The International Journal of Life Cycle Assessment
https://doi.org/10.1007/s11367-024-02301-1

GREEN CHEMISTRY

# Life cycle assessment as a driver for process optimisation of cellobiose lipids fermentation and purification

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Received: 23 August 2023 / Accepted: 19 March 2024


## Abstract

**Purpose** Cellobiose lipids (CL) are biosurfactants produced by various *Ustilaginaceae* species in aerobic fermentations. They show high potential for application as alternatives to conventional oleochemical- or petrochemical surfactants. To ensure their environmentally friendly performance, we aimed to assess CL production from a life cycle perspective at an early developmental stage to identify process steps that have the highest impact on the environment. With this information, optimisation approaches can be derived.

**Materials and methods** Following a cradle-to-gate approach, we modelled the CL fermentation and purification process based on experimental data from the lab scale and process simulation data at a 10 $m^3$ scale. For LCA, the impact categories (IC) abiotic depletion potential (ADP), eutrophication potential, photochemical ozone creation potential, global warming potential, acidification potential, and the primary energy demand were calculated for all process steps. Based on the obtained results, process bottlenecks were identified, and alternative process scenarios varying the related process parameters were simulated. These were used to assess the environmental impact reduction potential (EIRP) of an optimised process and draw recommendations for experimental process optimisation.

**Results and discussion** The obtained results showed that the fermentation caused ~ 73% of ADP and more than 85% of all other ICs. The major contributor was the electricity consumption for continuous fermenter aeration. Thus, reducing the fermentation duration from the initial 14 to 5 days would result in a decrease in all investigated ICs of up to ~ 27–52%. An increase in CL concentration results in a decrease in all ICs of a similar magnitude due to the higher yield per batch at comparable energy and material consumption. Although the share of purification process steps to all ICs is overall relatively small, implementing foam fractionation for in situ product recovery showed an additional EIRP of 18–27% in all purification IC shares.

**Conclusions** The conducted LCA showed that overall, more EIRP can be achieved by optimising fermentation process parameters compared to purification process steps. This is mainly due to the long fermentation duration and large energy consumption for fermenter aeration. This highlights the importance of using LCA as a driver for process optimisation to identify process steps with high EIRP. While some of the results are specific to CL, other obtained results can be transferred to other fermentations.

**Keywords** Fermentation · Life cycle assessment · Cellobiose lipids · Process optimisation · Biosurfactants

Communicated by Guido W. Sonnemann.

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Published online: 24 May 2024

## 1 Introduction

Cellobiose lipids (CL) are one class of glycolipid biosurfactants with high potential as substitutes to chemically synthesized surfactants currently used in the market. The global surfactants market was estimated at 36 bn. US$ in 2020, with an estimated compound annual growth rate of more than 4% in 2022–2027 (Mordor Intelligence 2021). The current application is distributed amongst most commodity sectors, such as detergents, personal care products, paints, lubricants, textiles, pharmaceuticals, food and many more. While environmental regulations in many countries (e.g. EU Regulation No. 648/2004 (European Parliament 2004)) have led the surfactant industry to develop non-toxic and biodegradable petroleum- and plant-oil-based surfactants, there is still room to enhance the environmental footprint of surfactants. Biosurfactants that are produced by microorganisms using renewable substrates are promising candidates for better environmental performance. However, while in general, an enhanced environmental impact is attributed to biosurfactants, for example in Banat et al. (2000) and Banat and Thavasi (2018), an enhanced environmental impact may not be obtained per se and must, therefore, be systematically evaluated. This was recently highlighted in Briem et al. (2022), where we reviewed several life cycle assessment studies on biosurfactants.

Due to the early developmental stage of biosurfactant production, previous life cycle assessment (LCA) studies have tended to show significantly higher environmental impacts compared to traditional surfactants, but the different scales and development stages prevent comparability. To illustrate an example, Kopsahelis et al. (2018), for instance, calculated a global warming potential (GWP) of 735 kg $CO_2$ Eq./kg of sophorolipids and 567 kg $CO_2$ Eq./kg of rhamnolipid, whereas the GWP of market average surfactants ranged between 0.5 kg $CO_2$ Eq./kg and 2.7 kg $CO_2$ Eq./kg surfactant (Schowanek et al. 2018). At the same time, the number of publicly available LCAs for biosurfactant production is rather limited, especially with regard to the evaluation of upscaling and process optimisation towards industrial production. Thus the literature does not yet show a complete picture of the environmental aspects of biosurfactant production. Available LCA publications of biosurfactants are limited to the biosurfactants Sophorolipids and Rhamnolipids only (Baccile et al. 2016, Aru and Ikechukwu 2018, Kopsahelis et al. 2018). For instance, Bacille et al. compare the production of Sophorolipids to other surfactants and examine the impact of the production phase, the use phase and the end-of-life phases as a whole. Specific production steps along the fermentation and purification process were not examined. In their work, they show that 90% of environmental impact is caused by the substrates rapeseed oil and glucose, and 7% by electricity. They conclude that all in all, the environmental impact was similar to that of chemical surfactants derived from fossil resources.

LCA studies are therefore crucial to obtaining a profound knowledge about the environmental impact of biosurfactants, especially in comparison with traditional, chemically synthesized surfactants. Traditional surfactants have been studied extensively and their production processes are continuously optimised, so data availability for conducting detailed LCA is present. This is shown, for example, in the results and datasets published in a recent study of the *ERASM surfactant life cycle and ecofootprinting project*, in which aggregated life cycle inventories of 15 surfactants and 17 precursors are presented (Schowanek et al. 2018). Further, some industrial surfactant producers own internal LCI data which are not publicly available but were used to assess their surfactant production sites (Schowanek et al. 2018).

LCA is often used to calculate the overall environmental impact of a certain product/substance and compare it to other products/substances. Standardised methods (e.g. ISO 14040/44) ensure the comparability of the obtained results, provided all assumptions are comparable. Based on an inventory analysis of a product's life cycle, its impact on different environmental categories can be quantified using certain chemical substances as equivalents. For instance, the GWP is calculated based on an equivalent of $CO_2$, also widely known as *$CO_2$ footprint*. The abiotic depletion potential (ADP) is calculated as equivalent to Sb. It is defined as the ratio of a consumed resource and the square of the ultimate reserve for this resource, divided by the same ratio for Sb (van Oers et al. 2020). The acidification potential (AP) represents the contribution of acidic emissions, such as $SO_2$, $NO_x$, HCl, $NH_3$, and HF, to form $H^+$ and is calculated as an equivalent relative to the AP of kg $SO_2$ Eq. (Azapagic et al. 2003). Similarly, the eutrophication potential (EP) represents emissions that may cause over-fertilisation of water and soil and are calculated relative to the same effect of a kg of $PO_4^{3-}$ Eq. (Azapagic et al. 2003). The photochemical ozone creation potential (POCP) sums up the contribution of emissions that can cause photochemical smog, such as volatile organic carbons, represented by $C_2H_4$ Eq. The primary energy demand (PED) in MJ sums up all energy demand needed to operate the production of the analysed product. Other environmental impact categories addressing different environmental issues are, for instance, the aquatic ecotoxicity potential.

Depending on the assessed product, different system boundaries and impact categories become more or less relevant. A cradle-to-grave approach, for instance, is appropriate for established products with a defined application. Here, both their production process, as well as their disposal are being considered. On the other hand, for less developed products, where the specific application is still not defined yet, a cradle-to-gate approach may be more reasonable. Setting the system boundaries as cradle-to-gate would focus on the production process itself. Impact categories that are highly dependent on the disposal of a product, such as aquatic ecotoxicity, cannot be adequately assessed here, though.

This is often the case during early development stages: no specific application is defined, and the production process usually still needs optimisation. Further, data availability is usually

scarce at such a stage, and thus the model accuracy is not very high. At this stage, using LCA for an absolute assessment of a product would be associated with high uncertainty. It can, however, be used as a tool to identify optimisation potential within the production process and thus contribute to a faster process development that considers environmental impacts from the early development stage. That way, developing an optimised production process from an experimental or economic perspective, which may, however, have a negative impact on the environment, is prevented. Using LCA, the environmental impact of individual process steps along the production cycle of a product is calculated. These hereby-obtained results can be used to conduct a so-called hot spot analysis and identify process bottlenecks that cause the highest impact on the environment. Based on these bottlenecks, recommendations for experimental process optimisation can be drawn, and process optimisation can be realised at an early development stage, where process alteration is still possible at low costs. The potential technology space for environmental sustainability improvement includes for instance enhanced agricultural practices and sustainable farming solutions for substrate production for the fermentation, or the usage of secondary feedstock. Further, the bioreactor design could be adjusted to enable a more sustainable fermentation process, or fermentation yields could be increased. Considering common purification processes, optimisation potential may include recycling of used solvents, the usage of solvents from renewable resources, or more efficient separation processes. A general measure for achieving an overall optimised process from an environmental perspective is the use of renewable energy and energy integration.

A similar integrated process development approach was followed and described by Biwer in his dissertation and illustrated for different biotechnological processes (2003). By comparing different process scenarios of a modelled enzymatic α-cyclodextrin production process, he concluded, for instance, that an increase in the yield of the enzymatic conversion has the biggest EIRP, whereas using thermostable enzymes would lead to small ecological improvements only. A similar approach was followed by Gasafi et al. where they used life cycle assessment to identify process bottlenecks of the supercritical water gasification of organic feedstock with the highest environmental impact to improve environmental performance through process optimisation (2008). Munagala et al. also used LCA and economic assessment to identify process optimisation potential for sugarcane bagasse valorization to sugar lactic acid based on the analysis of different scenarios (2021). Both approaches were specific to their described process and used environmental hotspot analysis to derive recommendations for process optimisation.

Despite the high potential of integrated process development for biotechnological processes, only little research is done in this area. Especially for biosurfactant process optimisation, there are no available studies on using integrated process development for comprehensive process optimisation to our knowledge. Therefore, with this research, we aim to provide an LCA model of CL fermentation and purification to partly contribute to the research gap in this area. Using this model, we identified environmental process bottlenecks of the CL fermentation and purification process. Based on several process scenario simulations, we analysed the optimisation potential in terms of a reduced environmental impact and were able to propose process optimisation potential based on the hotspot analysis at an early development stage of CL fermentation. The obtained results can partly be transferred to other fermentation processes and are not limited to CL only.

## 2 Methods

Life cycle assessment of the CL fermentation and purification process was conducted based on ISO 14040/44, using the software *LCA for Experts (GaBi)* and the *Sphera's Managed LCA Content* database (formerly known as *GaBi professional* database) version CUP 2021.2 according to the four phases of LCA as follows: First, the goal and scope were defined, and the system boundaries were set. An inventory analysis of all process steps was conducted, and the impacts were calculated and assessed. The results were finally interpreted, and different scenarios were compared. This procedure is described for each of the four specific phases in detail in the next subsections.

### 2.1 Goal and scope

The goal of this life cycle assessment was to assess the environmental impact of different process steps along the CL fermentation and purification route to identify process bottlenecks that contribute the most to each impact category. These bottlenecks were analysed to propose changes in the fermentation and purification process that would lead to optimisation in terms of an enhanced final process with reduced impact on the environment. The assessment included all fermentation steps, starting from the seed culture to the production culture based on a 10 $m^3$ fermenter and the purification steps needed to obtain CL with purities > 90%. The followed *cradle-to-gate* approach is illustrated schematically in Fig. 1. This assessment scope was chosen because currently there is no specific application area for CL defined yet. The functional unit is defined as 1 kg purified CL (with > 90% purity).

### 2.2 Inventory analysis

For the CL fermentation and purification process model, material balances were calculated based on experimental data from the lab scale. Energy and utility balances were obtained from a process simulation in the 10 $m^3$ scale (Oraby et al. 2022b) or calculated based on thermodynamic equations. This pilot plant scale was chosen because of the availability of primary process data

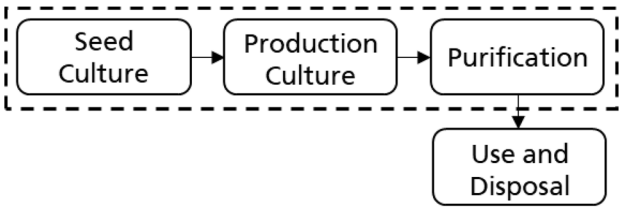


**Fig. 1** Schematic illustration of the scope of the LCA study of the CL production process. The dotted rectangle indicates the process steps within the examined cradle-to-gate scope

from a 10 $m^3$ pilot-plant fermenter. Thus, a higher model accuracy could be achieved, although fermentation data were only obtained from a 10 L scale. A restriction within the chosen scale is that the obtained absolute values of the environmental impact do not reflect a larger industrial-scale fermenter. However, since the model is based on material and energy balances, they can be mostly scaled up linearly. Exceptions where the assumption of linear scalability does not apply are stated individually in the results' discussions. Therefore, the scale analysed is considered sufficient for deriving recommendations for process optimisation.

The modelled process steps are illustrated in Fig. 2. A first seed culture in shake flasks is followed by a second seed culture in a 300 L fermenter. This seed culture serves to cultivate a certain amount of biocatalyst (the CL-producing microorganism) to inoculate the production culture in the 10 $m^3$ fermenter. A shift in cultivation medium and pH triggers the microorganisms to produce CL. All fermenters are agitated and aerated with pressurized ambient air. For each fermenter, all media components in the reactor, as well as energy consumption needed for agitation and aeration, are modelled. Further, fermenter cleaning (CIP-cleaning in place) and sterilisation (SIP-sterilisation in place) are simulated in specific models. In addition, sterilisation of each media component group is considered as part of the media preparation unit, either by heat sterilisation for heat-stable media components (mineral salts, urea, sucrose) or by filtration for heat-labile components (vitamins).

In the purification model, the culture broth from the fermenter, containing CL crystals, biomass and remaining media components, is first separated in a separator to obtain a pellet that consists of biomass and CL crystals, with a liquid content of 80%. In the case of an operation with foam fractionation (Oraby et al. 2022a), only the separated foamate fraction is purified. In this operation mode, the foam that is created during fermentation is directed from the fermenter headspace into a foam tank without additional external power, due to existing overpressure in the fermenter. A product-rich fraction, the foamate, amounting to approx. 7% of the fermenter content is then transferred to the DSP unit. In a first approximation, no additional energy input is modelled for this. The product-poor fraction, the retentate, with media components and biomass, is pumped back to the fermenter. Therefore, an additional pump is modelled for the foam fractionation unit.

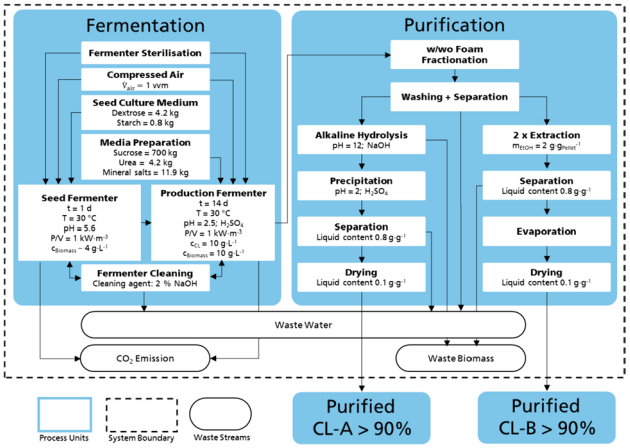


**Fig. 2** Schematic illustration of the modelled CL fermentation and purification process within the cradle-to-gate system with the different purification scenarios. The main process parameters for the modelled standard scenario are indicated for each process unit

For approximation purposes, an ideal foam fractionation process with no biomass remaining in the foamate is modelled.

In both operation modes, with or without foam fractionation, the separated pellet is washed with acidic water to remove the remaining sugars (Oraby et al. 2020). A second switch in the modelled purification enables the choice between two purification methods, defining the proceeding purification steps:

1. Extraction with ethanol to obtain CL from the pellet after separation, followed by a separation step to remove the biomass and finally, evaporation and drying of the purified CL: CL-B
2. pH-shift from 2.5 to 12 to solubilise CL and hydrolyse it to obtain the hydrophilic variant CL-A, followed by a separation step to remove the biomass, then precipitation with sulphuric acid and a final separation and drying step to obtain CL: CL-A

The hydrolysis of CL-B to CL-A results in a weight loss by a factor of approx. 0.2, due to the cleavage of side chains. This means that out of 1 kg CL-B, only 0.8 kg CL-A can be obtained. A detailed description of both the fermentation and purification process was previously published by Oraby et al. (2022b). Liquid waste, potentially containing remaining culture media components, such as non-consumed sugar or vitamins, is assumed to be dispensed into the municipal wastewater system and treated in a wastewater treatment plant. Solid waste, e.g. the microorganisms after fermentation, is modelled as being combusted in a waste-to-energy incineration plant to generate electricity (Tables 1 and 2).

The individual input data sources used in the described model are presented in the following:

**Table 1** Main input material streams and the modelled input processes, along with assumptions made if the modelled materials did not match the real material completely

| Process unit | Item | Modelled input/output | Amount per batch [kg] | Assumption/data source |
|---|---|---|---|---|
| | | **Seed culture medium** | | |
| **Seed culture medium** | Dextrose | US: crystalline dextrose monohydrate (from corn) | 4.2 | The used PD medium (Becton Dickinson, Le Pont de Claix, France) was approximated with 20 $g \cdot L^{-1}$ dextrose and 4 $g \cdot L^{-1}$ starch. |
| | Starch | DE: dried starch (corn wet mill) | 0.8 | |
| | | **Media preparation** | | |
| **Mineral salt medium** | Mono-potassium phosphate | EU-28: raw phosphate (32% $P_2O_5$) | 7 | The four media components with the largest shares were modelled. The remaining components contribute to less than 0.35% of the used medium. |
| | Calcium chloride | DE: calcium hypochlorite | 0.7 | |
| | Sodium chloride | DE: sodium chloride (rock salt) | 0.7 | |
| | Magnesium sulfate | DE: magnesium sulphate (agrarian) | 3.5 | |
| **Substrate** | sucrose | EU-28: sugar (from sugar beet) | 700 | |
| | urea | DE: urea/stamicarbon process | 4.2 | |
| **Acid for pH adjustment** | $H_2SO_4$ | DE: acetic acid (96%) | 1.1 | Calculated to achieve a pH of 2.5 in the production culture media. |
| **DI water for media preparation** | $H_2O$ | GLO: deionised water (reverse-osmosis/electro-deionisation, from groundwater, for regionalization) | $203^a + 6423^b$ | "DE: tap water from groundwater" was used as input. |
| **Filter for media sterilisation** | Filter cartridge | EU-28: polyester (PET) fabric | 0.8 | Only the consumables (filter cartridges) were modelled and approximated gravimetrically |
| **Steam for media sterilisation** | Steam | GLO: steam conversion (vlp) | 266 | External heat sterilisation of cultivation media in the media preparation vessels. Values are calculated using thermodynamic equations assuming a sterilisation temperature of 121 °C and an initial temperature of 21 °C. |
| | | **Fermenter** | | |
| **$CO_2$ emission by the microorganisms** | $CO_2$ | Emission flow "carbon dioxide (biogenic)" | $5^a + 221^b$ | Emitted $CO_2$ was calculated based on reaction stoichiometry and biomass concentrations. |
| | | **Fermenter sterilisation** | | |
| **Steam for reactor sterilisation (300 L + 10 $m^3$ fermenter)** | Steam | GLO: steam conversion (vlp) | $40^a + 1330^b$ | Filled fermenter sterilisation. Values are calculated using thermodynamic equations assuming a sterilisation temperature of 121 °C and an initial temperature of 21 °C. |
| **SIP procedure (300 L + 10 $m^3$ fermenter)** | Steam | GLO: steam conversion (vlp) | $15^a + 500^b$ | Values obtained from the SuperPro Designer simulated process published in (Oraby et al. 2022b). |

Table 1 (continued)

| Process unit | Item | Modelled input/output | Amount per batch [kg] | Assumption/data source |
|---|---|---|---|---|
| | | **Fermenter cleaning (CIP)** | | |
| **CIP procedure** | NaOH | DE: sodium hydroxide (caustic soda) mix (100%) | 20 kg per 10 $m^3$ | Calculated based on 0.1 L cleaning agent (2% NaOH) per L vessel volume. $H_2SO_4$ was used to neutralize the washing liquid before disposal. All material streams were adjusted to the individual fermenter and vessel volumes. The amount of modelled waste water was adjusted to the biomass content in the real amount of generated waste water. |
| | $H_2SO_4$ | DE: acetic acid (96%) | 25 kg per 10 $m^3$ | |
| | DI $H_2O$ | GLO: deionised water (reverse-osmosis/ electro-deionisation, from groundwater, for regionalization) | 2990 kg per 10 $m^3$ | |
| | Disposed waste water | DE: municipal waste water treatment | 174 kg | |
| | | **Washing + separation** | | |
| **Acidic wash of the CL/biomass pellet** | $H_2SO_4$ | DE: acetic acid (96%) | 3.2 | Calculated to achieve a pH of 2. |
| | DI $H_2O$ | GLO: deionised water (reverse-osmosis/ electro-deionisation, from groundwater, for regionalization) | 6300 | |
| | | **Alkaline hydrolysis** | | |
| **Hydrolysis of CL-B to CL-A** | NaOH | DE: sodium hydroxide (caustic soda) mix (100%) | 12 | Experimental data |
| | $H_2SO_4$ | DE: Acetic acid (96%) | 16 | Experimental data |
| | Correction factor CL-A to CL-B | - | 0.8 kg/kg | Experimental data |
| **Alkaline hydrolysis** | Steam | GLO: steam conversion (vlp) | 386 | Calculated using thermodynamic equations to obtain a reaction temperature of 50 °C. |
| | | **Extraction** | | |
| **CL extraction with ethanol** | Ethanol | DE: ethanol (96%) | 246 | Extraction was done twice with 2 $g_{EtOH}/g_{Pellet}$; however, only the not recycled amount of ethanol was calculated as an input stream. |
| | | **Evaporation** | | |
| **Evaporation** | Steam | GLO: steam conversion (vlp) | 779 | The operation parameters were estimated based on common evaporators and the values were obtained from the SuperPro Designer simulated process published in Oraby et al. (2022b). |

All media components were scaled based on a lab-scale process in a 10 L fermenter and were previously described in Oraby et al. (2022b). The presented amounts were calculated for the standard fermentation scenario and both purification methods (CL-B and CL-A) and are categorized according to the corresponding processing steps in Fig. 2

[a]Values calculated for the seed culture

[b]for the production culture

**Table 2** Main input and output energy and utility streams and the modelled input processes along with the data sources or the used calculations

| Process unit | Process step | Modelled input/output | Amount per batch | Assumption/data source |
|---|---|---|---|---|
| | | **Seed culture and compressed air** | | |
| **Seed culture** | Generation of pressurised air for aeration | Electricity (DE: electricity grid mix) | 92 MJ | Calculated based on a consumption of 0.303 MJ·$Nm^{-3}$ for the generation of 4 bar compressed air. |
| | Agitation | | 20 MJ | Calculated based on a power input of 1 kW·$m^{-3}$ stirred volume. |
| | Circulation of cooling water | | 0.4 MJ | Estimated based on data obtained from a 10 $m^3$ pilot plant. |
| | | **Media preparation** | | |
| **DI-$H_2O$** | Deionisation of tap water | Electricity (DE: electricity grid mix) | 0.0037 MJ/kg $H_2O$ | Required input of GLO: deionised water (reverse-osmosis/electro-deionisation, from groundwater, for regionalization) |
| | | **Fermenter and compressed air** | | |
| **Production culture** | Generation of pressurised air for aeration | Electricity (DE: electricity grid mix) | 42,800 MJ | Calculated based on a consumption of 0.303 MJ·$Nm^{-3}$ for the generation of 4 bar compressed air. |
| | Agitation | | 8543 MJ | Calculated based on a power input of 1 kW·$m^{-3}$. |
| | Circulation of cooling water | | 13 MJ | Estimated based on data obtained from a 10 $m^3$ pilot plant. |
| | | **Fermenter cleaning (CIP)** | | |
| **CIP procedure of the 10 $m^3$ fermenter** | Recirculation of washing liquids and mixing via agitation | Electricity (DE: electricity grid mix) | 17.4 MJ | Estimated based on data obtained from a CIP unit of a 10 $m^3$ pilot plant fermenter. |
| | Operation of different valves during the CIP procedure | Pressurised air | 1.82 $Nm^3$ | Estimated based on data obtained from a CIP unit of a 10 $m^3$ pilot plant fermenter. |
| | | **Foam fractionation** | | |
| **Foam fractionation** | Recirculation of foamate fraction | Electricity (DE: electricity grid mix) | 8.8 MJ | Calculated for an estimated continuous conveyance of 7 L of foamate per minute back to the fermenter. |
| | | **Washing + separation** | | |
| **Separation** | Separation | Electricity (DE: electricity grid mix) | 22 MJ | Separators were used after each purification step to separate the liquid from the solid fraction to a water content of 80% (value approximated based on experimental results). The operation parameters were scaled up based on laboratory data and the values were obtained from the SuperPro Designer simulated process published in Oraby et al. (2022b). |
| | Circulation of cooling water | | 0.2 MJ | Calculated based on an estimated consumption of 420 L of cooling water per operation hour. |
| **Acidic wash of the CL/biomass pellet** | Pellet suspension via agitation | Electricity (DE: electricity grid mix) | 12.6 MJ | Calculated based on a power input of 1 kW·$m^{-3}$. |
| | Electricity for pellet separation | | 22 MJ | Calculated as in the separation step and adjusted to the input amount. |

Table 2 (continued)

| Process unit | Process step | Modelled input/output | Amount per batch | Assumption/data source |
|---|---|---|---|---|
| | | **Alkaline hydrolysis** | | |
| **Alkaline hydrolysis** | Pellet suspension via agitation | Electricity (DE: electricity grid mix) | 202 MJ | Calculated based on a power input of 1 kW·m$^{-3}$ and a reaction duration of 8 h. |
| | Electricity for biomass separation | | 22 MJ | Calculated as in the separation step and adjusted to the input amount. |
| | | **Precipitation** | | |
| **Acidic precipitation** | Mixing via agitation | Electricity (DE: electricity grid mix) | 24 MJ | Calculated based on a power input of 1 kW·m$^{-3}$ and a reaction duration of 1 h. |
| | Electricity for CL separation | | 24 MJ | Calculated as in the separation step and adjusted to the input amount. |
| | | **Extraction** | | |
| **CL extraction with ethanol** | Pellet suspension via agitation | Electricity (DE: electricity grid mix) | 8.1 MJ | Calculated based on a power input of 1 kW· m$^{-3}$. |
| | Electricity for pellet separation | Electricity (DE: electricity grid mix) | 6.1 MJ | Calculated as in the separation step and adjusted to the input amount. |
| | | **Evaporation** | | |
| **Evaporation** | Electricity | Electricity (DE: electricity grid mix) | 24.7 MJ | The operation parameters were estimated based on common evaporators and the values were obtained from the SuperPro Designer simulated process published in Oraby et al. (2022b). |
| | | **Drying** | | |
| **Drying** | Thermal energy | Thermal energy (DE: thermal energy from natural gas) | 2410 MJ | Air drying process from the *Sphera's Managed LCA Content* database to a final liquid content of 10% was used. |

All energy streams were calculated/estimated for the standard scenario based on a simulated pilot scale process in a 10 m$^3$ fermenter, as published in Oraby et al. (2022b). The presented amounts were calculated for the standard fermentation scenario and both purification methods (CL-B and CL-A) and are categorized according to the corresponding processing steps in Fig. 2

Material or energy consumption that contributes to < 1% of an individual process was not considered as long as their sum did not exceed 5%, in accordance with ISO14040/44 and as suggested by Heinrich (2009). Examples, therefore, are energy consumption for heating/cooling of the fermenter during fermentation (30 °C) or electricity for control units, such as computers. For background processes like electricity generation and production of materials, as well as common process steps like drying or waste treatment, models from the *Sphera's Managed LCA Content* database from Sphera were used.

### 2.3 Impact assessment and scenario generation

For impact assessment, the following impact categories (IC) were chosen based on the CML2001 (August 2016) methodology:

- ADP, elements: abiotic depletion potential [kg Sb Eq.]
- EP: eutrophication potential [kg $PO_4^{3-}$ Eq.]
- POCP: photochemical ozone creation potential [kg $C_2H_4$ Eq.]
- GWP: global warming potential [kg $CO_2$ Eq.]
- AP: acidification potential [kg $SO_2$ Eq.]
- PED: primary energy demand [MJ]

All impact indicator results were calculated relative to the functional unit of 1 kg of purified CL. The selection is based on the relevance and robustness of these impact categories as well as the focus on the production phase with the cradle-to-gate approach. Our aim was to address multiple environmental aspects with a high relevance for bioprocess optimisation. Impact categories related to eco- and human toxicity were not considered, as the application of CL and thus its use phase and disposal, where the majority of relevant emissions in these categories are to be expected, are yet to be defined.

Based on the evaluation of the standard scenario, process parameters that largely contribute to the individual ICs were varied to simulate process scenarios with the highest environmental impact reduction potential (EIRP). The contribution of the standard scenario to all ICs is normalised (100%) and used as a benchmark to compare the environmental impact of all the other scenarios. All analysed scenarios are summarised in the following table (Table 3).

Scenario 1 is the main scenario modelled based on experimental data and calculations as stated in Section 2.2. Variations in the different parameters were chosen based on the following upper/lower bounds:

- Fermentation duration: a hypothetical minimal duration of 5 days was chosen based on the higher CL productivity within the first fermentation phase, as described in Oraby et al. (2023)
- CL concentration: the maximal CL concentration currently described in the literature is 33 $g \cdot L^{-1}$ (Günther 2014). With advancing fermentation optimisation, a concentration of up to 50 $g \cdot L^{-1}$ seems plausible and was thus chosen as the upper bound to evaluate the potential ecological impact of this increase in CL concentration.
- Aeration and agitation rates: A lower bound of 10% of the values modelled in the main scenario was chosen to assess the EIRP that could be achieved if agitation and aeration were to be reduced.
- Liquid content in CL/biomass pellet: The liquid content in the CL/biomass pellet is highly dependent on the used separation method. To assess the EIRP of a "dryer" pellet, a value of 0.5 was chosen arbitrarily in comparison to the experimentally determined value of 0.8. A further decrease in the liquid content would complicate the proceeding resuspension of the pellet in the liquid phase (EtOH or water, depending on the purification method).

## 3 Results and discussion of impact assessment

Aspiring for a better environmental footprint, we used LCA as a decision-making tool for process optimisation of both the CL fermentation and purification process. Based on an initial impact assessment (Section 3.1), environmental bottlenecks were identified, and hypothetical optimisation scenarios were derived. By evaluating these hypothetical scenarios and their impact on the environment, we were able to suggest prospective experimental optimisation approaches that would lead to optimised CL fermentation (Section 3.2) and purification (Section 3.3).

### 3.1 Overall environmental impact of the CL fermentation and purification process

The modelled CL production process was divided into the main sections of seed culture, fermentation, and purification and relative values were used to assess the contribution of each process unit (Fig. 2) to the overall process impact. While the impact of the seed culture can be neglected (0.6–1.6% in all ICs), the production culture contributed to more than 85% in all ICs, except for the ADP with 73% (Fig. 3 and Supplementary Table 1). The remaining impact is caused by operations within the purification process of CL.

The relatively low impact of the seed culture is explained by the large-scale proportion between the production culture fermenter and the seed fermenter (300 L vs. 10,000 L, equivalent to a factor of 33). Further, the seed culture is operated

**Table 3** Analysed process scenarios along with the varied parameters for each scenario

| Scenario | 14 d | 10 d | 5 d | 20 g·L$^{-1}$ | 50 g·L$^{-1}$ | 0.1 vvm | 0.1 kW·m$^{-3}$ | CL-A | W | 0.5 |
|---|---|---|---|---|---|---|---|---|---|---|
| Scenario number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Fermentation duration [d] | 14 | 10 | 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| $c_{CL}$ [g·L$^{-1}$] | 10 | 10 | 10 | 20 | 50 | 10 | 10 | 10 | 10 | 10 |
| Agitation rate [kW·m$^{-3}$] | 1 | 1 | 1 | 1 | 1 | 1 | 0.1 | 1 | 1 | 1 |
| Aeration rate [vvm] | 1 | 1 | 1 | 1 | 1 | 0.1 | 1 | 1 | 1 | 1 |
| CL-B | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| CL-A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Foam fractionation (FF) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Liquid content in CL/ biomass pellet [g·g$^{-1}$] | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.5 |

For categorical parameters (e.g. CL-A vs. CL-B), the numbers 0 and 1 indicate the active process parameter

for 24 h, whereas the production culture is fermented for up to 336 h, adding a factor difference of 14 by which all time-dependent input and output streams of the fermentation are scaled. Hence, it can be concluded that the seed culture is not a process bottleneck of the CL production process and will thus be neglected in further analysis and discussions within this work.

Within the fermentation process units, aeration of the fermenter contributes the most to all ICs, followed by its agitation. Both operations consume the highest shares of PED caused by the agitation rate of 1 kW·m$^{-3}$ and the compression of air used for aeration at 1 vvm. These parameters are both time-dependent. Therefore, the EIRP due to a decrease in fermentation duration was assessed in scenarios 1–3 (Section 3.2.1). In addition, due to the potentially high EIRP by the reduction of both agitation and aeration rates, scenarios 6 and 7 were analysed (Section 3.2.3). Both fermentation duration and oxygen input due to varying aeration and agitation rates can highly affect microbial growth and CL production. Therefore, further scenarios 4 and 5 with hypothetical CL concentrations were simulated (Section 3.2.2).

Besides aeration and agitation, the used carbon source has a large impact on the EP (Fig. 3). This is mainly due to emissions to the environment during the cultivation of sugar beet used for sugar synthesis. Further, the conversion steps within the sugar refinery process also contribute to these emissions. One approach to reducing this impact is the use of sugar obtained from 2$^{nd}$ generation feedstock, like agricultural waste streams. Using these streams as the substrate would result in valorising these waste streams while reducing the environmental impact attributed to the used carbon source. While, to our knowledge, there is no known production of CL using 2$^{nd}$ generation feedstock, the potential fermentation of other biosurfactants, like rhamnolipids using lignocellulosic substrates, was presented in Joy et al. (2020), for instance. A further measure to reduce the impacts caused by the C-source is the usage of sugar intermediates along the sugar refinery route. This would save conversion steps and thus reduce the impacts caused by sugar production. The usage of such intermediates was demonstrated for two CL-producing strains, as presented in a project report (Oraby and Baron-Nunez 2021). However, a detailed assessment and comparison between the used substrates is crucial for an accurate estimation of EIRP. A limitation here is the uncertainty in data collection and the assumptions made during the assessment of the substrates of agricultural origin (for example regarding land use and land use change) and the scarcity of background models for different sugar intermediates and lignocellulosic substrates.

A further notable impact on ADP within the fermentation process units, besides aeration and agitation, was caused by the CIP processes of the fermenter (18%), caused by the usage of NaOH and $H_2SO_4$. These process steps, however, cannot be avoided and would gain more importance at reduced fermentation durations. Cleaning the fermenter between each batch is crucial for successful fermentation. An option to reduce these impacts could be to change the cleaning chemicals and use more environmentally friendly substances instead. Further, while reducing the fermentation duration would result in more CIP cycles, switching to a continuous fermentation process would decrease the needed CIP and SIP cycles. To our knowledge, there is currently no described process for continuous CL fermentation. However, operating the DSP units continuously could be achieved easily with appropriate fermentation scheduling, thus resulting in a potential reduction of CIP cycles of the DSP units.

Looking at the DSP process units, there is no single process step that contributes the most to all ICs. However, the extraction step has a comparably large impact on all ICs (7% to ADP, 3% to EP, 10% to POCP, 5% to GWP, 4% to AP, and 7% to the PED), due to the consumption of ethanol. In the modelled scenario, ethanol recirculation is already taken into account. Thus, the calculated impact is caused solely by the amount of ethanol lost after extraction and

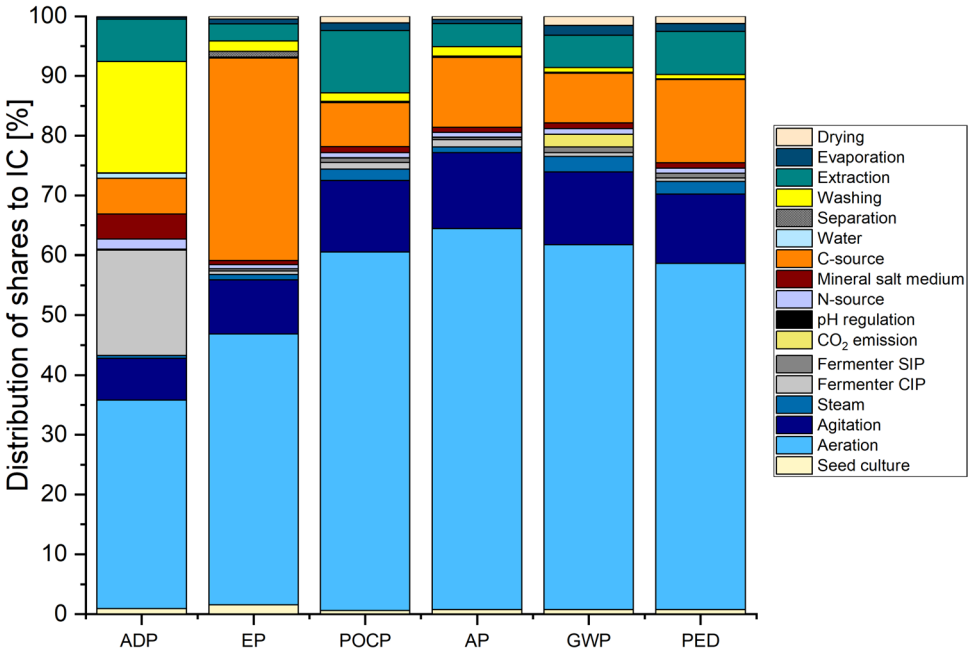


**Fig. 3** Distribution of IC shares on all process units of the CL fermentation and purification process within the cradle-to-gate approach

evaporation. Therefore, in terms of EIRP, a reduction in ethanol consumption is very limited, especially since the calculated amount for this model corresponds to the already optimised lower limit of 2 $g_{EtOH} \cdot g_{Pellet}^{-1}$ (Oraby et al. 2023). Another measure to decrease this impact is the usage of an alternative purification process (scenario 8), as discussed in Section 3.3.1. Further, the application of more innovative purification methods that can simplify CL purification and the overall amount of solvent used for extraction could be considered. An example is given in Section 3.3.2, where an in situ product recovery method via foam fractionation is simulated (scenario 9).

The environmental impact caused by the evaporation step can mainly be attributed to the energy consumption for steam generation. However, the overall impact is below 2% in all ICs. The separation and drying steps show an overall comparably low environmental impact. While the washing step also has a small impact on EP, POCP, GWP, AP and PED, it causes 19% of the ADP of the CL production process. This contribution is mainly caused by the usage of $H_2SO_4$ to neutralise the washing solution (NaOH) used for CIP processes. In general, the used amounts of $H_2SO_4$ and NaOH caused the major impact of the washing step. Wastewater treatment after the pellet wash also contributes to the EP. One approach to minimise this impact is the utilisation of washing agents containing acids and bases that have a lower environmental impact.

A further EIRP worth examining is the separation efficiency. While the separation processes themselves do not have a high impact on any IC, the separation efficiency and thus the amount of separated pellet and its liquid content affect the proceeding purification steps. For the extraction step, the amount of ethanol used is calculated based on the pellet mass. A more compact pellet with less liquid content would result in a decrease in the used ethanol amount and should, consequently, result in a decrease in the overall environmental impact of CL purification. Therefore, a purification process with an enhanced separation efficiency (scenario 10) was simulated and is discussed in Section 3.3.3.

Overall, based on the evaluation of our LCA model, and the assessment of the relative impact of each process unit to all IC, fermenter aeration and agitation, as well as the process parameters fermentation duration and CL concentration were identified as the major process bottlenecks with the highest EIRP for CL fermentation. Using alternative purification methods (other than ethanol extraction) and a more efficient separation process may further reveal EIRP for the purification process. A limitation to our obtained results may be the different assumptions made during data assessment and the uncertainties related to the current early development stage and laboratory scale of CL fermentation and the herewith associated lack of experimental data from large-scale fermentations. Therefore, only the comparison of relative values is feasible at this point, whereas absolute values of IC are expected to gain accuracy with further advancement in CL research and the availability of more process data. Nevertheless, these relative values were sufficient to identify potential measures for process optimisation.

## 3.2 Environmental process bottlenecks of CL fermentation and scenario analysis

To get more insight into the theoretical EIRP of an optimised fermentation process, hypothetical scenarios with a reduced

fermentation duration, an increased CL concentration and lower agitation and aeration rates were simulated. The results are presented and discussed in detail in the following.

#### 3.2.1 Fermentation duration

Fermentation duration was previously shown to be the major economic bottleneck for CL production (Oraby et al. 2022b). A proportional relation between the fermentation duration and all analysed ICs was observed from an environmental perspective as well (Fig. 4). This is mainly caused by the two major contributors to most ICs: agitation and aeration of the fermenter. Both are time-dependent process parameters. Thus, their impact is directly proportional to the fermentation duration. The major cause of environmental impact by both process parameters is their electricity consumption. Emissions caused by and resources consumed for energy generation are reflected in all IC and the PED. All other process steps are independent of the duration of the fermentation, and thus their contribution to the IC results does not decrease with a shorter fermentation duration. This emphasizes the importance of fermentation duration as a parameter with high EIRP since varying this parameter results in a significant reduction in all ICs.

At the same time, the fermentation duration is restricted by the metabolism of the microorganisms during fermentation and cannot be chosen freely, which highly limits the technology space for optimisation here. However, means to reduce the fermentation duration without compromising the obtained CL concentrations, i.e. to increase the space–time-yield, are amongst others media optimisation, genetic modification of the producing microorganisms, or the application of feeding strategies. These need to be examined further for CL fermentation.

Although none of the known LCA studies of biosurfactants identified the fermentation duration as a parameter with high EIRP, Kopsahelis et al. attributed the lower GWP of rhamnolipids, compared to sophorolipids, to a reduced time-dependent energy consumption, due to their shorter fermentation duration (Kopsahelis et al. 2018). Our results show that a decrease in fermentation duration from 14 to 5 days can result in a decrease in all ICs of up to ~ 27–49%. Since the fermentation duration proved to be one of the main process bottlenecks from an environmental perspective and a shorter duration of 10 days seems to be plausible (Oraby et al. 2022b), all scenarios in the following chapters are compared to the 10-day scenario. Furthermore, the change in the relative impact of other process steps among the analysed scenarios can be illustrated more clearly this way.

#### 3.2.2 CL concentration

In biological processes, the fermentation duration is often proportional to the synthesised product concentration, i.e.

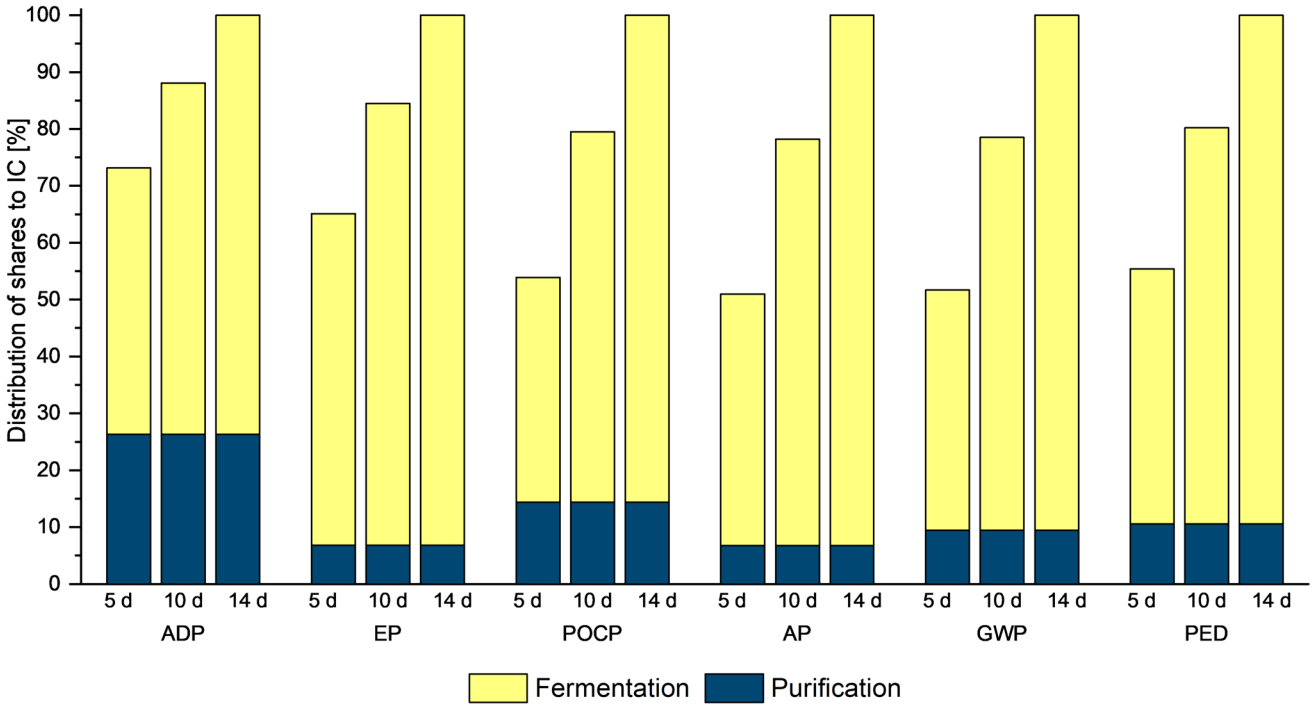


**Fig. 4** Distribution of shares to ADP, EP, POCP, AP, GWP, and PED of the CL fermentation and purification processes at different fermentation durations (5–14 days). A detailed overview of the contribution of each of the major process units of the fermentation process to the ICs is listed in Supplementary Table 2

decreasing the fermentation duration would lead to lower product concentrations if all other parameters remained the same. Therefore, when considering a variation in fermentation duration, it is crucial to also evaluate the effect of concentration variation on the ICs. Increasing the CL concentration would result in a decrease in the ICs per kg CL since the emissions of the fermentation batch would remain almost the same. This can be seen with all ICs, where the contribution of the fermentation to each IC is reduced by ~ 50% at a doubled concentration of 10 g·L$^{-1}$ and by ~ 20% at a fivefold concentration of 50 g·L$^{-1}$ (Fig. 5). Since the fermentation process steps have the largest share of all ICs, compared to the purification process steps, an increase of CL concentration from 10 to 50 g·L$^{-1}$ would result in an overall decrease in all ICs by ~ 68–77%.

The environmental impacts caused by the purification steps, on the other hand, do not decrease with the same significance with increasing CL concentration. A larger amount of CL requires more ethanol for its extraction from the culture broth but also more energy for the evaporation of the ethanol extract and the drying of the extracted CL per batch. However, when normalised and divided by the larger amount of produced CL, the share to all ICs decreases still.

While the obtained results are expected, the quantification of the EIRP is of high importance for the experimental process optimisation. As previously mentioned, higher CL concentrations are achieved with increasing fermentation durations. However, CL production rates are not constant during the fermentation duration. They would rather reach their maximum at an early time point and decrease with the declining availability of glucose in the cultivation medium (Oraby et al. 2023). At the same time, longer fermentation durations are associated with an increase in environmental impact. For instance, decreasing the fermentation duration from 10 to 5 days would result in a decrease in GWP by 34%, while increasing the CL concentration from 10 to 20 g·L$^{-1}$ would result in a larger decrease in GWP by 46%. Similar results are observed for all ICs when comparing both scenario variations. Therefore, when optimising a fermentation process in the lab, an optimum between maximising the obtained concentration and minimising the fermentation duration should be found. The decision on the optimal time for the termination of fermentation can be made based on the results shown here.

### 3.2.3 Aeration and agitation rate

The major process parameters contributing to most ICs are electricity consumption for fermenter agitation and aeration, as previously mentioned. These parameters were also identified as major contributors to most ICs in an organic acids fermentation process with a duration of 100 h (Thompson

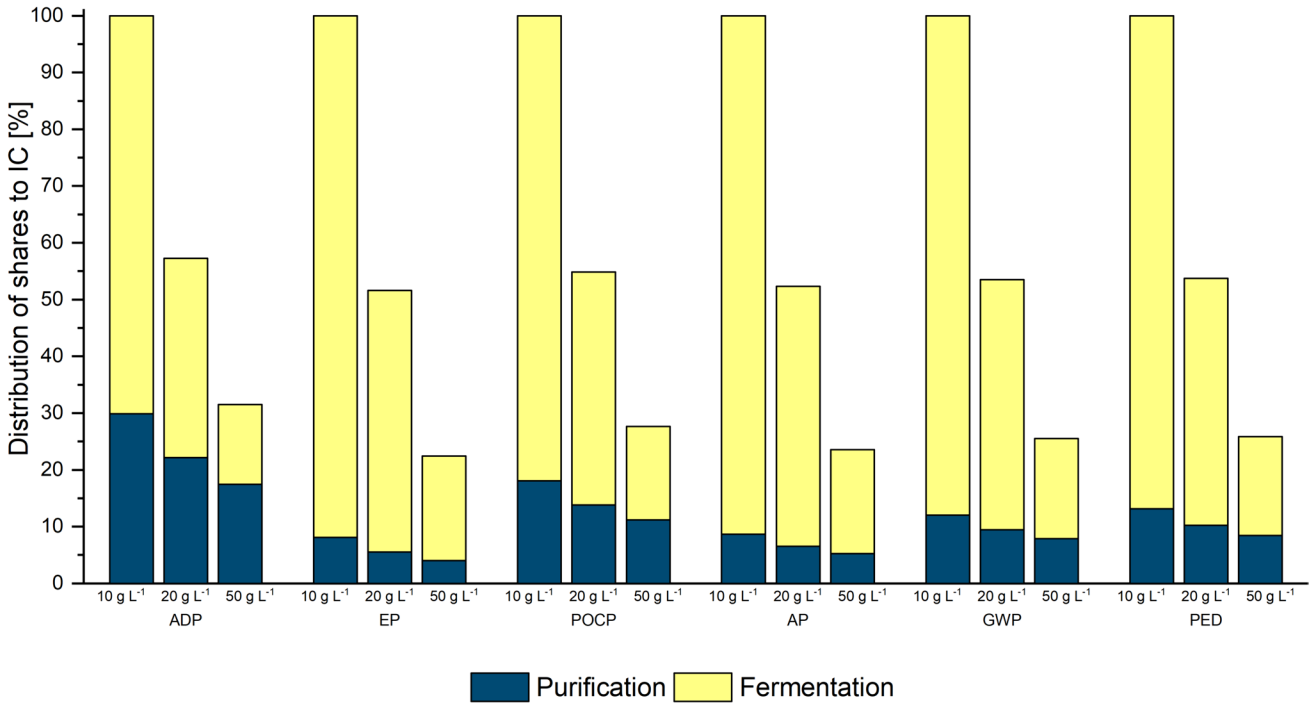


**Fig. 5** Distribution of shares to ADP, EP, POCP, AP, GWP, and PED of the CL fermentation and purification processes at different CL concentrations (10–50 g·L$^{-1}$). A detailed overview of the contribution of each of the major process units of the fermentation process to the ICs is listed in Supplementary Table 3

et al. 2018). In the few published LCA studies on biosurfactants, only (Aru and Ikechukwu 2018) identified overall electricity consumption as a significant contributor to GWP, but they did not include electricity consumption due to aeration and agitation in their calculations. The lack of analysis of electricity consumption due to aeration and agitation in biosurfactant LCA studies in the literature is also reflected in the lack of experimental optimisation studies in this field.

Although common fermentation optimisation approaches aim at maximising space–time yields and thus minimising the fermentation duration, barely any attention is given to decreasing energy input for aeration and agitation, especially in early development stages. At the same time, our results show that reducing the power input (underlying electricity mix: German grid mix) from 1 to 0.1 kW $m^{-3}$ at a 10-day CL fermentation would result in a decrease in the PED by approx. 9%. Decreasing the aeration rate from 1 to 0.1 vvm would even result in a decrease of PED by approx. 46% (Fig. 6). This large EIRP is reflected in all analysed ICs as well, emphasising that the results obtained in this study are of major importance when it comes to process optimisation.

During fermentation, reactor agitation is necessary to ensure a homogeneous distribution of biomass and substrates in the fermenter broth. Further, it helps distribute and disperse gas bubbles introduced to the fermenter via aeration. Fermenter aeration is crucial to cover the oxygen demand of the growing microorganisms in aerobic fermentations. Thus, both operations are crucial for a fermentation process. Therefore, to maintain the required broth homogenisation and oxygen supply while reducing the power input to a minimum, the adjustment of both agitation and aeration rates to just cover the demand without any excess is crucial. This can be achieved by adequate process control and online measurement of the dissolved oxygen levels in the culture broth. Another way to ensure a reduction in power supply would be to increase the efficiency of air compressors used to supply fermenters with compressed air for aeration. This is a common approach since air compressors have a significant impact on energy consumption in manufacturing systems in general (Mousavi et al. 2014). A further technology space for process optimisation is using air-spargers with small pores that increase oxygen transfer at the same aeration rates. This presents an alternative approach that can be applied to CL fermentation to decrease the environmental impact caused by aeration and agitation. An experimental demonstration of such an air sparger was previously presented by our group (Oraby et al. 2022b).

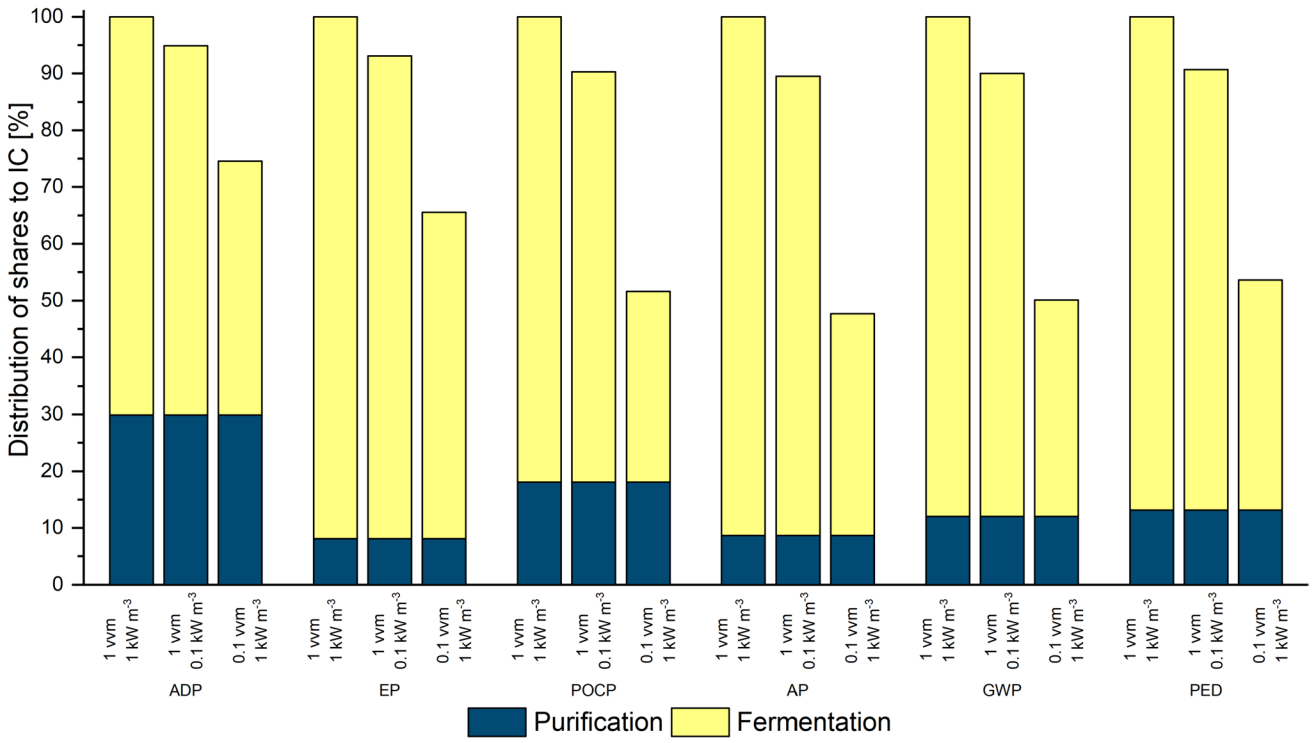


**Fig. 6** Distribution of shares to ADP, EP, POCP, AP, GWP, and PED of the CL fermentation and purification processes at different aeration (0.1–1.0 vvm) and agitation (0.1–1 kW·$m^{-3}$) rates. A detailed overview of the contribution of each of the major process units of the fermentation process to the ICs is listed in Supplementary Table 3

Overall, the obtained results emphasise the importance of detailed analyses of the microorganisms' oxygen demand pattern along the fermentation. In the case of CL, more oxygen is consumed by the microorganisms during the initial growth phase, and then the oxygen uptake decreases during the stationary phase and major fermentation duration (Oraby et al. 2020). Further, the application of adequate process control and efficient air compressors can result in a significant reduction of the overall environmental impact of CL fermentation. These conclusions can be transferred to other aerobic fermentation processes as well. A more straightforward measure to reduce the high environmental impact of aeration and agitation is to use renewable energy resources for electricity supply. However, this measure does not involve optimisation of the fermentation process itself and is thus not discussed here. It has to be noted though, that the choice of the LCI data used to model electricity supply would impact the obtained results accordingly.

## 3.3 Environmental process bottlenecks of CL purification and scenario analysis

While the major EIRP for the CL production was identified for parameters during fermentation, a notable amount of environmental impact can be reduced by optimising some purification steps, as presented and discussed in the following.

### 3.3.1 Solvent extraction and CL purification via acidic precipitation

Among all purification steps, no individual step showed a significantly higher contribution to all ICs compared to the other DSP steps (Fig. 3), but ethanol consumption for CL extraction largely contributed to all ICs. As previously discussed in Section 3.1, a reduction in the amount of used ethanol is not possible, and recirculation of the used ethanol is already considered in this model. However, the purification of CL via pH shift and hydrolysis (see method 2, Section 2.2) would completely prevent the usage of ethanol. Instead, NaOH is used to apply the adequate pH value for CL hydrolysis and $H_2SO_4$ to precipitate the hydrolysed CL-A. While both CL-B and CL-A show different characteristics, due to their different structure, which may be of interest for different applications, the hydrolysis of the molecule results in a reduction of the produced amount per batch. 1 kg of fermented CL-B results in approx. 0.8 kg of hydrolysed CL-A.

This is reflected in the normalised shares of ICs per kg CL. While the impact of the fermentation process steps remains the same per produced batch (see Fig. 7a) compared to the CL-B scenario, the normalised impact per kg CL for CL-A fermentation is increased by approx. 8–52% for all ICs (see Fig. 7b). On the other hand, despite the reduced total amount of produced CL-A per batch, compared to the native CL-B, a reduction in the impact of the purification process steps per kg CL-A to most ICs is observed for this process scenario. An exception is seen for ADP. This increase in ADP is explained by the larger amount of acid and base used during the CIP procedure after CL hydrolysis and precipitation compared to CL extraction. While for the extraction, a ratio of 2 kg EtOH is used per kg pellet; for hydrolysis, a ratio of 9 kg $H_2O$ is added per pellet. This results in a larger volume. Thus a larger vessel is needed for the hydrolysis. After both the hydrolysis and consequent precipitation of CL, the used vessels are washed in different cycles with water and 2% NaOH. This washing water has to be neutralised before disposal. Thus, a significant amount of acid is used. For a larger vessel, more washing agents and, thus, neutralising agents are consumed. In total, these additional acids and bases result in an overall increase of ~ 23% in ADP per batch and ~ 47% per kg CL-A, thus resulting in a total impact exceeding 100%, compared to CL-B.

This significant increase in ADP for CL-A could theoretically be decreased by using more environmentally friendly washing and neutralising agents for the CIP procedure and consequent waste water neutralisation. Further, through adequate scheduling and continuous operation, washing cycles could be reduced. While both recommendations do not affect the CL hydrolysis process itself, it is advisable to experimentally explore other CIP procedures, to achieve a reduction in the overall environmental impact of CL purification.

Except for ADP, the reduction in all other analysed ICs per batch shows that although CL purification via pH shift and hydrolysis may show a considerable EIRP compared to CL purification with ethanol extraction, the decreased CL yield per batch reverses the EIRP. In fact, a comparison of IC based on a mass-based functional unit (per kg of produced CL) may be a limited approach, since CL-B and CL-A are different molecule groups with different characteristics. Therefore, they may be considered as different biosurfactants, and a comparison based on the amount necessary for an individual application (i.e. fulfilling a specific function as addressed with the concept of a functional unit) may be the more adequate approach in this case. However, this comparison is currently not possible, due to the absence of sufficient data for CL application in certain products.

### 3.3.2 In situ product recovery via foam fractionation

In the first purification step for both CL purification processes, the culture broth undergoes a separation process, as previously described in Section 2.2, to obtain a pellet containing CL and biomass. The remaining culture media residues are then washed from this moist pellet, and CL is extracted/hydrolysed consequently. In recent years, foam fractionation has been described as a first separation step/in situ product recovery during several biosurfactant

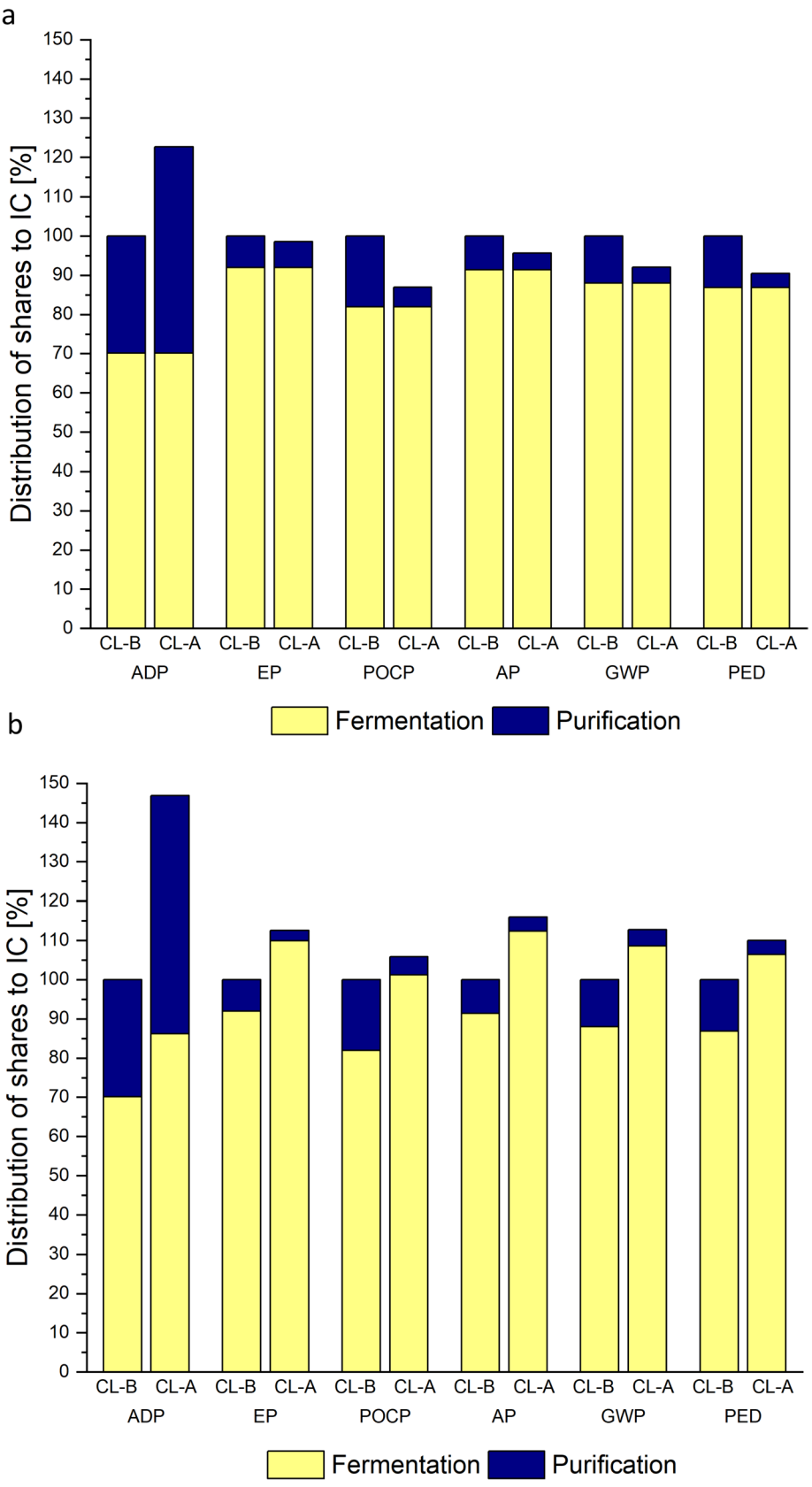


**Fig. 7** Distribution of shares to ADP, EP, POCP, AP, GWP, and PED of the CL fermentation and purification processes for CL-B purification via EtOH extraction and via hydrolysis to CL-A and acidic precipitation. **a** normalised per batch; **b** normalised per kg CL

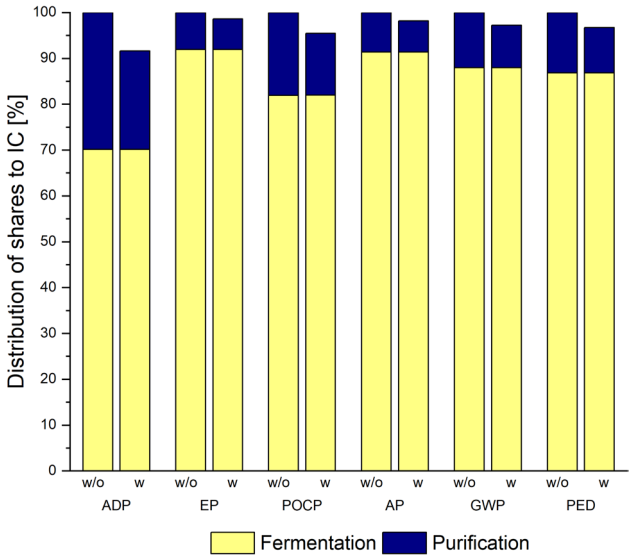


**Fig. 8** Distribution of shares to ADP, EP, POCP, AP, GWP, and PED of the CL fermentation and purification processes for CL fermentation without (w/o) or with (w) foam fractionation

fermentations (e.g. Díaz De Rienzo et al. 2016; Anic et al. 2018; Blesken et al. 2020; Chen et al. 2021). Biosurfactants being surface active agents, usually cause foam formation during aerobic fermentations and accumulate in the foam phase. By separating this foam, one can obtain a fraction with a higher biosurfactant concentration (foamate) compared to the culture phase. Ideally, this fraction should be biomass-free.

Assuming a biomass-free foamate, the fraction that undergoes the washing and proceeding purification steps is much smaller (approx. 7%, see Section 2.2). This is reflected in the overall reduced share of the purification steps to all ICs (Fig. 8). When foam fractionation is operated, the first separation step is not needed because the CL crystals are separated automatically in the foamate fraction. Thus, there is no impact calculated for the initial separation step. However, a slight increase in electricity consumption is calculated for operating the pump recirculating the remaining culture media and biomass back to the fermenter. Further, wastewater disposal of the culture broth is considered in the foam fractionation step in this scenario, not in the separation step as in the standard scenario. Thus, the impact of the first separation step is almost the same as the impact of the foam fractionation step for all ICs (Fig. 9). Also, the impact of the final CL drying step is the same for both scenarios since the same amount of CL is produced in the two cases.

All other purification steps show a reduced environmental impact in all ICs, varying in their EIRP. Especially the ADP can be reduced by approx. 27%, due to the smaller pellet obtained after foam fractionation that needs to be washed. After foam fractionation, the pellet contains only CL. On the other hand, after normal separation from the culture broth, the pellet contains both CL and biomass and is thus larger. Hence, more acidic water and a larger vessel are needed for the washing step. The same applies to the ethanol extraction step since the ethanol amount is calculated based on the pellet weight. This is also slightly reflected in the evaporation step where a smaller amount of ethanol extract needs to be evaporated.

All in all, up to 18–27% of the environmental impact of the purification steps can be reduced in all ICs if foam fractionation is implemented in the fermentation process. Although the chosen assumptions of a completely biomass-free pellet are idealised, the first experimental approaches for CL fermentation coupled with foam fractionation show very promising results in this regard. In our recent publication, we showed that with foam fractionation in a 10 L scale, CL recovery rates of up to 100% and thus complete separation from the culture broth, with only ~ 5% biomass separation, can be achieved (Oraby et al. 2023). Coupled with the shown EIRP, these results show that in situ product separation via foam fractionation can also contribute to a better environmental performance of the CL production process. Further, depending on the desired purity grade of CL, complete omission of the ethanol extraction step could be considered when applying foam fractionation. If a small amount of biomass in the purified CL is accepted, or if foam fractionation is optimised in such a way that the foamate does not contain any biomass, then the purified CL can be simply obtained by drying the foamate fraction directly after the washing step.

Also, applying foam fractionation could increase the utilisation of the fermenter since less head space for foam accumulation needs to be left empty. This would mean that the fermenter could be filled with a larger starting volume, and thus more CL would be produced at almost the same environmental impact as the fermentation process. While this still has to be demonstrated for CL at a larger scale, increasing the fermenter filling volume while applying foam fractionation was already shown at a 10 L scale for CL (Oraby et al. 2023). Further, Czinkóczky and Németh showed that applying foam fractionation with a surfactin fermentation has the potential to decrease the annual operation costs by 3% for the same reason (2020). So overall, applying foam fractionation seems to have the potential to reduce both the ecological, as well as economic impact of a fermentation process, due to the higher fermenter utilisation as well as the facilitated proceeding purification.

#### 3.3.3 The enhanced separation efficiency of CL during separation steps

As previously illustrated, for both CL purification via ethanol extraction and hydrolysis and precipitation, multiple

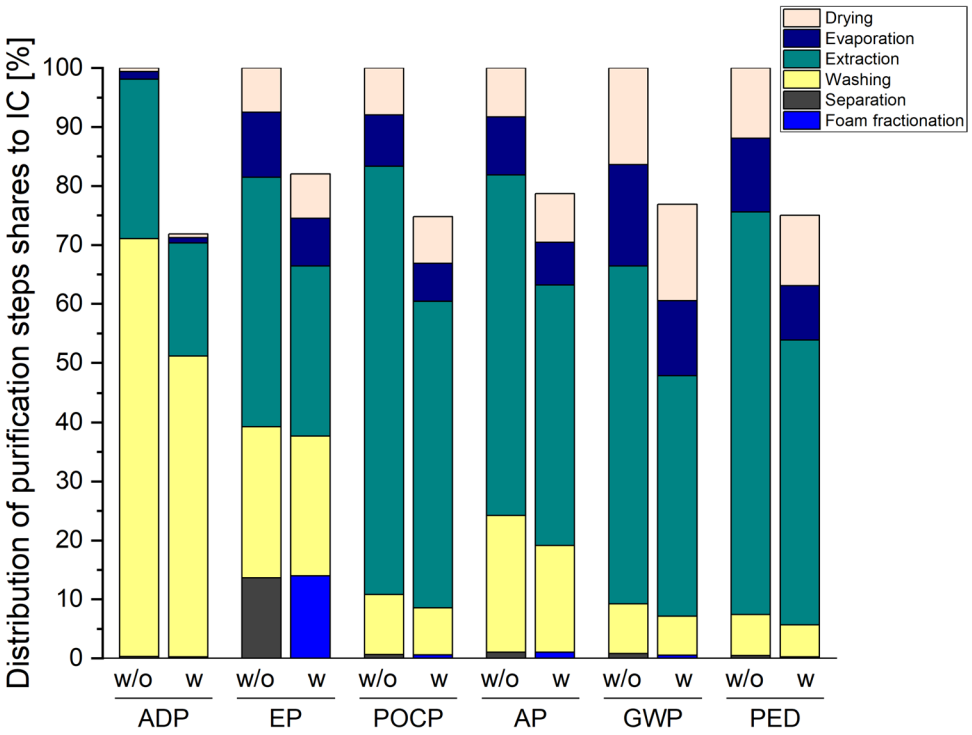


**Fig. 9** Distribution of shares to ADP, EP, POCP, AP, GWP, and PED of the CL purification steps for CL fermentation without (w/o) or with (w) foam fractionation

separation steps are needed. Increasing the separation efficiency of the utilised separator would thus affect several steps along the purification process. This is reflected in the reduced environmental impact for all ICs at higher separation efficiencies (liquid content in the pellet 0.5 vs 0.8 $g \cdot g^{-1}$, Fig. 10). A dryer pellet results in an overall smaller pellet amount. This means less water is needed for the pellet wash, and less ethanol is needed for CL extraction.

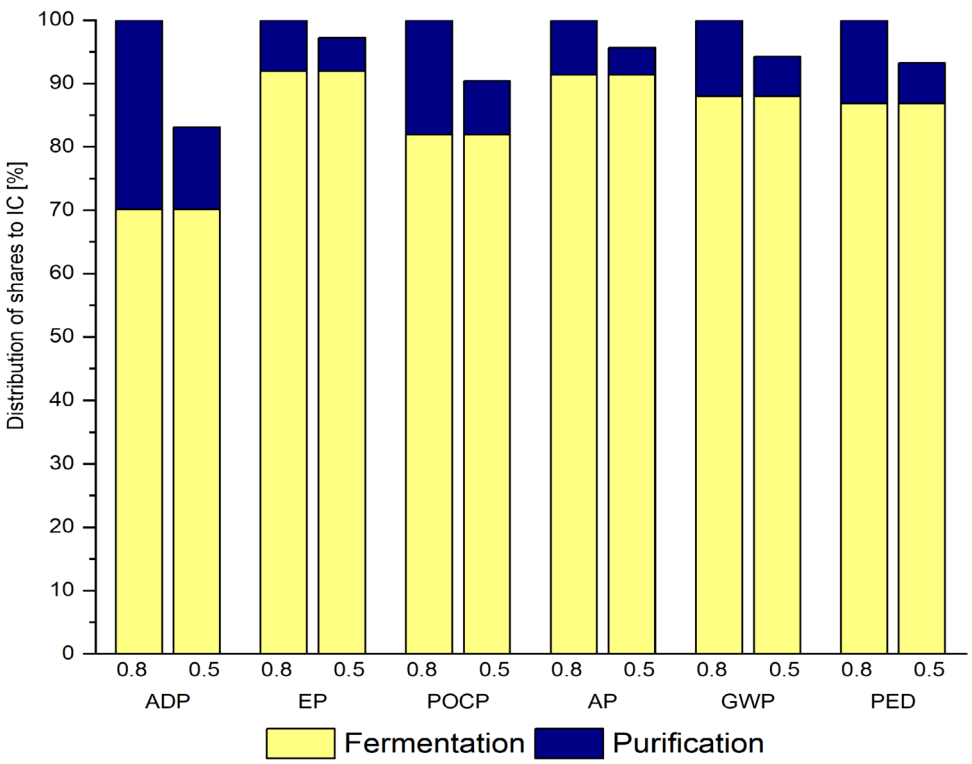


**Fig. 10** Distribution of shares to ADP, EP, POCP, AP, GWP, and PED of the CL fermentation and purification processes for different separation efficiencies during CL purification (liquid content 0.8 $g \cdot g^{-1}$ vs 0.5 $g \cdot g^{-1}$). A detailed overview of the contribution of each of the process units of the purification process to the ICs is listed in Supplementary Table 4

Thus, less energy is consumed for the proceeding evaporation step.

While such a basic process facilitation, like increasing the separation efficiency, can have a large EIRP, the present or potential technology space needs to be examined further. These results, however, show that when considering large-scale CL production, the adequate choice of a high-performing separator for biomass and CL separation can highly affect the environmental performance of the whole purification process.

## 4 Conclusion and future recommendations

Based on lab-scale and simulation data in a 10 $m^3$ scale, the LCA of CL fermentation and purification showed that the fermentation process was the major contributor to all analysed impact categories, with ~ 74% of ADP and more than 85% of all other ICs. Within the fermentation section, the environmental impact of the seed fermentation cascade was minimal, due to the short fermentation period and small volume, compared to the production culture. Here, a direct correlation between fermentation duration and environmental impact was evident, primarily due to fermenter aeration, contributing the most to all ICs. Other major contributors to some ICs were fermenter agitation, the used carbon source, the used ethanol for CL extraction, and $NaOH/H_2SO_4$ used for vessel cleaning. While some results, such as the dependency of EIRP on the obtained CL concentration, were expected and are usually considered in the context of process optimisation in the lab scale, other results were less apparent. Especially, the high EIRP associated with the fermenter agitation and aeration rates sheds light on the benefits of using LCA as a driver for process optimisation. These parameters are usually not focused on in traditional lab-scale fermentation process optimisation, but our results showed that they could significantly contribute to a better environmental performance of CL if optimised. Having this information in an early development stage provides flexibility in CL process optimisation and adaptation to design a fermentation process with minimal agitation and aeration rates. Further, simulating a hypothetical CL scenario with in situ product recovery via foam fractionation showed that in addition to the process facilitation, integrating foam fractionation can contribute to the reduction in the environmental impact of CL fermentation.

Overall it was shown that LCA and hot spot analysis can both lead to the identification of process optimisation potential, as well as provide data to evaluate the environmental impact of new process methods in their early development stages. This bears the advantage that obtained results can be integrated into further research and development work, and that the comparison of the environmental impact of different process options (for instance CL purification via ethanol extraction vs. alkaline hydrolysis) can help choose and develop the most "sustainable" process option. However, a downside to the followed approach lies in the limited data availability and herewith associated model uncertainties. This uncertainty hinders the calculation of accurate absolute values of the environmental impact of CL. Nevertheless, using relative values provides sufficient information that can be used to draw recommendations for process optimisation, which would lead to a more environmentally friendly biosurfactant and contribute to a more sustainable bioeconomy.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11367-024-02301-1.

**Acknowledgements** The authors would like to thank Lea Maerz, Isabell Weickardt and Daniel Hug for their assistance with the experimental work that contributed to the conceptualisation of the simulated scenarios. AO would also like to thank Prof. Dr. Günter Tovar for his support and supervision during her PhD research and Dr.-Ing. Michael Held for his tremendous support with troubleshooting and data analysis.

**Author contributions** Amira Oraby: conceptualisation; data curation; formal analysis; investigation; methodology; visualisation; validation; writing—original draft. Ann-Kathrin Briem: validation of the LCA model; writing—review and editing. Lars Bippus: trouble-shooting of the LCA model; writing—review. Steffen Rupp: writing—review. Susanne Zibek: funding acquisition; resources; supervision; writing—review and editing.

**Funding** Open Access funding enabled and organized by Projekt DEAL. This work was partly funded by a Ph.D.—scholarship from the German Federal Environmental Foundation (DBU) AZ: 80017/333 and by grants from the Federal Ministry of Education and Research (031B0469P, 031B0469H).

**Data availability** All relevant data generated or analysed during this study are included in this published article.

## Declarations

**Conflict of interest** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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