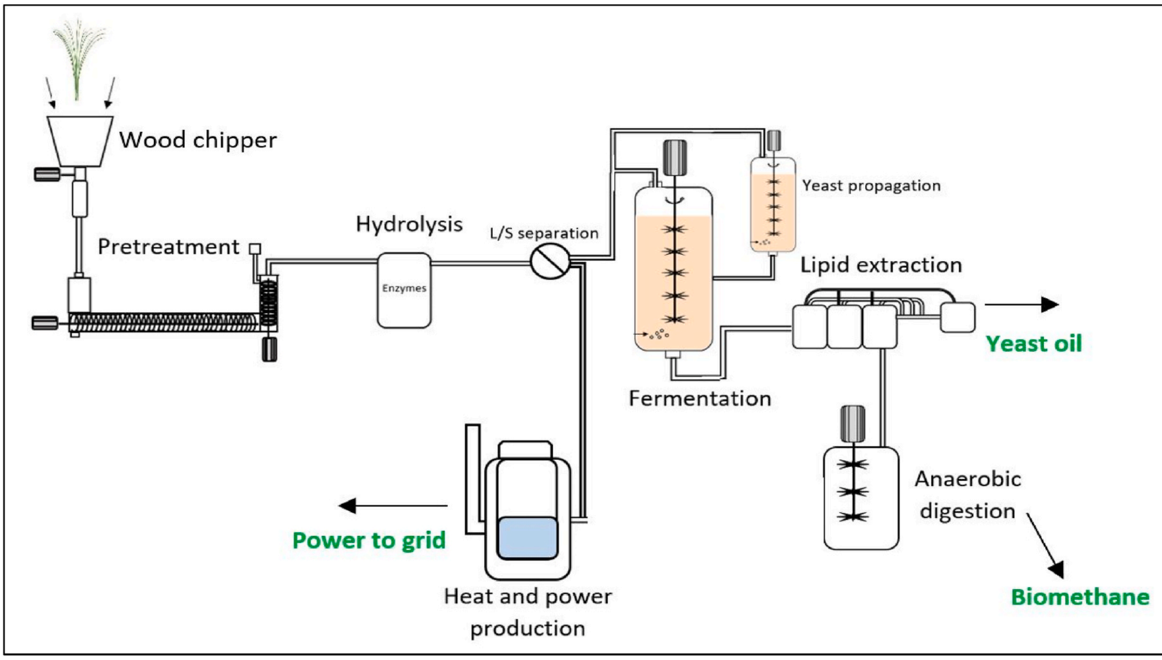


**Fig. 3.** *Process steps in the biorefinery from chipping of* Salix *bundles to fermentation of oil, biogas and excess power production.*

to soil, replacing mineral nitrogen fertilizer (1 kilo of digestate N was assumed to substitute 1 kilo of mineral N). The mineral nitrogen fertilizer replaced was assumed to be the same as in the *Salix* cultivation (see Table S1 in the Supplementary material). Sphera Database (former GaBi) and European datasets were used for calculating the emissions from the fertilizer. Emissions from transport and application of digestate in the field were included in the analysis, as were all avoided emissions from replacing the inorganic fertilizer. Changes in soil carbon from the application of digestate were not included in the systems analysis. The mass and energy balance for the biorefinery was modeled in Aspen Plus. Details on the energy use and GHG emissions from the individual inputs in the biorefinery process are listed in Table S7 in the Supplementary Material.

### 2.4. Life cycle assessment of climate impact

Climate impact was assessed in terms of two metrics, global warming potential during a time period of 100 years ($GWP_{100}$) and time-dependent climate impact. $GWP_{100}$ is the most commonly used climate metric and is a normalized value representing the cumulative warming potential of GHG emissions relative to $CO_2$ (Myhre et al., 2013). The GWP calculations were based on the characterization factors in Table 2 (AR6) (Intergovernmental Panel On Climate Change (IPCC) 2023) and on average data for *Salix* production (inputs and soil carbon change over 50 years). Time-dependent climate impact (ΔTs), or absolute global temperature change potential (AGTP), goes one step further in the cause-effect chain and represents the climate impact as a change in global mean surface temperature caused by a GHG flux at a specific time point (Shine et al., 2005; Myhre et al., 2013). Time-dependent climate modelling acknowledges the timing of the emissions of $CO_2$, nitrous oxide ($N_2O$), and methane ($CH_4$), and estimates the temperature response over time. The ΔTs methodology developed by Ericsson et al. (2013) was used to determine climate impact in this study.

**Table 2**
Characterization factors used for $GWP_{100}$.

| | $GWP_{100}$ (kg $CO_2$ eq/kg) |
|---|---|
| $CO_2$ | 1 |
| $N_2O$ | 273 |
| $CH_4$ – non fossil | 27 |

As biogas and power with different purposes and uses were produced alongside the yeast oil, economic allocation was used to differentiate the contribution of each product to the total climate performance of the biorefinery system. All prices used were 10-year averages (2010–2020) and the price of electricity (northern Europe) was set to USD 1.51 per GJ (USD 1 = 0.81 Euro). Biomethane was assumed to be priced like European natural gas (USD 7.32 per GJ) and yeast oil like rapeseed oil (USD 972 per tonne) (Nord Pool, 2023; The World Bank, 2023).

### 2.5. Biogenic carbon flux

Biogenic carbon flux between the atmosphere and biosphere was calculated for the SOC and live biomass compartments. The annual flux was based on the net annual C stock change in each compartment. The SOC changes were modeled using the ICBMr carbon model (Andrén and Kätterer, 1997; Andrén et al., 2004), which calculates the carbon flux based on variable annual inputs of biogenic material to the soil considering regional differences in climate and soil type. The ICBMr model conceptualizes soil carbon in two compartments, representing young (Y) and old pools (O) of SOC. Annual fresh carbon inputs (*i*) enter the Y pool, a part of which moves to the O pool according to the humification coefficient (*h*) and the rest is emitted to the atmosphere. The O pool is more stable and undergoes slower decomposition. Belowground carbon (roots and stumps) has ~2.3-fold higher *h* value than aboveground carbon residues (leaf litter) (Kätterer et al., 2011). Therefore, aboveground and belowground inputs are modeled separately in ICBMr using different *h* values (Ericsson et al., 2013). The ICBMr equations and parameters used to calculate the carbon flux are provided in Tables S4 and S5 in Supplementary Material.

The SOC changes in our analysis were limited to the 0–20 cm soil layer, based on available data from the field study. The assessment included soil carbon changes occurring during the 50-year period defined in the study. The soil carbon flux for the reference green fallow cultivation was based on similar modeling as in previous studies (Ericsson et al., 2013; Hammar et al., 2014). The annual change in carbon stock in live biomass was calculated based on yield levels, annual growth rate, and biomass carbon allocation patterns in *Salix*. Biomass production, allocation between different plant parts, for the different Salix varieties were based on a previous study by Kalita et al. (2021) and are presented in Table S6 in the Supplementary Material. The carbon in *Salix* stems, which ended up in the biorefinery products, was assumed to be released in the same year as production.

### 2.6. Soil $N_2O$ emissions

Nitrogen from mineral fertilizers and biomass inputs to the soil cause $N_2O$ emissions through the processes of nitrification and denitrification. In the present analysis, direct and indirect $N_2O$ emissions were calculated using IPCC Guidelines for National Greenhouse Gas Inventories with the Tier 1 approach and default parameters (as site-specific emission factors and activity data were unavailable), using the following equations:

$$N_2O_{direct} = EF_N \cdot (N_{applied} + N_{litter} + N_{fine\ roots}) \cdot \frac{44}{28}$$

$$N_2O_{indirect} = N_{applied} \cdot (F_A \cdot EF_D + N_{leached} \cdot EF_L) \cdot \frac{44}{28}$$

where $N_{applied}$ is nitrogen applied with fertilizer; $N_{litter}$ and $N_{roots}$ is nitrogen contained in aboveground litter and roots, respectively; $N_{leached}$ is nitrogen lost by leaching; EFN, EFD, and EFL are emission factors for direct emissions from applied nitrogen, indirect emissions from volatilization and re-deposition, and leaching, respectively; and $F_A$ is the fraction of applied nitrogen emitted as ammonia.

### 2.7. Sensitivity analysis

A sensitivity analysis was made to assess how variations in input data and choice of allocation method would affect the results. From a previous study on straw-derived yeast oil (Sigtryggsson et al., 2023), the economic value of the products involved and the allocation method chosen, proved to have a substantial impact on the result. Whilst most of the input data used is based on literature or on well-established and documented chemical processes, more uncertain lab-scale data has been used for modelling the pH-regulation. Moreover, *Salix* is bulky and to supply a biorefinery with feedstock, the impact of transport distance may also be highly relevant in case the feedstock catchment area would need to be expanded. For this sensitivity analysis, the following variables were analysed.

- A price increase of yeast oil by 10 %
- Allocation based on the energy content of the products (yeast oil, methane and electricity) rather than of their economic value
- A reduction in volume of acid and base for pH-regulation by 20 %
- An increase in transport distance to the biorefinery from 35 to 70 km.

## 3. Results

### 3.1. Mass and energy balance

The outcome of the system analysis was a mass and energy balance showing inputs and outputs of the biorefinery system for all six *Salix* varieties studied (Table 3). Cultivation of *Salix* (on average 30%) made a large contribution to the total fossil primary energy demand $(PED)_{fossil}$ of the system, which varied widely between the varieties. As the fertilization strategy and machinery inputs were the same for all varieties, $PED_{fossil}$ per tonne *Salix* was inversely related to biomass yield. There were small variations in material and energy inputs between the different *Salix* varieties based on their compositional values. Accordingly, overall $PED_{fossil}$ was higher for varieties with lower biomass production. Yield of yeast oil, biomethane, and excess power per tonne of *Salix* input was on average 113 kg, 2090 MJ, and 740 MJ, respectively. The economic allocation factor per tonne of yeast oil was found to be 0.81–0.84, meaning that oil production on average was considered to be responsible for 82% of the fossil energy used. $PED_{fossil}$ per tonne of oil was estimated to be 13.9–16.6 GJ.

The $GWP_{100}$ value in kg $CO_2$-eq per kg oil for the six varieties is presented in Fig. 4. When soil carbon sequestration potential was not taken into account, $GWP_{100}$ varied between 1.53 and 2.35 kg $CO_2$-eq (mean 1.85 kg $CO_2$-eq $kg^{-1}$) and followed the same trend as $PED_{fossil}$, i.e., the climate impact was inversely related to biomass yield. These values are comparable to the 1.62 kg $CO_2$-eq estimated for Swedish rapeseed oil (Bernesson, 2004), but much lower than the 2.49–4.25 kg $CO_2$-eq $kg^{-1}$ reported for different vegetable oils globally (Alcock et al., 2022). On average, *Salix* cultivation contributed almost 60% of the total GWP of the system, where the single largest contributor was production and use of mineral fertilizer and related $N_2O$ emissions following application. The biorefinery was the second largest emitter of greenhouse gases, with use of ammonia for yeast propagation being

**Table 3**

*Mass and energy balance showing inputs and outputs of the biorefinery* per *tonne of dry matter (DM) for the six* Salix *varieties considered. Inputs are presented both in kg product used and in MJ fossil primary energy demand ($PED_{fossil}$) except for power use, which refers to absolute energy demand. The outputs are presented in kg product and based on energy content.*

| Inputs per tonne of *Salix* (DM) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Inputs** | Björn | | Gudrun | | Jorr | | Loden | | Tora | | Tordis | |
| *Feedstock supply* | | | | | | | | | | | | |
| Cultivation (MJ) | 534 | | 847 | | 578 | | 922 | | 566 | | 495 | |
| Transport (MJ) | 124 | | 124 | | 124 | | 124 | | 124 | | 124 | |
| *Biorefinery* | *kg* | *MJ* | *kg* | *MJ* | *kg* | *MJ* | *kg* | *MJ* | *kg* | *MJ* | *kg* | *MJ* |
| Sulfuric acid | 22.1 | 69 | 22.1 | 69 | 22.1 | 69 | 22.1 | 69 | 22.1 | 69 | 22.1 | 69 |
| Enzymes | 16 | 208 | 16 | 208 | 16 | 208 | 16 | 208 | 16 | 208 | 16 | 208 |
| Ammonia | 15.5 | 645 | 14.7 | 612 | 14.3 | 595 | 14.4 | 599 | 14.3 | 595 | 15.3 | 637 |
| pH adjustment | 10.7 | 187 | 10 | 174 | 9.8 | 171 | 9.8 | 171 | 9.8 | 171 | 10.5 | 183 |
| Hexane | 7.1 | 289 | 6.7 | 272 | 6.6 | 268 | 6.6 | 268 | 7.0 | 285 | 7.0 | 285 |
| Avoided energy – biofertiliser (MJ) | −91 | | −87 | | −85 | | −86 | | −85 | | −91 | |
| $PED_{fossil}$ total (MJ) | 1970 | | 2220 | | 1930 | | 2280 | | 1930 | | 1910 | |
| Power use (MJ) | 1790 | | 1710 | | 1670 | | 1680 | | 1670 | | 1780 | |
| **Outputs per tonne of *Salix* (DM)** | | | | | | | | | | | | |
| **Outputs** | | | | | | | | | | | | |
| Yeast oil (kg) | 119 | | 112 | | 110 | | 111 | | 109 | | 117 | |
| Biomethane (MJ) | 2200 | | 2080 | | 2030 | | 2050 | | 2030 | | 2170 | |
| Excess power (MJ) | 502 | | 762 | | 869 | | 839 | | 883 | | 568 | |
| **$PED_{fossil}$ per tonne of oil** | | | | | | | | | | | | |
| Allocation factor oil | 0.84 | | 0.82 | | 0.81 | | 0.81 | | 0.81 | | 0.84 | |
| $PED_{fossil}$ (GJ)/tonne oil (allocated) | 13.9 | | 16.3 | | 14.2 | | 16.6 | | 14.3 | | 13.7 | |

$GWP_{100}$ and Time-dependent climate model.

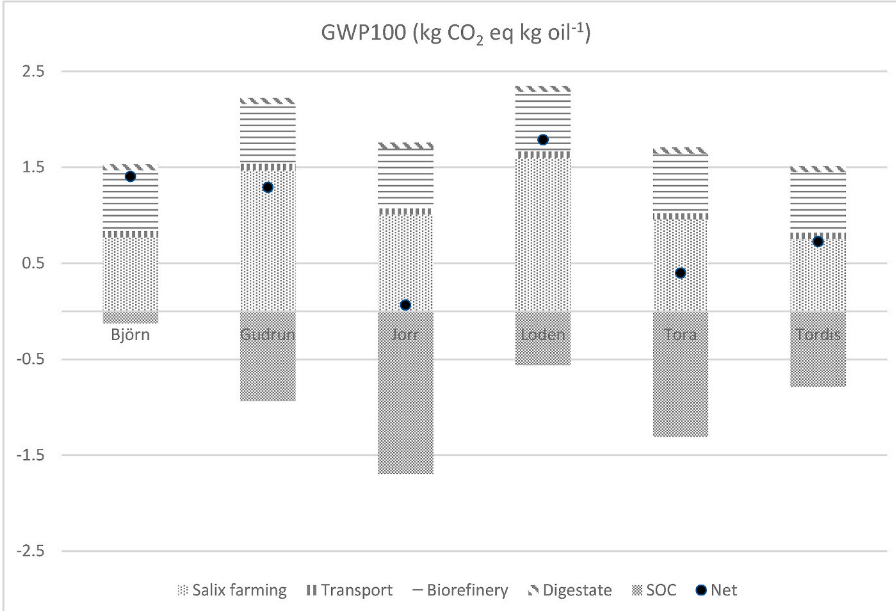


**Fig. 4.** $GWP_{100}$ for the six Salix varieties considered, representing emissions from cultivation, transport, and biorefinery and the negative climate impact of soil organic carbon sequestration and biomass. The net GWP effect of each variety is marked with a dot.

responsible for about half of the emissions (48%), followed by enzymes (18%) and methane losses in biogas upgrading (14%). Transport and spreading of digestate from the biogas reactor made a surprisingly high net contribution to GWP, through $N_2O$ emissions from soil, and was of a similar level to the contribution from transport of *Salix* from field to biorefinery, even after subtracting the avoided emissions from mineral nitrogen fertilizer. The GWP contributions from transport and the biorefinery remained on a relatively similar level for all varieties. However, the climate impact of *Salix* cultivation varied between the varieties depending on their productivity. Low-yielding varieties Gudrun and Loden had lower biomass production efficiency, which meant that the material and energy inputs per unit of biomass produced were comparatively higher, leading to a greater climate impact from the *Salix* cultivation phase for these varieties.

The soil carbon modelling results showed an increase in SOC stocks from *Salix* cultivation under the specific study conditions across all varieties. However, the magnitude of increase in SOC was dependent on the *Salix* variety. The sequestration of $CO_2$ by increase in SOC contributed to a mitigating effect on climate (shown as darker negative emissions in Fig. 4). Net GWP, calculated by adding positive and negative emissions, was relatively low for all varieties and varied from 0.1 (Jorr) to 1.80 kg $CO_2$ eq kg $oil^{-1}$ for Loden with an average (0.96) considerably lower than for most vegetable oils reported.

The climate impact of yeast oil production was also charted as a temperature response curve, to better predict the effect on the atmospheric temperature over time. The temperature effect obtained, as $\Delta Ts$ in degrees Kelvin (K) per kg yeast oil, for all six varieties over 50 years is presented in Fig. 5. Emissions from the '*Salix* supply chain' (Salix farming and transport) and from the biorefinery had a net warming effect, as indicated by a temperature rise in the graph. Conversely, carbon sequestration in biomass and soil (represented as 'Biogenic carbon') had a cooling effect that varied widely between the varieties (Fig. 5). As *Salix* is a fast-growing and relatively productive crop, the rapid increase in biomass after establishment of the plantation leads to high uptake of $CO_2$ in live biomass. This has a significant climate mitigation effect in terms of $\Delta Ts$ in the short term and does not change significantly after the first rotation period. In the $GWP_{100}$ climate metric, cumulative emissions over the life cycle of a product are considered. As there was no net live biomass accumulation during the study period, this led to a climate neutral effect in terms of the $GWP_{100}$ of live biomass. However, the $\Delta Ts$ metric captures the temporal variation between the uptake and release of GHGs. The cooling effect from live biomass is temporary and will be quickly lost in the years after ending the cultivation. The temperature mitigation effect of SOC sequestration gradually increases with time, with the build-up of soil carbon stock. As carbon sequestered in the soil has a longer residence time before being released as $CO_2$, its contribution to $\Delta Ts$ is longer-lasting than that of live biomass. Jorr, Tora, and Tordis had higher SOC sequestration than the other varieties according to our carbon modeling, which led to a greater negative temperature response. Jorr and Tora had a net negative $\Delta T$ during the study period, which infers that oil production from these varieties would have a net cooling effect on the climate. Björn and Loden, with a lower SOC sequestration effect, had a warming effect on the climate after about 25 years (end of the first rotation period) (Fig. 5).

### 3.2. Sensitivity analysis

A sensitivity analysis was performed for one of the varieties with average performance (Tora), to test the impact of variations in four factors: yeast oil price, economic allocation method, acid-base use, and transport distance presented in Table 4. First, an increase of 10% in the price of yeast oil was tested, affecting the economic allocation of the oil, and was found to have a minor impact on the results (GWP of yeast oil increasing by less than 2%). However, applying allocation based on energy content (37 MJ per kg of oil assumed) resulted in an almost 30% reduction in GWP. The amount of acid and base assumed to be used for regulating pH during fermentation was based on laboratory data and could have been overestimated. However, a 20% reduction in the amount had a marginal effect on GWP (−2.3% in the biorefinery and −0.9% for the total system). Transport distance is known to be critical when transporting biomass and could in reality easily increase. Doubling it from 35 to 70 km in sensitivity analysis led to an overall GWP increase of 3.9%.

## 4. Discussion

Although production of microbial oils in many aspects is a far more complex process than conventional rapeseed oil production, it showed

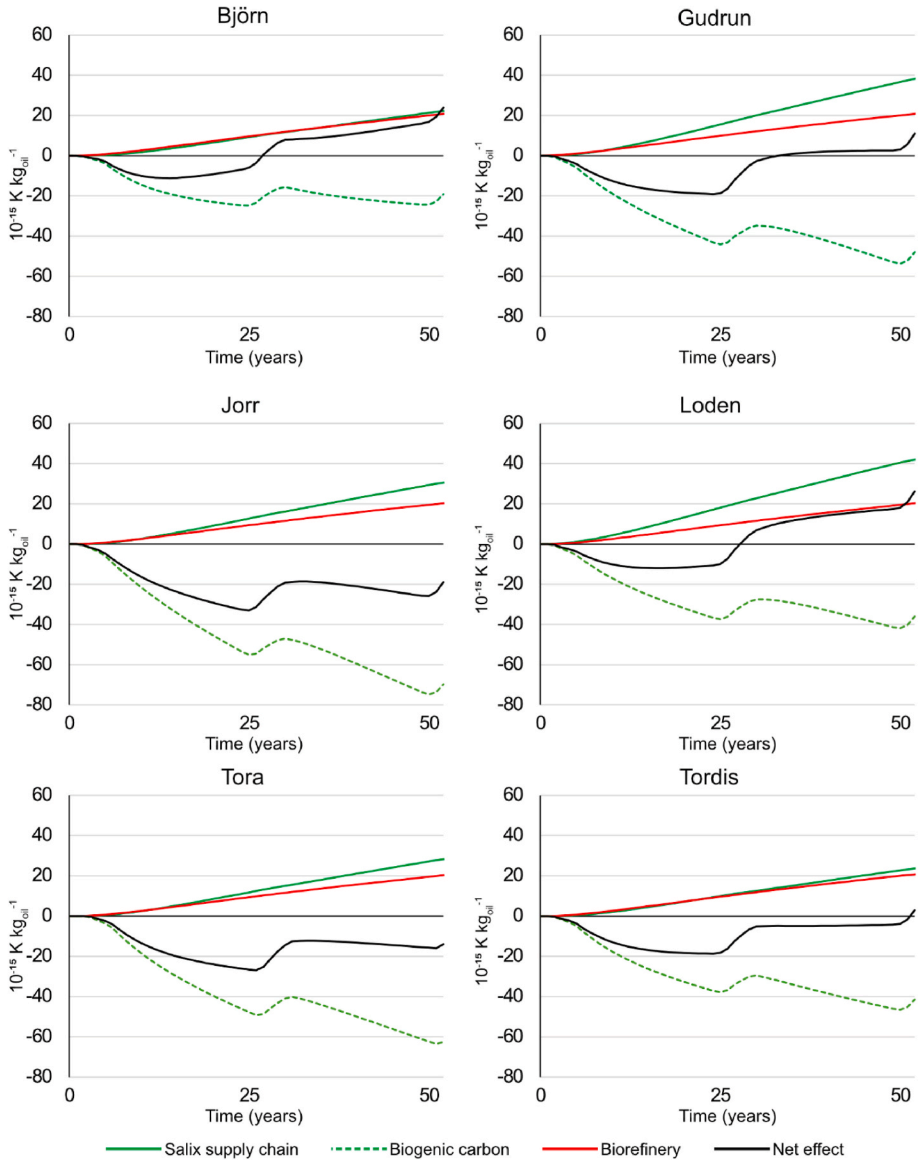


**Fig. 5.** Temperature response curves for the six Salix varieties considered, showing the warming effect of cultivation and production of oil over 50 years, and the cooling effect of carbon sequestration in soil and uptake in biomass. The net temperature effect for each variety is marked with a black solid line.

**Table 4**
Results of sensitivity analysis in which the price of yeast oil was increased by 10%, the amount of acid and base was reduced by 20 % and the transport distance doubled. Applying allocation based on energy content instead of economic allocation was also analysed.

| | Yeast oil +10% | Energy allocation | pH adjust - 20 % | Transport +100% |
|---|---|---|---|---|
| $GWP_{100}$ yeast oil | 1.7% | −27.5% | −0.9% | 3.9% |

comparable fossil primary energy and climate performance. The overall energy demand, which was covered by combustion of lignin and therefore bio-based, was high. A major challenge for global societies is to decrease the current dependence on fossil resources, meaning that energy consumption must be reduced, irrespective of its origin. As 15.2 GJ of "green" process electricity were used per tonne of microbial oil in our system, it could be argued that this electricity could just as well have been used to replace fossil energy on the grid. As noted in previous studies (Karlsson et al., 2016; Sigtryggsson et al., 2023), the hotspot in terms of process energy demand was stirring and aeration of the yeast culture. An aeration rate of 1 vvm (1 L of air per 1 L of medium and minute) was assumed in the present analysis, but large-scale fermentation experiments are necessary to fully optimize the stirring and aeration requirements and further improve the energy performance.

The $GWP_{100}$ values obtained in our analysis varied between 1.53 and 2.36 kg $CO_2$-eq per kg oil (average 1.86 $CO_2$-eq $kg^{-1}$), indicating a relatively low carbon footprint associated with yeast oil production from *Salix* biomass. When soil carbon sequestration potential was included in the analysis, the net GWP was relatively low for all varieties and even negative for Jorr, suggesting a potential cooling effect on the climate. Furthermore, the temperature response curves (Fig. 5) demonstrated a significant impact of biomass and soil carbon sequestration on the net temperature response over 50 years. The rapid increase in biomass after plantation establishment contributed to a substantial climate mitigation effect in the short term, while the SOC sequestration effect gradually increased with time, leading to a longer-lasting negative temperature response, particularly for the varieties Jorr and Tora.

The carbon footprint of oil production depended strongly on the *Salix* variety cultivated. The single most important factor appeared to be yield, where varieties with higher yield had lower climate impact and energy use. However, it should be noted that the amount of inputs used in the field experiment remained the same for all six varieties, whereas fertilizer rates and harvest intervals would have been adapted to actual plantation growth in real cultivation. *Salix* generally has an impressive capability to store carbon in the ground, due to its many fine roots. For some of the varieties considered here, soil carbon sequestration and carbon storage in stems and coarse roots (biomass) was at a level that seemed to compensate for emissions from cultivation and biorefinery production of oil. However, this was a temporary effect during the study period, for as long as production continued. It was also the reason for the time frame chosen (50 years), as the net increase in soil carbon sequestration is likely to decline with time. On ending *Salix* cultivation, the "biomass effect" stops almost immediately, while the soil carbon content slowly decreases to reach a new steady state. For the same reason, the positive climate effect of growing *Salix*, i.e., having a cooling effect on the climate, should be seen as a bonus to already superior climate performance in comparison with conventional vegetable oil production, and not that the system will have a permanent cooling impact on the climate.

Biogenic carbon fluxes from SOC sequestration and live biomass had a strong mitigating effect on the climate impact. However, the effect of live biomass was not seen when using the cumulative $GWP_{100}$ metric, as there was no net live biomass left at the end of the study period, whereas $\Delta T$ captured the effect of timing of emissions on global mean surface temperature (Ericsson et al., 2013). Soil carbon sequestration potential is dependent on initial soil conditions and climate effects, so the magnitude of SOC change will differ for *Salix* cultivated in different soils and climate conditions. However, all varieties can be expected to show similar trends in their SOC sequestration potential. Soil carbon is not an endless sink and sequestration potential can be expected to decrease over time, which will reduce its climate mitigation potential.

The suitability of *Salix* feedstocks for fermentation is also dependent on their composition, recalcitrance, and accessibility to yeast (Baker et al., 2022). There is potential to optimize the physical and chemical characteristics of *Salix* wood, besides biomass productivity and SOC sequestration, by genetic improvements (Rönnberg-Wästljung et al., 2022). Breeding programs can play a crucial role in developing *Salix* varieties that are well-suited for sustainable yeast oil production. Previous assessments of straw as a feedstock for both yeast oil and biodiesel production by Karlsson et al. (2017) and Sigtryggsson et al. (2023) assumed that harvest residues that would otherwise be left in the field were instead used as feedstock, meaning there would be no need for more land. In this study, we assumed instead that *Salix* was cultivated on soil not used for ordinary crop production (due to low profitability, low soil fertility, or non-rational location for growing annual crops) and thus did not compete for arable land. Growing *Salix* for oil production on soil that is not suitable for ordinary crop production could also convert relatively poor soils into perennial plantations with fairly good oil yields. At a *Salix* yield of 11.3 tonnes per hectare, a *Salix* system produces a similar amount of oil as an average Swedish rapeseed field (assuming rapeseed yield of 2.56 tonnes per hectare and 50% oil content). In addition to this, cultivation of *Salix* will bring the positive effects of a perennial crop providing permanent land cover, reduced risk of soil erosion, lower management requirement, and habitat for insects and animals. Moreover, the substantial potential for SOC sequestration shown by several of the varieties included in our analysis presents an opportunity to improve soil quality. Future studies could examine techniques for enhancing carbon sequestration in the soil, potentially through agricultural practices or soil management strategies that promote long-term carbon storage.

In this study, we assumed continuous production of oil and thus a constant inflow of feedstock to the chipping site at the biorefinery, in order to avoid mold development during long-term storage of wet woodchips. By harvesting and storing *Salix* in bundles, it is also possible to run the chipper on electricity rather than a diesel engine. The power was assumed to come from internal sources and, as generation of electricity in the plant exceeded demand, the overall climate impact of production in the biorefinery would not be affected, even though the chipping needed to produce finer fractions and thereby consumed more energy. Like stirring and aeration, the size of wood chips needs to be adjusted to ensure efficient steam explosion.

Yeast oil has similar properties to many vegetable oils and could be used for similar purposes. The raw yeast oil extracted in this study could either be used as is in animal feed or further refined for human consumption. The oil is naturally red in color, due to its betacarotene content, but the carotenoids could be removed to produce a clear, transparent oil. Extraction and separation could be achieved effectively with supercritical $CO_2$, either as a single method to recover other valuable components like proteins without damaging their structure or as a second step to remove impurities from the oil. It is also possible to use the yeast biomass without separating the components, as demonstrated for the case of fish feed for Arctic char (Blomqvist et al., 2018; Brunel et al., 2022, 2024). In industrial feed production, however, fats are added to the feed after pellet formation, so extracting the oil increases its usability for many purposes.

The size of the biorefinery assumed in our analysis would correspond to annual production of 9500 tonnes of oil when operating 96% of the time. The plant would consume 84,100 tonnes of *Salix* feedstock per year, means that its location would need to be carefully planned to ensure a feedstock supply within reasonable transport distance. Assuming annual *Salix* yield of 12 tonnes per hectare, around 7000 ha of *Salix* would have to be grown within an average 35 km transport distance (one-way). For comparison, the current area of *Salix* plantations in Sweden is 12,000 ha (Jordbruksverket, 2013). Thus the amount of *Salix* needed for a biorefinery of the size assumed in our analysis is not unrealistic, but the plant would need to be located in an area with a high proportion of agricultural land unsuitable for ordinary crop production. In Sweden, an estimated 100,000 ha of land are suitable for *Salix* cultivation (Jordbruksverket, 2013). With an assumed *Salix* yield of about 12 tonnes per hectare, the production potential would be 136,000 tonnes of oil, compared with 314,000 tonnes of rapeseed (approx. 50% oil content) grown in Sweden in 2023 (SCB, 2023). Thus, large-scale cultivation of *Salix* on land currently not used for other agricultural production could substantially increase overall production of oil in Sweden.

## 5. Conclusions

This analysis showed that *Salix* biomass can be a viable feedstock for yeast oil production, with GWP comparable to that of conventional rapeseed oil. Including SOC effects reduced the climate impacts even further, making the system an interesting conversion pathway for feed and food oil. The particular *Salix* variety cultivated determined the net climate impact of the yeast oil produced, since characteristics such as SOC sequestration, productivity, and response to fertilization were variety-specific, which affected the overall energy demand and climate impact of the system.

In conclusion, yeast oil production from *Salix* biomass has potential in mitigating future climate change through the significant climate mitigation effects of biomass uptake and SOC sequestration. However, our findings underscore the importance of considering both short-term and long-term climate impacts when evaluating the sustainability of bioenergy systems. They also highlight the need for continuous research and development efforts to further enhance the environmental performance of yeast oil production from *Salix* biomass.

## CRediT authorship contribution statement

**Christian Sigtryggsson:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Conceptualization. **Saurav Kalita:** Writing – review & editing, Writing – original draft, Software,

Formal analysis, Conceptualization. **Hanna Karlsson-Potter:** Supervision, Formal analysis, Conceptualization. **Volkmar Passoth:** Funding acquisition, Formal analysis, Conceptualization. **Per-Anders Hansson:** Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2025.144881.

## Data availability

Data will be made available on request.

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