Environmental impacts of biosurfactants production based on substrates from sugar industry

Andreas Schonhoff[†,⸸,*], *Nina Ihling*[‡,⸸], *Andrea Schreiber*[†,⸸], *Petra Zapp*[†,⸸]

[†]Institute of Energy and Climate Research - Systems Analysis and Technology Evaluation (IEK-STE), Forschungszentrum Jülich GmbH, Wilhelm-Johnen-Straße, 52428 Jülich, Germany

[‡]Aachener Verfahrenstechnik - Biochemical Engineering (AVT.BioVT), RWTH Aachen University, Forckenbeckstraße 51, 52074 Aachen, Germany

[⸸]Bioeconomy Science Center (BioSC), Forschungszentrum Jülich GmbH, Wilhelm-Johnen-Straße, 52428 Jülich, Germany

[*]Corresponding author: a.schonhoff@fz-juelich.de; +49 (0) 2461 61 3731

Keywords: Life cycle assessment; Bio-based surfactants; Fermentation; Rhamnolipid; Mannosylerythritol lipid; Bio-economy

ABSTRACT

Regarding the omnipresent topic of climate change, establishing a bio-economy appears reasonable, but requires critical analysis of its products. This project-specific study (project Bio²) presents previously unknown environmental impacts caused by the novel production of biosurfactants (rhamnolipids (RL) and mannosylerythritol lipids (MEL)) based on substrates from sugar industry (molasses and sugar beet pulp) using Life Cycle Assessment (LCA). Identifying critical impacts and processes (e.g., extraction agent production) reveals optimization potentials for the considered forward-looking process designs. Based on surfactants' specific cleaning performance, environmental impacts vary substantially for RL and MEL. Primary causes of MEL productions' lower environmental impacts are advantageous microbial properties and process designs. Substrate choice does not play an essential role. An analysis of realistic yield changes and comparisons with conventional surfactants sharpens the view on the development position of the chosen surfactants. In particular MEL shows environmental benefits compared to today's oleo-/petrochemical produced surfactants. Identified optimization options (e.g., increased agent recycling) and yield increases could strengthen especially the advantages of MEL. Summarizing, the results show advantages of MEL compared to RL to some degree, indicate weak points of current processes and highlight favorable options for future design of RL and MEL production, regarding their environmental impact.

INTRODUCTION

In the context of an increasingly important bio-economy, the aspect of sustainability and its analysis is steadily growing in relevance. Action plans like the "European Green Deal" and its ascertainment, as well as civic actor movements such as "Fridays for Future" or "Extinction Rebellion" demand an increase in more sustainable production.[1-5] Besides climate neutrality by 2050, another aim of the green deal is resource efficient use of materials.[6]

In terms of these goals, the project Bio² (2017-2020) aimed at the production of sustainable biosurfactants.[7] Biosurfactants are herein defined as totally bio-based surfactants in contrast to partly or mainly bio-based surfactants (e.g., oleochemical surfactants; definition in DIN EN 17035).[8] The project focused on the development of novel microbial production processes for rhamnolipids (RL) by *Pseudomonas putida* and mannosylerythritol lipids (MEL) by *Ustilago maydis*.[9-12] Surfactants are mainly used for household detergents ($>50\%_{\text{total production}}$), cosmetics or textiles, but also in the food sector or the paints and coatings industry.[13]

As a large global market (17 m Mg annual production in 2019, related revenues of 35 bn €) and good prospects for a future yearly market growth of >3% in relation to mass and >5% in sales, the surfactants market offers a huge potential for sustainable products.[14-17] Although today's overall share of biosurfactants is below 2% and that of microbial produced surfactants even <0.05% (own calculations), an considerable future increase in this segment is expected.[18,19] This is also indicated by Evonik Industries' current investment in the industrial production of RL in Slovakia.[20]

To show the implied environmental superiority of biosurfactants against conventional petro-/oleochemical-based surfactants, Life Cycle Assessment (LCA) studies are required.[21,22] So far, hardly any LCA of biosurfactants is available. In Adlercreutz et al. the production of alkanolamides (mainly bio-based surfactants) was investigated in a techno-economic analysis.[23]

Kopsahelis et al. have shown LCA results for the biosurfactants sophorolipids and rhamnolipids.[24] However, they limited the scope of the work to a short process section (gate-to-gate system) at pilot scale. A further LCA of biosurfactants for a 150 L fermentation was conducted by Bacille et al.[25] It includes the fermentation and purification process to produce sophorolipids (cradle-to-gate system) as well as their use for hand washing.

Conventional surfactants (oleo-/petrochemical production) have a dominating market share of more than 90%.[13,21] Nevertheless, the number of LCAs and databases for these surfactants is also limited. Zah et al. provided datasets for the production of various surfactants such as anionic fatty alcohol sulfates (FAS), anionic linear alkylbenzene sulfonate (LAS) and non-ionic alcohol ethoxylates (AE) for the ecoinvent database.[26,27] Aspects of land occupation, chemical and formulation processing, and a so-called "end life phase" (includes use and recycling) of alkyl polyglucosides (APG) were studied by Guilbot et al.[28] A dataset for the non-ionic APG, which are produced from coconut oil, can be found in the CPM-LCA-database.[29] By the ERASM database, a variety of datasets for petrochemical (e.g., alcohol ether sulphates) and oleochemical (e.g., AE) surfactants is offered for use in LCA software.[30]

As part of holistic assessments of novel products, the LCA has become established in terms of the products' environmental sustainability. To add further knowledge to biosurfactants' LCA, the present study examines environmental impacts caused by using two different substrates to produce two different surfactants. Earlier results have shown the limited influence of substrate choice on the environmental impact for RL production on a small pilot scale (112.5 L fermentation operation volume).[31] Based on this assessment, the question arises whether a scale-up to industrial scale (5,000 L fermentation) would reduce environmental impacts. Additionally, the comparison of RL and MEL can show which product has lower environmental impacts at industrial scale.

Therefore, this study considers process chains of two different substrates molasses (MOL) and sugar beet pulp (SBP) and the two products (RL, MEL), based on experimental outcomes. Aspects and results of microbiological activities (e.g., efficient screening of production titers), mechanisms adaptation of natural microbial production (e.g., implementation utilization pathways), (microbiologic-) technical aspects related to fermentation, and downstream processing are considered (e.g., bubble-free aeration, downstream concepts).[31-36] In addition to the direct use of the results within the project Bio², findings are usable for future optimization and improvement options. Continuous result updating enables a further iterative proceeding of process development. For possible follow-up projects, the won information offers starting points towards sustainable development and practical implementations. Looking at the biosurfactant-community, the outcomes provide knowledge on critical points and information on this rarely explored area of biosurfactants' LCAs.

MATERIAL AND METHODS

The attributional LCA was carried out in accordance with the established ISO 14040/14044 standards.[37,38] For the technical implementation GaBi 10.5 was used, which is an well-established and frequently used LCA software in industries and science.[39]

STUDY GOALS

Aim of the study was to evaluate the environmental performance of RL and MEL process chains based on MOL or SBP substrates and to benchmark the processes to conventional oleo-/petrochemical surfactant production. Lab and technical scale experiments have shown product purities of >98% for RL (amount includes share of max. 10-20% of HAA (=3-(3-

hydroxyalkanoyloxy)alkanoate)) and similar values for MEL.[36] The assessed process chains differ in terms of products (RL, MEL) and substrates used (MOL, SBP), resulting in the four process chains RL_MOL, RL_SBP, MEL_MOL, and MEL_SBP (product_substrate). These cradle-to-gate systems contain all stages from the supply of raw materials (cradle) to the dried biosurfactants in a storage (gate). The use and end-of-life phases of the products are not included, as it is common in LCAs for the provision of intermediate products for further processing. Additionally, parts of the process chains for surfactant production, which are highly energy and material intensive, were identified. To compare the developed surfactants with products already on the market, exemplarily three comparative surfactants conventionally produced were chosen. The choice of these alternatives (ALT) depended strongly on the data availability for the required critical micelle concentration (CMC) value of the surfactants (for justification of choice see section FUNCTIONAL UNIT). The considered conventional surfactants are “C10-13 Linear alkylbenzene sulphonic acid” (ALT_01_HLAS; petrochemical), “Cocamide diethanolamine” (ALT_02_CDEA; oleochemical), and “C8-18 Alkyl amidopropyl betaine” (ALT_03_AAPB; oleo/petrochemical). Based on the project framework, the geographical and time-related system boundaries are Germany and Europe (depending on dataset availability) today.

FUNCTIONAL UNIT

The comparison of the environmental impacts of different surfactants’ production is only feasible based on the same functional unit. The predominant function of surfactants is to reduce the surface tension for realizing purposes like cleaning. To achieve this reduction, different surfactants require different amounts of mass. In reverse, comparison on a fixed mass or volume basis (e.g., 1 kg surfactant 1 vs. 1 kg surfactant 2) is not meaningful for different products. To determine the amount of surfactant necessary to fulfill this purpose, a specific cleaning performance (SCP), defined by the critical micelle concentration (CMC), is chosen. It presupposes the finding, that "microbial surfactants are most effective and efficient at their CMC (...), thus less surfactant is necessary to get a maximum decrease in surface tension" (see Rodrigues, derived from Ghista).[40,41] As the surface tension is not further reduced above the CMC, the CMC specifies the appropriate concentration for meaningful use (surfactant specific mass per volume (g/L)). This means, a comparison is made based on a defined SCP (impact per SCP) instead of the often-used mass based comparison (impact per one kg) in LCA studies. Therefore, in the present study, the functional unit is defined as the production of the mass of surfactant that is required to fulfill the same SCP as 1 kg of MEL. The choice of MEL as a reference was motivated retrospective by its lower environmental impacts and the resulting clearer relations to environmental impacts of RL.

As no project specific CMC values were available, average values were derived from literature. A data collection of 15 sources for RL (e.g., Kaczorek, Sharma et al.) as well as for MEL (e.g., Rau et al., Morita et al.) (see "Supporting Information Table S1.") were used to define average values.[42-45] The CMC values for RL vary from 31 mg/L to 200 mg/L resulting in an arithmetic average value of 96 mg/L. The arithmetic average value of MEL is 27 mg/L (variation from 5.5 mg/L to 100 mg/L). The ratio of these values implies that 3.6 kg of RL are required to fulfill the same purpose as 1 kg MEL (for the same application).

Datasets of the conventional surfactants were not equipped with a CMC value. Therefore, CMC data of ALT_01_HLAS (CMC = 650 mg/L), ALT_02_CDEA (CMC = 1,480 mg/L), and ALT_03_AAPB (CMC = 250 mg/L) was taken from available sources (see "Supporting Information Table S2.").[46-48] This results in the reference values of 1 kg MEL, 3.6 kg RL, 24.2 kg ALT_01_HLAS, 55.1 kg ALT_02_CDEA, or 9.3 kg ALT_03_AAPB, to reach the same SCP. It should be noted that the required amounts of biosurfactants (MEL, RL) are much smaller compared to conventional surfactants.

DATA SOURCES

Essential data for the modeled foreground processes was determined by laboratory tests and larger experimental set-ups. The underlying data and knowledge is much more advanced for RL than for MEL due to previous RL-focused work by project partners.[31,49-51] Besides project data, background processes like electricity supply or production of auxiliary materials, was taken from the established and frequently used databases ecoinvent 3.7 and GaBi professional database. [26,52] Furthermore, literature (e.g., Spoerri et al., Knoll), statistical data (e.g., animal feed from sugar beet), and own calculations (e.g., tank design) were used.[53-55] Data for the substrates' supply was taken from an existing LCA on sugar beet production and processing, since MOL and SBP are co-products of the sugar industry.[53] In conclusion, fermentation and the downstream are based on primary data, while the used data of the upstream (substrate supply, storage and preparation) is secondary data. An economic allocation was applied for the substrates' supply, so that the environmental backpack of the substrate is related to the share of its economic value. The considered mass flows and updated economic data with current time reference are shown in the "Supporting Information Table S3.". The datasets of the benchmarked system for ALT_01_HLAS, ALT_02_CDEA, and ALT_03_AAPB were taken from the ERASM database that is providing data from the European detergents and surfactants industry.[30]

ENVIRONMENTAL IMPACT CATEGORIES

The Environmental Footprint methodology EF 3.0 and its recommendations were applied for the Life Cycle Impact Assessment (LCIA).[56-58] The 16 used midpoint indicators, underlying single impact assessment methods, and their specific units are shown in the "Supporting Information Table S4.". In addition, a normalization step was carried out by the EF 3.0 normalization method (EF normalization factors) to compare the different impact categories.[58] This normalization allows an overall impact comparison. Normalized results are expressed in person equivalents (PE), referring to annual global emissions per average citizen (reference base: global values, population 2010).

LCIA results can be presented in absolute values and normalized values (PE) for single impact categories (e.g., acidification, climate changes, eutrophication). To compare different impacts expressed in different units, normalization is a useful tool. Normalized values reflect a comparison of category-specific impact results with region specific reference scores using so-called "normalization factors".

LIMITATIONS

Limitations of the study can be identified with the basic design of the industrial-scale and the transferability of data from laboratory to pilot scale. Furthermore, the assumption of same purposes for compared surfactants can be classified at least as worthy of discussion and is a subject to uncertainties.

PROCESS CHAINS

Based on an earlier LCA for a pilot scale RL production (112.5 L fermentation, see Tiso et al.), industrial scale fermentation (5,000 L fermentation) is considered for both products.[31] The process chains differ mainly in the associated downstream processing (isolation and purification after fermentation). All process chains are considered as batch processes, which cover a time span of 5 days per fermentation volume (5,000 L). The Life Cycle Inventory (LCI) depends mainly on microbiological and technological design aspects. The different yield coefficients (product per substrate unit in $kg_{Product}/kg_{Glucose}$) of the two microorganisms and conversion rates (share of converted sugars in %) of the two substrates influence the efficiency of production, and consequentially energy and material flows.

The technical systems include the basic process modules (e.g., fermentation, extraction) and the necessary infrastructure (e.g., extraction agent tank, pumps) of the foreground processes (developed RL and MEL process chains), as well as background processes like the supply of electricity, heat, water, and other auxiliary materials (e.g., solvents or mineral medium). In addition, the utilization of waste or residues (e.g., purge flow) is included.

PROCESS CHAINS RL

The process chain of RL production is divided into 9 process stages (see Figure 1) which aggregate several processes and associated supply and waste treatment chains. A more detailed process flow chart can be found in "Supporting Information Figure S1.". The first process stage "sugar beet production & processing" covers all process modules from sugar beets seeding to sugar preparation and its by-products (MOL and SBP).

Depending on the substrates considered, the second process stage "storage and preparation" is designed. While for MOL only storage tanks and pumps need to be considered, SBP requires a more complex system of substrate preparation and storage. After the delivery of SBP, conveyors ensure the transport to an intermediate storage, before being forwarded to a disintegration unit followed by hydrolyzation of the SBP.

For both substrates, part of it is directly conveyed to the following main fermentation, while the other part is used for seed fermentation. The "seed fermentation"-stage is modeled in a three-step tank layout with 5 L, 50 L, and 500 L operation volume. After seed fermentation, the produced inoculum for the main fermentation process is then pumped to the subsequent stage. The seed fermentation tanks include aeration modules, which were developed within the project Bio².[49]

In the following "fermentation"-stage, besides the tank (operation volume 5,000 L), a centrifuge (separation of solids and liquid phase) and pumps (e.g., substrate, water) are considered. Moreover, an aeration module is also included. Inputs for the fermentation are water, substrates (MOL or SBP), steam for sterilization, the inoculum from seed fermentation as well as a mineral medium to supply nutrients that are not or not sufficiently present in the complex substrates. Amount and composition of the mineral medium depend on the targeted product (Table 1). The mix ratio (volume ratio Ψ) between substrates and water for dilution varies between 1:1 (MOL) and 1:1.5 (SBP). Depending on the throughput, data of a centrifuge available on the market is considered, which separates the fermentation broth into solid and liquid shares (including RL).[59] The separated solid biomass is assumed to be treated as bio-waste for now. However, in the further developments of the processes, it should ideally be returned to the fermentation for further utilization and metabolization.

The liquid fermentation broth is forwarded to a "precipitation"-stage, where it is mixed (Ψ = 1:1) with acetone (precipitation agent). Afterwards the precipitated proteins are separated by centrifugation and treated as bio-waste.[31,36] The subsequent "precipitation agent recovery"-stage includes the recovery of acetone (buffer tank, heating unit, cooling unit, pumps) and the storage of the recovered acetone. A purge flow and the recycling of precipitation agent (default setting: 80% recycled) are considered. Furthermore, the fill-up with new precipitation agent is included. The protein-free liquid as the main output from this stage is passed to the “extraction”-stage.

Extraction comprises storage, supply and use of an acidification agent (0.1%$_{Vol}$; pH-value adjustment) and a mixer-settler. The added extraction agent (Ψ = 1:5; ethyl acetate; 20 % of protein-free liquid) is recycled from the product containing phase in the “extraction agent recovery” process. The same assumptions as for the “precipitation agent recovery” are considered. The aqueous phase from extraction is treated as wastewater. The organic phase from the mixer-settler is considered as the relevant output from this step.

In the “final conditioning”-stage, a centrifuge and a drying unit (natural gas operation) remove remained liquid components. The storage of the final powdery product and the necessary conveyer infrastructure (main material transport between process modules) are also included.

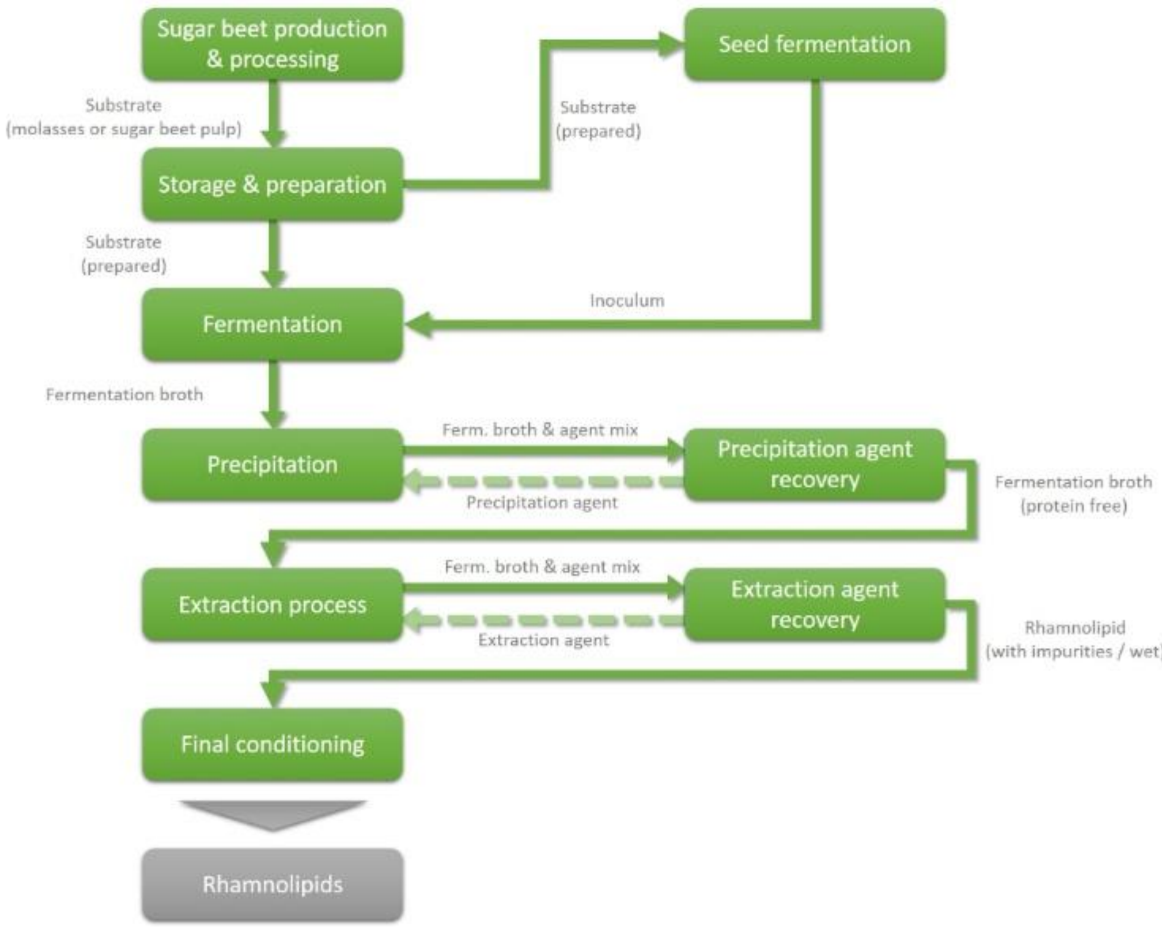

Figure 1. Simplified flow diagram of the process chain for rhamnolipid (RL) production; dotted lines represent recyclable flows.

PROCESS CHAINS MEL

As shown in Figure 2, the process chain of MEL is similar to the RL process chain up to fermentation. Following fermentation, the two process chains vary substantially. The entire production is divided into 11 process stages. As in RL production, differences in "storage and preparation" (quantity and design) rely on the substrate.

The outcoming separated liquid fermentation broth from the "fermentation"-stage is forwarded to the first of three "extraction"-stages (extraction process #1). In a mixer-settler, ethyl acetate (extraction agent I) is added (Ψ = 1:3 (v/v); ethyl acetate) to separate the intermediate product from the fermentation broth. The aqueous phase is treated as wastewater. The light phase (ethyl acetate, MELs, fatty acids) is pumped to a recycling unit, which is modeled like the recovery units for RL for the extraction agent I (default setting: 80% recycling).

The separated mixture of MELs and fatty acids is conveyed to the "extraction process #2". This mixture is again dissolved in water (50% of previous extraction agent amount (v/v)) and mixed with n-hexane (extraction agent II; Ψ = 1:3) as well as an acidification agent (0.1% (v/v)) in the mixer-settler unit II. Solvents and the fatty acids are partly separated from the MEL-water-mixture (separation of approx. $50\%_{Vol}$ fatty acids) which is pumped to the "extraction process #3". The solvent-fatty-acid-mixture is fed into the "extraction agent #2 recovery"-stage, where the fatty acids are thermally separated. A further utilization of fatty acids is not considered, even though it might well be possible to use them in industrial applications.

A further addition of n-hexane (extraction agent II; Ψ = 1:3) enables the separation of the remaining fatty acids (50%) from the MEL-water-mixture in the third extraction stage. The solvent-fatty-acid-mixture is fed to "extraction agent #3 recovery"-stage. The MEL-water-mixture is separated by a heating/drying unit and the purified powdery MELs are transported to a storage.

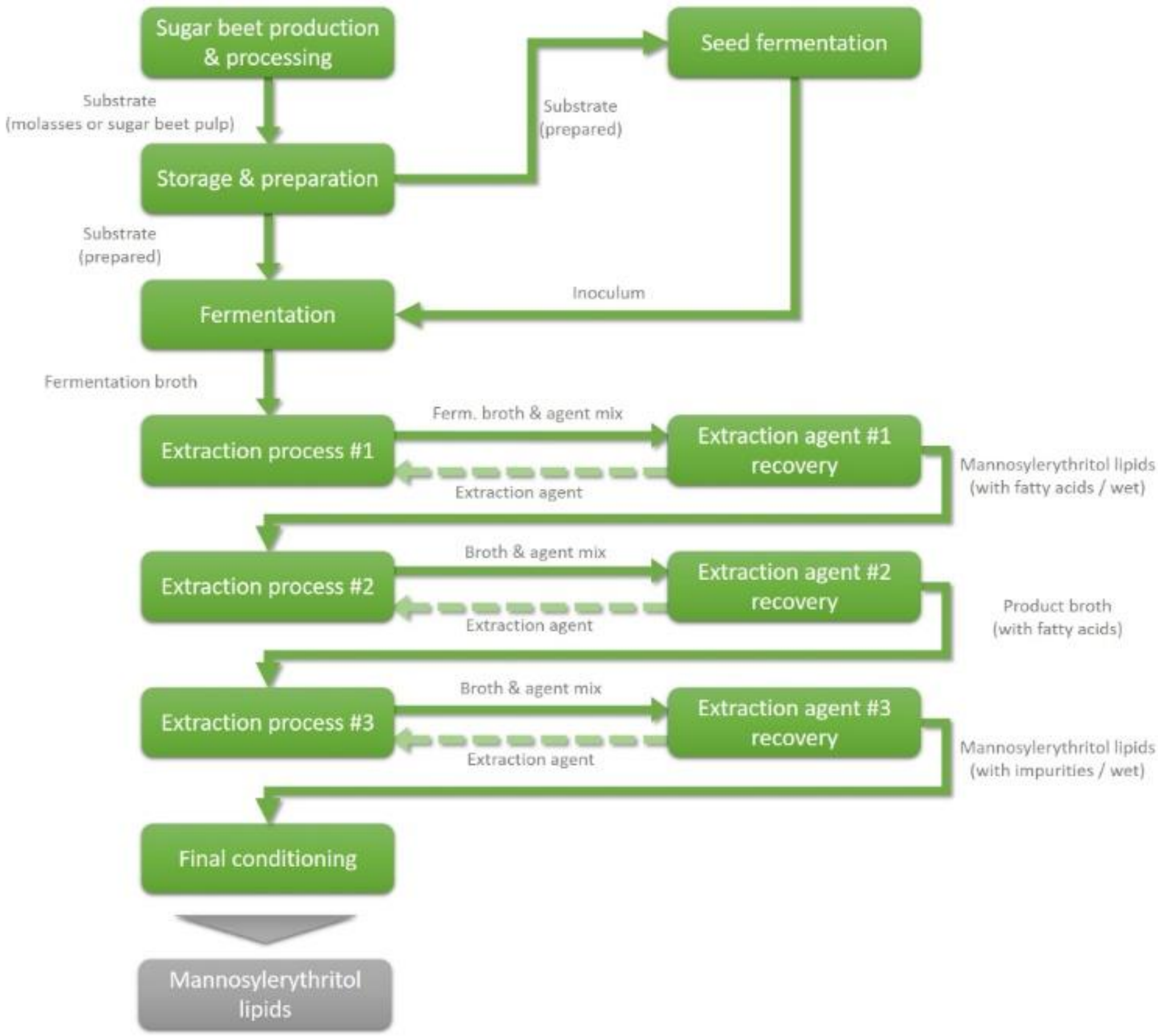

Figure 2. Simplified flow diagram of the Bio²-process for the mannosylerythritol lipids (MEL) production; dotted lines represent recyclable flows.

SENSITIVITY ANALYSIS YIELDS

A change in the yield coefficient of microbial production gives an impression about the potential for improvement in the field of microbial development. Furthermore, it allows an estimation of unknown consequences of setting yields too high in the industrial scale. In a sensitivity analysis, the yield coefficient for the process chains RL_MOL and MEL_MOL is varied exemplarily. The yield variation ranges in consistent steps from 0.1 $kg_{product}/kg_{glucose}$ (yield variation 0) to 0.4 $kg_{product}/kg_{glucose}$ (yield variation 3) around the actually achieved yields (see section LIFE CYCLE INVENTORY). The conversion rate of the processes (share of the sugar effectively metabolized) remains unaffected by this analysis.

Attention must be paid to whether changes in yield lead to non-linear dependencies. A varying yield necessitates different throughputs, which result in modified design of process modules (e.g., pumps or tanks). By these modifications, the shares of specific components can change in a non-linear way.

LIFE CYCLE INVENTORY (LCI)

The main inputs and outputs as well as intermediate flows of the four process chains for a 5,000 L fermentation unit size and an aeration rate of 0.1 $L_{Gas}/L_{ferm.\ broth}$/min are shown in Table 1. They illustrate the dependence on the basic process chain parameters: usable sugar content (substrate), conversion rate and yield coefficient of used microorganism, as well as the CMC (product).

The usable sugar content, which is more than 40% higher in hydrolyzed SBP compared to MOL, results in lower substrate demands for the same amount of sugar. Thus, the amount of extraction agent necessary for MOL-based processes is 5 to 10% higher compared to SBP. The lower usable sugar content results in a 20% higher amount of fermentation broth for the MOL process chains. Furthermore, MOL use shows higher product losses (ca. 35%), higher amounts of liquid waste (25-30%) but lower amounts of solid wastes (15-20%).

The specific yield (0.235 $kg_{product}/kg_{glucose}$) and sugar conversion rate (21%) for *Ustilago maydis* (MEL) is more than twice as high as for *Pseudomonas putida* (RL, 0.1 $kg_{product}/kg_{glucose}$, 10%). As illustrated in the section FUNCTIONAL UNIT, the CMC for MEL is lower compared to RL, so less MEL is needed to achieve the same SCP. Due to different microbial requirements, the fermentation of RL requires significantly less mineral medium (3.5-4 times). However, as these are different mineral media, a mass comparison has only limited relevance.

The production of RL and MEL requires different agents (e.g., acetone, ethyl acetate, n-hexane). Additionally, RL production requires a large amount of precipitation agent for the removal of proteins, while MEL production requires extraction agent processes exclusively.

Table 1. LCI and basic parameters of the four considered process chains RL_MOL, RL_SBP, MEL_MOL, and MEL_SBP.

basic parameter	**unit**	**RL_MOL**	**RL_SBP**	**MEL_MOL**	**MEL_SBP**	**source**
basic parameter						
product	[-]	RL	RL	MEL	MEL	-
substrate	[-]	MOL	SBP	MOL	SBP	-
content usable sugar	[$kg_{Glucose}/kg_{Substrate}$]	0.47	0.67	0.47	0.67	Bio²
yield coefficient	[$kg_{Product}/kg_{Glucose}$]	0.1	0.1	0.235	0.235	Bio²
conversion rate	[%]	10	10	21	21	Bio²
critical micelle concentration (CMC)*	[Nm/m]	96	96	27	27	ØLit.
inputs						
substrate	[$kg_{Substrate}/kg_{Product}$]	241.0	171.0	43.5	30.9	C
substrate per 5,000 L fermentation	[$kg_{Substrate/Fermentation}$]	3,396.4	1,761.9	3,204.9	1,686.2	C

water per 5,000 L fermentation	[$kg_{Water/Fermentation}$]	2,447.0	2,936.6	2,309.0	2,810.3	C
mineral medium per 5,000 L fermentation	[$kg_{Mineral\ medium/fermentation}$]	65	65	287	234	Bio²
inoculum per 5,000 L fermentation	[$kg_{Inoculum/fermentation}$]	29	29	28	28	Bio²
total operation volume	[$L_{Fermentation\ content}$]	5,000	5,000	5,000	5,000	A
specific aeration rate	[$L_{Gas}/L_{ferm.\ broth}$/min]	0.1	0.1	0.1	0.1	Bio²
precipitation agent	[kg]	3,554.7	3,357.4	0	0	Bio²
recycling rate	[%]	80	80	80	80	Bio²
extraction agent I	[kg]	801.8	757.3	1,349.8	1,275.0	C
recycling rate	[%]	80	80	80	80	A
acidification agent	[kg]	0.535	0.505	0	0	C
extraction agent II	[kg]	0	0	360	335	C
recycling rate	[%]	80	80	80	80	C
intermediate products						
fermentation broth	[kg]	5,937.4	4,792.5	5,828.9	4,758.4	C
theoretical contained RL or MEL	[kg]	16.0	11.7	74.3	55.4	C
product losses	[kg]	1.9	1.4	4.8	3.6	C
output						
product	[kg]	14.0	10.3	69.6	51.9	C
solid residues	[kg]	683.0	793.3	582.8	713.8	C/Bio²
liquid residues	[kg]	5,646.0	4,369.7	5,268.3	4078.5	C/Bio²

* average value generated from fifteen different data sources for each type of surfactant

Bio² - data from project Bio²; C - calculated value; A - Assumption; ØLit. - average literature based value

LIFE CYCLE IMPACT ASSESSMENT (LCIA) RESULTS AND DISCUSSION

This section shows the normalized environmental impacts of the individual process chains. An overview of impact category-specific results with absolute and normalized (PE) values is given with “Supporting Information Table S5.”.

ENVIRONMENTAL IMPACTS OF RL PRODUCTION

The two process chains for RL show normalized (see section ENVIRONMENTAL IMPACT CATEGORIES) environmental impact results in the same order of magnitude ($\Delta_{PE} \approx 12.0\%$, see Figure 3). The total normalized impact is 0.487 PE to produce 1 kg of RL with MOL, which corresponds to an SCP-based value of 1.740 PE/SCP. In the case of SBP use, 0.549 PE per kg are caused by RL production, which correlates to 1.960 PE/SCP. Largest impacts are generated for “resource use, fossils” (share of total impact: RL_MOL: 24.7%, RL_SBP: 25.8%), “ecotoxicity, freshwater - total” (RL_MOL: 22.3%, RL_SBP: 20.8%), and “climate change - total” (RL_MOL: 10.5%, RL_SBP: 10.9%). The share of the first and third category is slightly higher in the case of RL production based on SBP.

The impact “resource use, fossils” is dominated by the resource use of natural gas (category specific share: RL_MOL: 44.2%, RL_SBP: 43.7%), which is mainly used during acetone production in the “precipitation”-stage. Crude oil resource consumption is the second most impacting flow (RL_MOL: 28.8%, RL_SBP: 28.6%). Its origin is also the acetone production related to the “precipitation”-stage.

With 26.3% for RL_MOL and 27.1% for RL_SBP, “ecotoxicity, freshwater - total” is dominated by inorganic emissions to freshwater by chloride. This is mainly emitted by the utilization of solvent wastes from the precipitation stage. As a second main driver of the “ecotoxicity, freshwater

- total”-category in the RL_MOL-process chain, the inorganic emissions of sulphur were identified (RL_MOL: 22.3%). These are caused by the potassium chloride production of the “sugar beet production and processing”-stage. In the case of RL_SBP the second ruling flow is the inorganic emission of hydrogen sulphide (RL_SBP: 17.7%) by the biowaste treatment in the “fermentation”-stage.

The impact "climate change - total" is significantly determined by carbon dioxide emissions (RL_MOL: 83.5%, RL_SBP: 83.7%) from the acetone production (precipitation). As the second less significant flow, methane emissions (RL_MOL: 13.0%, RL_SBP: 13.4%) are responsible in the “climate change – total”-category. Their origin can also be traced back to the acetone production.

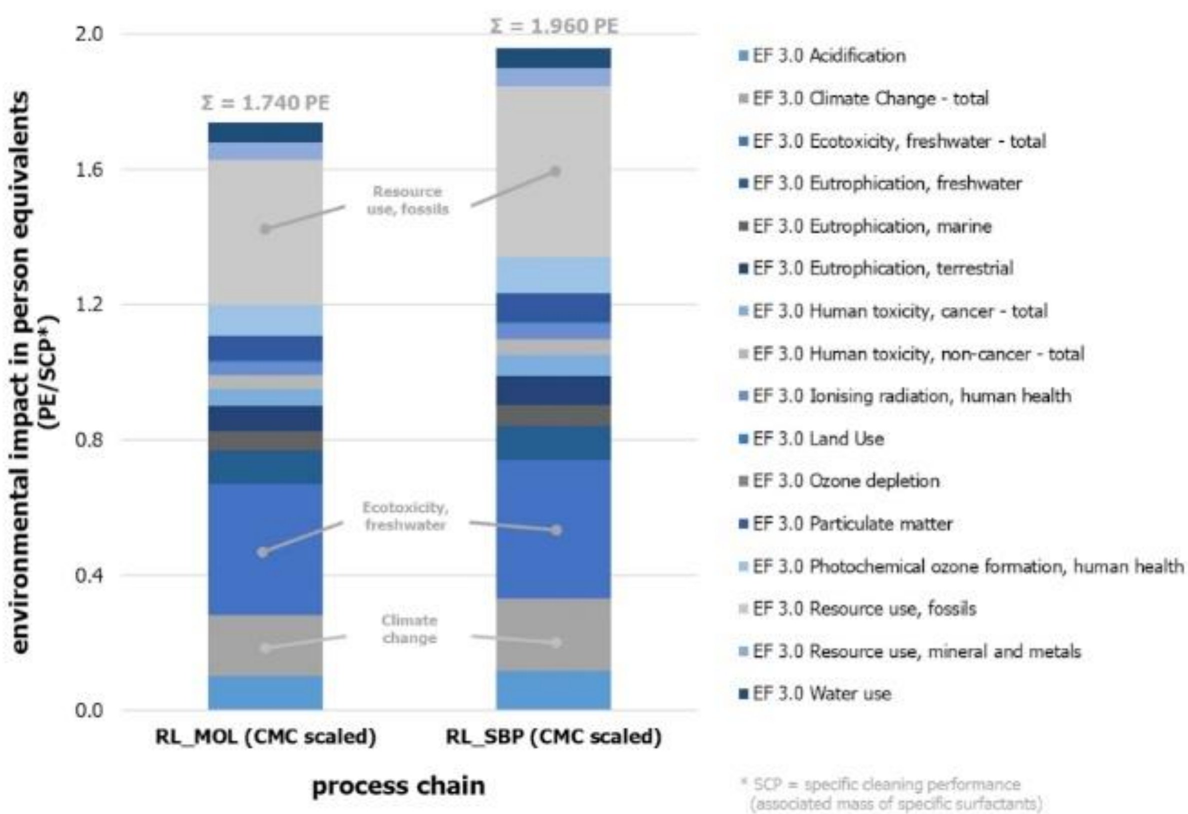

Figure 3. Environmental impacts per impact category for the production of RL by process chains RL_MOL and RL_SBP in PE/SCP; scaled by SCP-based CMC-factor (SCP ≙ production of 3.6 kg RL).

As shown in Figure 4, the highest environmental impacts of RL_MOL come from the "precipitation agent recovery"-stage (38.2%), the "fermentation"-stage (24.5%) as well as the sugar beet production and processing (21.1%). Most contributing categories are "resource use, fossils" (precipitation recovery, fermentation) and "ecotoxicity, freshwater - total" (fermentation, sugar beet production and processing). For RL_SBP, the contribution of the "precipitation recovery"-stage and the "fermentation"-stage are even a bit higher (43.3% and 29.3%, respectively) (Figure 4). The sugar beet production and processing contributes 13.2% to RL_SBP. The influence of other process stages is lower than 10% in each case. As the most effective contributing categories "resource use, fossils" (precipitation recovery) and "ecotoxicity, freshwater - total" (fermentation, sugar beet production and processing) could be identified.

The results provide a hint to optimization potentials. Some process stages can be influenced by an appropriate process design. While the production of substrates as well as of solvents (acetone) are beyond the influence of the project Bio², both "fermentation"-stage as well as "precipitation"-stage can be improved. Especially the reduction of acetone use during RL purification represents an important point for process optimization, so minimizing use or an increase of recycling rates appear as effective measures of impact lowering. Moreover, the use of MOL causes lower impacts than SBP.

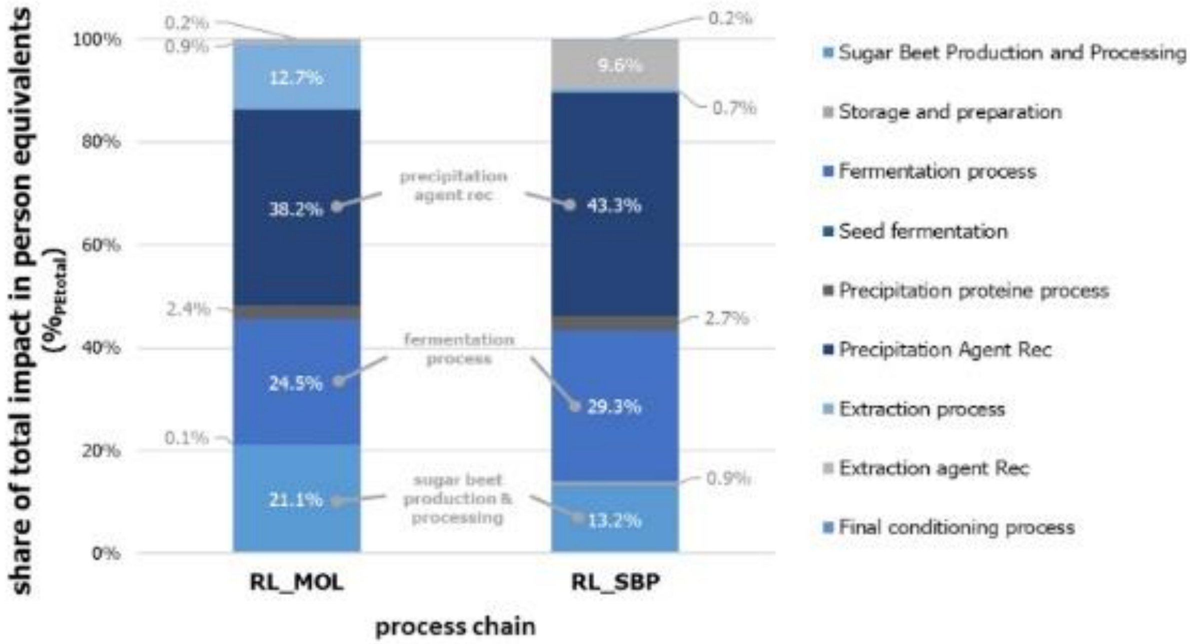

Figure 4. Relative share of environmental impacts per process stage for the production of RL by process chains RL_MOL and RL_SBP.

ENVIRONMENTAL IMPACTS OF MEL PRODUCTION

To fulfill the defined SCP, the production of 1 kg of MEL is needed and causes an environmental impact of 0.073 PE (MEL_MOL) and 0.085 PE (MEL_SBP), respectively (Figure 5). The two process chains are also quite similar. Mainly generated impacts are “ecotoxicity, freshwater – total” (MEL_MOL: 29.5%, MEL_SBP: 28.5%), “resource use, fossils” (MEL_MOL: 18.8%, MEL_SBP: 19.5%), and “climate change - total” (MEL_MOL: 8.5%, MEL_SBP: 8.6%).

Mainly responsible flows for the “ecotoxicity, freshwater – total”-category are inorganic emissions to freshwater by sulphur (MEL_MOL: 20.5%) and chloride (MEL_SBP: 19.2%). Sulphur-emissions can be traced back to potassium chloride production in the “sugar beet production and processing”, while chloride is mainly released in the treatment of solvents from extraction. The second most influential flows are inorganic emissions to freshwater by chloride (MEL_MOL: 20.3%) from the potassium chloride production and inorganic emissions to air by aluminum (MEL_SBP: 18.6%) from ethyl acetate production (extraction process #1).

The largest contribution to the category “resource use, fossils” originates from the use of natural gas (MEL_MOL: 34.2%, MEL_SBP: 32.5%). Responsible process modules are mainly the compressed air supply of the fermentation (MEL_MOL) and the production of ethyl acetate production (extraction process #1). As second contributing flow, crude oil from the ethyl acetate production for the extraction could be identified (MEL_MOL: 25.7%, MEL_SBP: 25.3%).

Effects of the “climate change - total”-category are attributable to flows of carbon dioxide (MEL_MOL: 87.1%, MEL_SBP: 87.7%). These inorganic emissions to air are connected to the compressed air supply of the fermentation in both process chains. A second relevant flow for both chains is the methane emission (MEL_MOL: 7.8%, MEL_SBP: 8.0%) related to the ethyl acetate production (extraction process #1).

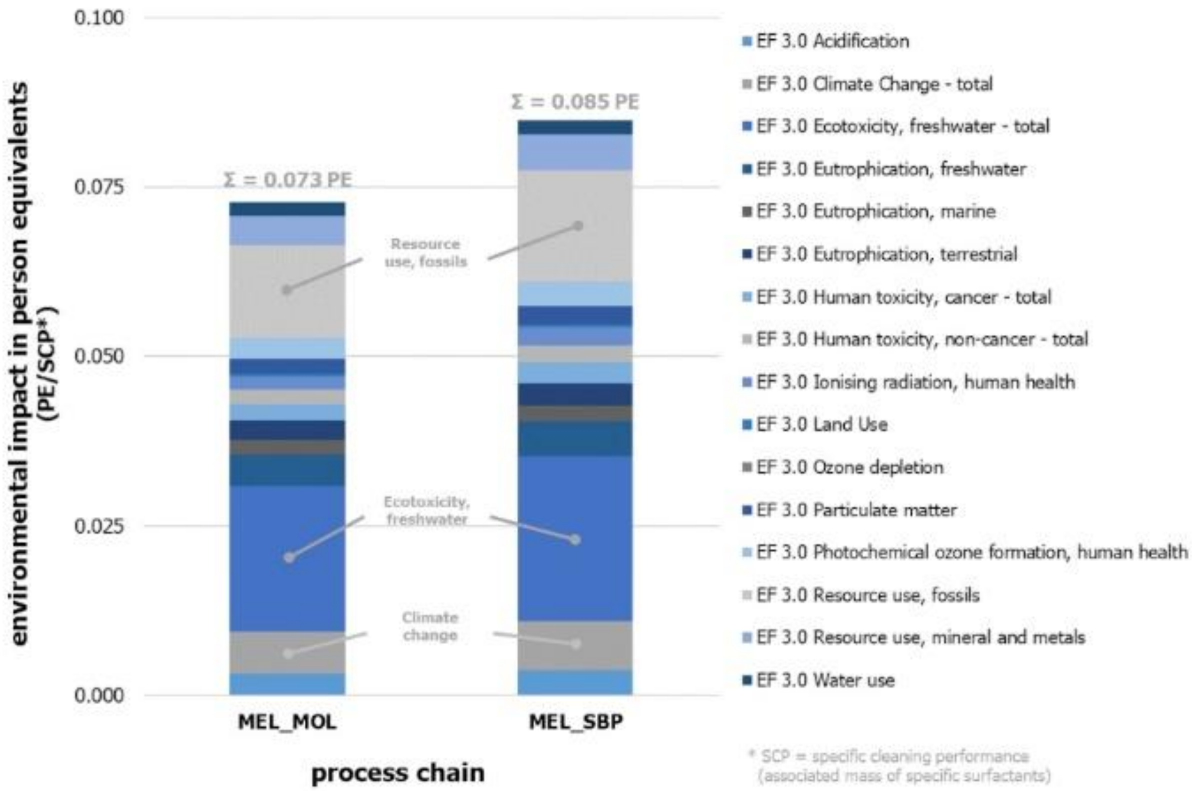

Figure 5. Environmental impacts per impact category for the production of MEL by process chains MEL_MOL and MEL_SBP (SCP ≙ production of 1 kg MEL).

Figure 6 reveals that the "extraction process #1" contributes the largest impact share in both process chains (MEL_MOL: 35.6%, MEL_SBP: 38.6%). Further remarkable contributions could be found in the "fermentation"-stage (MEL_MOL: 30.8%, MEL_SBP: 36.4%) and the "sugar beet production and processing"-stage (MEL_MOL: 25.5%, MEL_SBP: 15.4%). The dominating impact category, "ecotoxicity, freshwater - total" was found in all three stages and both process chains.

The results of MEL production show the process stages with the largest optimization potential. The "extraction process #1"- stages show large environmental impacts, which implies the possibility of decreased impacts by minimizing the use of ethyl acetate and an increase of its recycling. Further, use of ethyl acetate produced from non-fossil sources, could be envisaged.[60] So, in contrast to the dominating precipitation of RL production, the extraction is dominating for MEL production. The effect of substrate choice is similar to RL production in the case of MEL production, so that MOL is more advantageous.

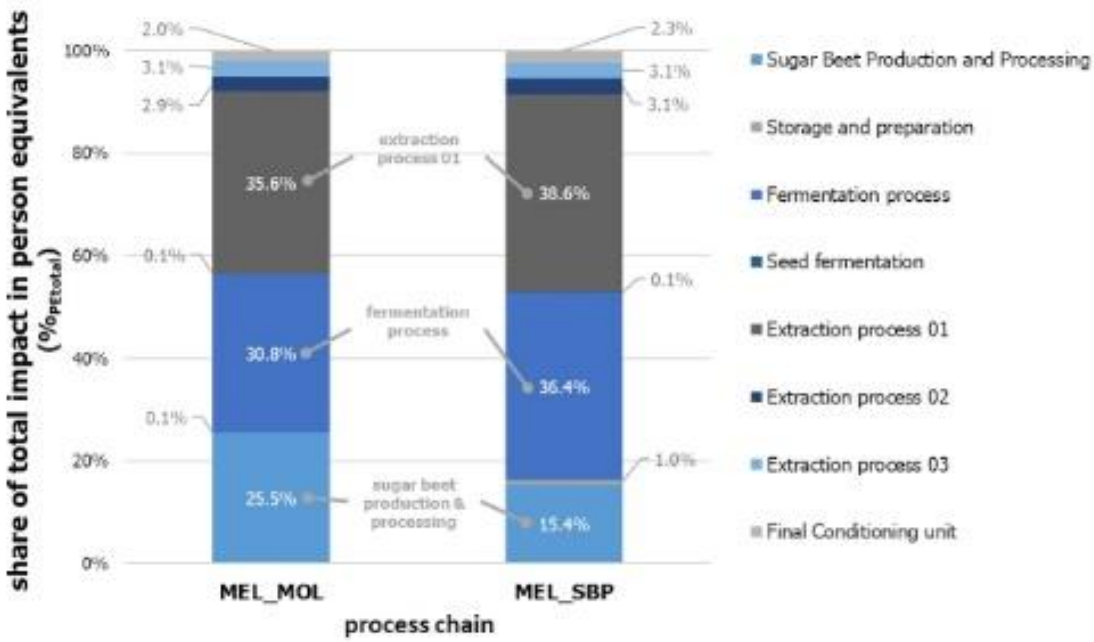

Figure 6. Relative share of environmental impacts per process stage for the production of MEL by process chains MEL_MOL and MEL_SBP.

The identification of the most influential impact categories and impacts' origin in RL and MEL production processes enable direct influence within the project development presented here. They may also give external process developers the opportunity to determine the focus of their developments and investigations.

COMPARISON TO PILOT SCALE AND CONVENTIONAL SURFACTANTS

To improve the classification of the previous results, the comparison of RL and MEL production with each other and with conventional surfactants was carried out. As defined in the section FUNCTIONAL UNIT, the comparison is made on the amount of surfactants needed for a SCP. To illustrate any scaling effects, a comparison was also made with previous study results of pilot scale implementation [31].

RL vs. MEL production - industrial scale

As it can be seen in Figure 3 and Figure 5, the environmental impacts of RL production are approximately 23 to 24 times larger than MEL production. The choice of substrates influences this ratio only minimally. Striking similarities are the most influencing impact categories and their shares (resource use, fossils; ecotoxicity, freshwater - total; climate change - total). Moreover, the dominating parts of each process chain are similar in their qualitative pattern (Figure 4, Figure 6). "Fermentation" and "sugar beet production and processing" stages are the second and third most impacting sections in all process chains. In contrast, the most affecting stages (RL: precipitation; MEL: extraction) differ for RL and MEL but share the characteristic that both include the use and production of auxiliary materials (agents). The lowest impacts can be found in the "land use" and "ozone depletion" categories for each process chain.

A deeper view into results of single impact categories shows that none of the 16 impact categories results in lower impacts for RL production. Including results of all process chains, the specific category values per kg product differ by factor 2.8 ("resource use, mineral and metals";

RL_MOL : MEL_SBP) to 10.7 (“particulate matter”; RL_SBP : MEL_MOL). The consideration of the SCP reinforces this effect.

Main reasons for the higher environmental impacts of RL production are the significantly lower specific yield coefficient and conversion rate of RL production in comparison to MEL, which results in a much higher throughput of flows such as fermentation broth. Therefore, this requires higher specific energy and material flows per kg product and increases the environmental impacts. In addition, the necessary higher amount per SCP leads to further increases for RL compared to MEL. The adjustment and optimization of further parameters (e.g., lower water input in fermentation) would probably not lead to a sufficient reduction of environmental impacts. A sensitivity analysis of changing yield coefficients is shown in section YIELD VARIATION and gives indications for the required increases of this parameter to make RL production competitive.

Identified differences between RL and MEL show clear advantages for MEL production from different perspectives (total impact, single impact categories). In addition to the individual results in sections ENVIRONMENTAL IMPACTS OF RL PRODUCTION and ENVIRONMENTAL IMPACTS OF MEL PRODUCTION, the comparison illustrates the effect of different microbial yields. Regarding the comparison of RL and MEL, the different data and knowledge situation mentioned above, and possible parameter changes due to real upscaling should be kept in mind in the interpretation of the results.

RL vs. MEL production - pilot scale

The results for the industrial scale are confirmed by a similar magnitude of impact ratio per SCP (RL:MEL $\approx$ 22) for the pilot scale like previously presented in Tiso et al. for RL.[31] Looking at the results for the pilot scale in “Supporting Information Figure S2.” (updated results from Tiso et al.

with EF 3.0 method and same characteristics), the exemplary process chains RL_MOL and MEL_MOL (pilot scale) show similar characteristics like the industrial scale (ratio between most contributing categories, ratio between process chains). The total impact of the industrial scale processes investigated in this study are 9.7% (RL_MOL) and 17.9% (MEL_MOL) below the pilot scale results. Like shown in "Supporting Information Figure S2.", effects of scaling on single impact categories vary from 2% to 40% for RL_MOL and from 10% to 50% for MEL_MOL. The largest changes can be found in the categories "Ozone depletion" (RL_MOL, MEL_MOL), "Photochemical ozone formation, human health" (MEL_MOL), and "Ionising radiation, human health" (RL_MOL). Assuming similar dependencies and ratios in the case of SBP-based processes, shown results verify a positive effect of scaling on the environmental impacts.

RL and MEL production vs. conventional surfactant production

Starting point for the comparison to conventional surfactants were the calculated impacts of ALT_01_HLAS, ALT_02_CDEA, and ALT_03_AAPB, that amount to 0.002 PE/kg, 0.003 PE/kg, and 0.002 PE/kg, respectively. By considering specific amounts of surfactant to reach the SCP of one kg MEL, the used mass increase by factor 24.2 (ALT_01_HLAS), 55.1 (ALT_02_CDEA), and 9.3 (ALT_03_AAPB). The results of Figure 7 represent these amounts and show the SCP-related environmental impacts compared to MEL. While ALT_01_HLAS (0.046 PE/SCP; 54.4-63.3%$_{\text{MEL impact}}$) and ALT_03_AAPB (0.017 PE/SCP; 19.6-22.8%$_{\text{MEL impact}}$) represent lower impacts, ALT_02_CDEA (0.161 PE/SCP; 189.6-220.7%$_{\text{MEL impact}}$) shows a significant larger impact. The impacts of MEL are in a similar order of magnitude as for the conventional surfactant (MEL-production impact 2 to 5 times higher or half the amount lower in the present setting).

A look at the shares of different impact categories shows a diverse pattern for the conventional surfactants. For example, ALT_01_HLAS shows a large share related to “resource use, fossils” (ca. 49%$_{PE\ total}$), while ALT_02_CDEA shows only a share of 10%$_{PE\ total}$ for this impact. This fact and a further comparison with the distribution of the results of MEL show a certain dependence of the quantitative profile of specific categories on the product type (e.g., larger shares of “ecotoxicity, freshwater” for MEL or “eutrophication, marine” for ALT_02_CDEA). By increasing recycling rates and further optimization, MEL results could become even more advantageous. Further, partially lower shares of “climate change - total” (primarily CO_2-emissions) or “resource use, fossils” (non-renewable resources) of MEL-production can be highlighted. For RL, with the current parameters, the achievement of similarly low environmental impacts is not possible. A combination of process related optimization and yield increases appears to be a possibility to reach competitive results. Summed up, environmental competitiveness is given or reachable for MEL and conditionally also for RL.

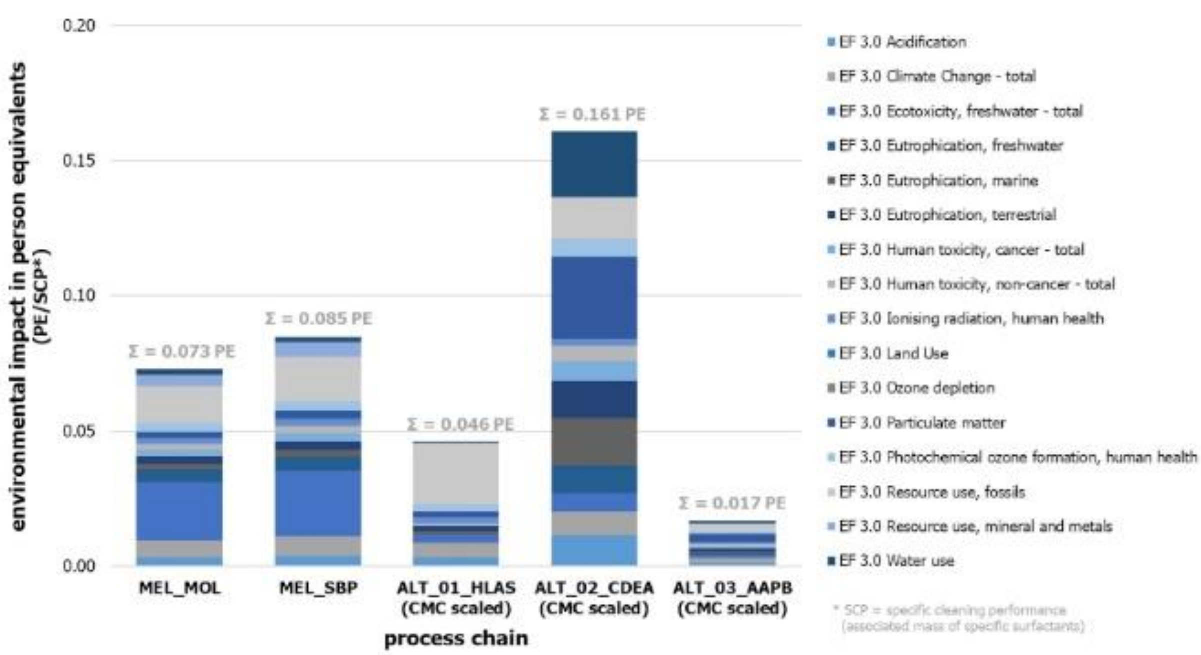

Figure 7. Environmental impacts per impact category for the production of MEL by process chains MEL_MOL and MEL_SBP in comparison to conventional surfactants in PE/SCP (SCP ≙ production of 1 kg MEL; MEL_MOL ≙ 1 kg; MEL_SBP ≙ 1 kg; ALT_01_HLAS ≙ 24.2 kg; ALT_02_CDEA ≙ 55.1 kg; ALT_03_AAPB ≙ 9.3 kg).

The results of the above section provide a rough idea of how industrial scale biosurfactant production compare to current surfactants' production in terms of their environmental impact. Furthermore, the results show that biosurfactants can be part of a potentially more sustainable bio-economy.

YIELD VARIATION

The environmental impacts regarding the variation of yield for RL_MOL (left) and MEL_MOL (right) are shown in Figure 8. A doubling of yield leads approximately to a halving of environmental impacts (RL_MOL: 50.4%, MEL_MOL: 50.7% of "yield variation 0"), a triplication leads to a third (RL_MOL: 33.6%, MEL_MOL: 34.1% of "yield variation 0"), the fourfold increase approximately to one quarter of impacts (RL_MOL: 25.2%, MEL_MOL: 25.4% of "yield variation 0"). The reason for this almost linear dependence is that the most influential processes behave linearly (e.g., extraction) or do not change, while some non-linear dependent processes (e.g., pumps or conveyors) have only a small influence on the overall results.

Assuming a linear reciprocal relation between yield and impact, the yield of RL cannot be increased in such a way that impacts reach the level of MEL process chains (considering the SCP). The theoretical maximum of yield ($1kg_{product}/1kg_{substrate}$) would still cause impacts of 0.174 PE/SCP (RL_MOL) and 0.194 PE/SCP (RL_SBP). Accordingly, there is a tendency for an advantage of

MEL compared to RL. Nevertheless, practical tests in industrial scale dimensions should be realized to verify these findings and to uncover unknown improvements.

In the case of MEL a doubling of yields would lead to impacts clearly below those of ALT_01_HLAS (0.046 PE/SCP). To reach the value of ALT_03_AAPB (0.017 PE/SCP), the yield of MEL would need to increase in a range that appears unrealistic (> 400% increase).

The results above show that the yield increase could contribute to a significant lower impact and an increasing competitiveness. For the comparisons in this section and section "RL and MEL production vs. conventional surfactant production" it has to be noted that the compared datasets for conventional production are black boxes and therefore differ in detail, which can influence the result further (negatively as well as positively).

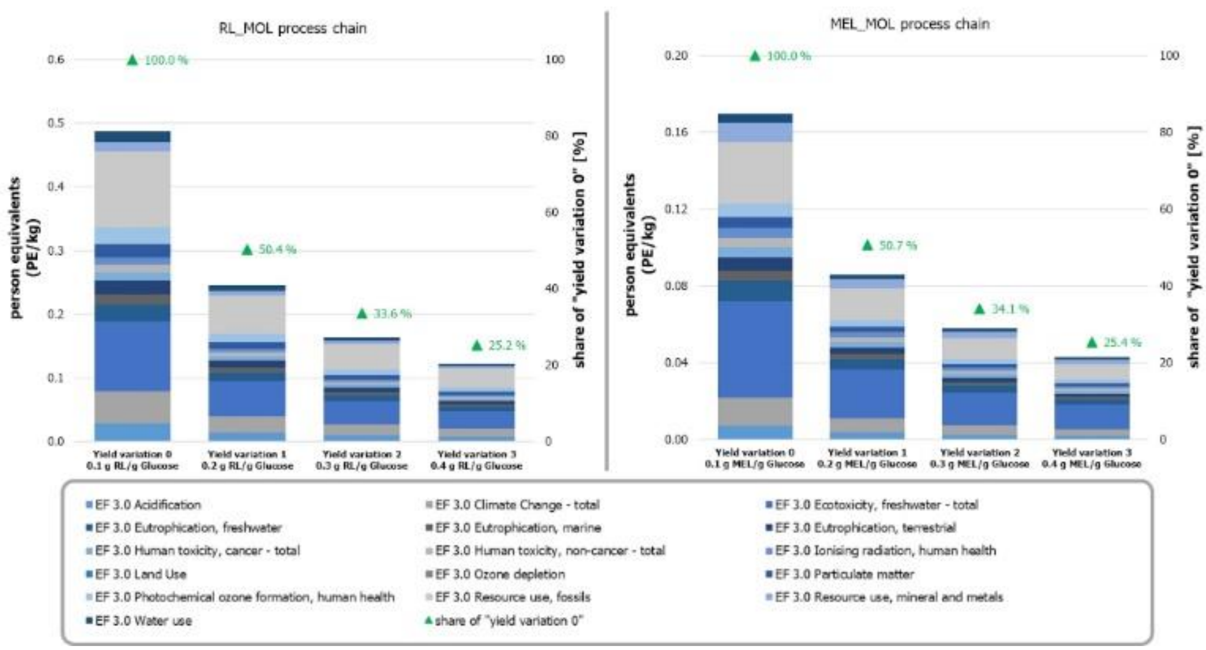

Figure 8. Variation of yield depending absolute results and relative shares of environmental impacts for RL and MEL production with molasses substrate (RL_MOL, MEL_MOL) in person

equivalents (PE); variation from “yield variation 0” (0.1 $g_{product}/g_{substrate}$) to “yield variation 3” (0.4 $g_{product}/g_{substrate}$).

IMPLICATIONS FOR FUTURE WORK

Regarding the environmental impact reduction of RL and MEL production improvement options are given by waste disposal adjustments, waste avoidance or replacements by recycling. Especially flows of biowaste should be studied with regard to further process-internal use. Furthermore, a demand-oriented aeration supply could be analyzed. Different dilution ratios and related water consumption decrease should be investigated, as the absolute throughput volume would influence dimensioning aspects. External effects of minimizing the supply of fertilizer in the biomass supply could be considered. Regardless of the given focal points of impact, future electricity mixes with lower CO_2 emissions can influence the results also. All these modifications potentially lead to significant reductions in specific impact categories and total environmental impacts and should be taken into account for future work. Further recommended research should also include the modeling of alternative downstream processes to identify possible potentials by technology changes.

After the implementation of described processing improvements, a revision of comparisons should be done. The combination of technological improvements and yield increases has to be assessed in detail to check joint effects. However, it has to be understood that yield increases cannot be made at will, but are limited and may be associated with further microbial behavior changes. Nevertheless, it can be assumed that yield changes are one of the main influencing factors and therefore core element of further research.

CONCLUSION

This LCA study compiles the environmental performance of RL and MEL production based on molasses and sugar beet pulp compared to each other and to conventional surfactants. The results identify hotspots such as the "fermentation" and "sugar beet production & processing"-stage for RL and MEL process chains. Decisive process modules of these stages are the treatment of organic wastes and compressed air supply (fermentation) as well as the production of fertilizer (sugar beet production and processing). The largest influence could be identified in the "precipitation recovery" (RL) and "extraction process #1"-stage (MEL). Main driver of their impacts is the agent supply (acetone production, ethyl acetate production), despite a recycling rate of 80%. Detected most relevant impact categories were "resource use, fossils" (largest contribution RL), "ecotoxicity, freshwater - total" (largest contribution MEL), and "climate change - total". Thus, the results allow the identification of optimization potential. Particular attention should be paid to influenceable parameters such as recycling rates. While the production and use of fertilizers is outside the foreground system, the role of agent production can be limited by less use. Furthermore, the development of an impact saving aeration concept (demand-oriented) is within the scope of influence of the foreground systems.

A comparison of the four process chains' results has shown clearly lower environmental impacts for MEL-production than for RL-production. This fact indicates that the targeted product (RL or MEL) can be described as the decisive influencing factor in terms of environmental impacts. The results offer, that the choice of substrate does not play a major role in the overall environmental performances. Nevertheless, it should be emphasized that use of molasses leads to lower environmental impacts due to easier handling and less technological requirements. Differences related to the chosen substrate (e.g., change of dilution rates) should be investigated in upcoming studies. These findings confirm previous findings in a pilot scale modeling, where similar impact ratios between the four process chains were observed. Moreover, it could be stated that an upscaling of the process chains led to lower impacts also.

By the exemplary comparison with conventional surfactants, it could be shown that the environmental performance of biosurfactant production is in a competitive range. The combination of technological improvements and yield increases could foster the position of RL and MEL compared to other surfactants. Follow-up studies and comparisons with further conventional surfactants (oleo-/petrochemical) should be used to confirm this recognition. This applies in particular to possible applications in areas that require higher purities (e.g., cosmetics or pharmaceuticals) and are therefore more valuable.

As a decisive factor, the increase of microbial individual yields could be identified by a sensitivity analysis. The almost linear dependence of environmental impacts on yields allowed the estimation of the feasibility and dimension of yield increases. While RL process chains seem to be out of feasibility range in the presented setup, MEL process chains could expand their advantage within a realistic range. Nevertheless, process conditions such as yields are limited in their extent. Real upscaling must verify the results and might reveal additional factors not considered here.

Process parts with large optimization potential such as aeration supply, the utilization of process wastes, or the recycling of agents (acetone and ethyl acetate) should be improved by design changes (process module design, processing modifications) to reduce the environmental impacts of the overall system. Future designs with further recycling options (e.g., fermentation broth) or different dimensioning of process chains, could be modeled to verify their effectiveness in terms of environmental impacts.

The presented results offer different options for process chain improvements and confirm the use of LCA as a useful tool in iterative process developments. In summary, the current LCA-results provide information on the development status and environmental compatibility of this novel production process options for next generation biosurfactants RL and MEL. From the perspective of environmental burdens, it can be assumed that conventional surfactants currently on the market could be replaced by biosurfactants in the future.

SUPPORTING INFORMATION

Illustration of biosurfactant production in project Bio², methodologically updated pilot scale results, average critical micelle concentration (CMC) data, alternative surfactants' properties, allocation data of sugar beet production and processing, impact categories from EF 3.0 method, process chain specific results of LCIA per impact category

CORRESPONDING AUTHOR

Andreas Schonhoff

Affiliation: Institute of Energy and Climate Research - Systems Analysis and Technology Evaluation (IEK-STE), Forschungszentrum Jülich GmbH, Wilhelm-Johnen-Straße, 52428 Jülich, Germany

E-Mail: a.schonhoff@fz-juelich.de

ORCID: 0000-0002-8937-6957

AUTHOR CONTRIBUTIONS

The conceptualization of the present study was done by A.Scho., A.Schr. and P.Z., whereas the data curation was carried out by A.Scho with data resources provided by acknowledged project partners. The investigation was made by A. Scho. together with acknowledged project partners and supervisors. Applied project-specific methods were (further) developed by A.Scho. The related project Bio² was supervised and administrated by N.I., while the presented LCA study was supervised by P.Z. A.Scho. was responsible for the writing of the original draft and visualization, while N.I., A. Schr., and P.Z. performed the duties of reviewing and editing. All authors have approved the final version of the manuscript.

PRESENT ADDRESSES

Nina Ihling is currently affiliated with GOTTSCHALD Patentanwälte Partnerschaft mbB, Klaus-Bungert-Straße 1, 40468 Düsseldorf, Germany.

FUNDING SOURCES

Scientific activities of the project FocusLab Bio2 - "Integration of next generation biosurfactant production into biorefinery processes." were financially supported by the Ministry of Culture and Research of the German federal state of North Rhine Westphalia within the framework of the NRW-Strategieprojekt BioSC (No. 313/323-400-002 13).

NOTES

The authors declare no competing financial interest.

ACKNOWLEDGMENT

The presented results were generated within the Project Bio², which is part of the NRW-Strategieprojekt BioSC and thus funded by the Ministry of Culture and Science of the German State of North Rhine-Westphalia. The used process engineering data was provided and generated by Maximilian Schelden (RWTH Aachen University, AVT - Chemical Process Engineering), Patrick Bongartz (RWTH Aachen University, AVT - Chemical Process Engineering), and Andreas

Biselli (RWTH Aachen University, AVT - Fluid Process Engineering) under supervision of N. Ihling. Essential data that enabled the process development was offered and generated by Silke Jankowski/Marius Terfrüchte (Heinrich-Heine-University Düsseldorf - Institute for Microbiology - Department of biology, supervised by K. Schipper), Isabel Bator (RWTH Aachen University - Institute of Applied Microbiology (iAMB), supervised by T. Tiso) as well as Sonja Kubicki (Heinrich-Heine-University Düsseldorf - Institute of Molecular Enzyme Technology (IMET), supervised by S. Thies) related to aspects of microorganism and microbiology.

ABBREVIATIONS

RL: rhamnolipids

MEL: mannosylerythritol lipids

LCA: life cycle assessment

MOL: molasses from sugar industry

SBP: sugar beet pulp from sugar industry

HAA: 3-(3-hydroxyalkanoyloxy)alkanoate

RL_MOL: process chain for RL production using MOL

RL_SBP: process chain for RL production using SBP

MEL_MOL: process chain for MEL production using MOL

MEL_SBP: process chain for MEL production using SBP

ALT...: alternative process for surfactant production

SCP: specific cleaning performance

CMC: critical micelle concentration

EF 3.0: environmental footprint; assessment method for environmental impacts

LCIA: life cycle impact assessment

PE: person equivalents

LCI: life cycle inventory

Ψ: volume mix ratio of two liquids (volume/volume)

ALT_01_HLAS: alternative surfactant - C10-13 Linear alkylbenzene sulphonic acid

ALT_02_CDEA: alternative surfactant - Cocamide diethanolamine

ALT_03_AAPB: alternative surfactant - C8-18 Alkyl amidopropyl betaine

REFERENCES

1. European Comission. *Communication from the Commission (...) - The European Green Deal - COM(2019) 640 final; COM(2019) 640 final*; last revised 12/2019. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=COM:2019:640:FIN (accessed 2022-02-28)

2. European Commission. *Communication From The Commission To The European Parliament, The Council, The European Economic And Social Committee And The Committee Of The Regions - 'Fit for 55': delivering the EU's 2030 Climate Target on the way to climate neutrality*, last revised 07/2021. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52021DC0550 (accessed:2022-02-28)

3. Brändlin, A.-S. The year of climate consciousness. *Deutsche Welle (Bonn, DE)*, December 27, 2019. https://p.dw.com/p/3V0j2 (accessed 2022-02-28)

4. Rankin, J. Greta Thunberg tells EU: your climate targets need doubling. The Guardian (London, UK), February 21, 2019. https://www.theguardian.com/environment/2019/feb/21/greta-thunberg-tells-eu-your-greenhouse-gas-targets-are-too-low (accessed 2022-02-28)

5. Beckh, P.; Limmer, A. The Fridays for Future Phenomenon. In *Strategies for Sustainability of the Earth System*; Springer International Publishing: Cham, 2022; pp 427-432. DOI: 10.1007/978-3-030-74458-8_28

6. European Union. *Making sustainable use of our natural resources - Factsheet: Nature and forests*. European Union, 2021. https://op.europa.eu/en/publication-detail/-/publication/93c08b45-00e6-11ec-8f47-01aa75ed71a1 (accessed 2022-02-28)

7. BioSC. *FocusLab Bio² - Integration of next generation biosurfactant production into biorefinery processes*. https://www.biosc.de/bio2_en (accessed 2022-02-28).

8. Deutsches Institut für Normung (DIN). *DIN EN 17035 - Surface Active Agents - Bio-based surfactants - Requirements and test methods; German version CEN/TS 17035:2021*. Berlin, DE, 2021. DOI: 10.31030/3157001

9. Tiso, T.; Thies, S.; Müller, M.; Tsvetanova, L.; Carraresi, L.; Bröring, S.; Jaeger, K.-E.; Blank, L. M. Rhamnolipids: Production, Performance, and Application. In *Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Production of Fuels and Chemicals*. Springer International Publishing, 2017; pp 1-37. DOI: 10.1007/978-3-319-31421-1_388-1

10. Loeschcke, A.; Thies, S., Pseudomonas putida-a versatile host for the production of natural products. *Applied Microbiology and Biotechnology* **2015,** 99 (15), 6197-6214. DOI: 10.1007/s00253-015-6745-4

11. Arutchelvi, J.; Doble, M., Mannosylerythritol Lipids: Microbial Production and Their Applications. *Biosurfactants: From Genes to Applications* **2011,** 20, 145-177. DOI: 10.1007/s10295-008-0460-4

12. Terfrüchte, M.; Wewetzer, S.; Sarkari, P.; Stollewerk, D.; Franz-Wachtel, M.; Macek, B.; Schlepütz, T.; Feldbrügge, M.; Büchs, J.; Schipper, K., Tackling destructive proteolysis of unconventionally secreted heterologous proteins in Ustilago maydis. *Journal of Biotechnology* **2018,** 284, 37-51. DOI: 10.1016/j.jbiotec.2018.07.035

13. Verband TEGEWA. *Die fleißigen Verbindungen Eine kurze Einführung in die Welt der Tenside*. Verband der Hersteller von Textil-, Papier-, Leder- und Pelzhilfs- und -farbmitteln,

Tensiden, Komplexbildnern, Antimikrobiellen Mitteln, Polymeren Flockungsmitteln, Kosmetischen Rohstoffen und Pharmazeutischen Hilfsstoffen oder verwandten Produkten, 2014. https://www.tegewa.de/wp-content/uploads/2018/09/Tensid_Broschuere_2014_deutsch.pdf (accessed 2022-02-28)

14. *IHS Markit - Assessing the sustainability and performance of green surfactants*. https://ihsmarkit.com/research-analysis/assessing-sustainability-and-performance-of-green-surfactants.html (accessed 2022-02-28).

15. *Industry ARC - Surfactants Market - Forecast (2020 - 2025).* ; https://www.industryarc.com/Report/15201/surfactants-market.html (accessed 2021-02-12).

16. *Research And Markets - Global Surfactants Market (Non-ionic, Anionic & Cationic): Insights, Trends and Forecast* (2019-2023). https://www.researchandmarkets.com/reports/4845555/global-surfactants-market-non-ionic-anionic-and (accessed 2020-01-31).

17. *Research And Markets - Surfactants Market - Growth, Trends, and Forecast (2020 - 2025)*. https://www.researchandmarkets.com/reports/4771655/surfactants-market-growth-trends-and-forecast#relb1-4845555 (accessed 2021-01-31).

18. Wiegmann, K.; Hünecke, K.; Moch, K.; Hennenberg, K. J.; Fehrenbach, H. *Implementierung von Nachhaltigkeitskriterien für die stoffliche Nutzung von Biomasse im Rahmen des Blauen Engel - Teil 4: PROSA - Biobasierte Wasch- und Reinigungsmittel Abschlussbericht*, Umweltbundesamt: Dessau Rosslau, DE, 2019. https://www.umweltbundesamt.de/publikationen/implementierung-von-nachhaltigkeitskriterien-fuer-2 (accessed 2022-02-28)

19. Spekreijse, J.; Lammens, T.; Parisi, C.; Ronzon, T.; Vis, M. *Insights into the European market for bio-based chemicals*. *Analysis based on ten key product categories*, Publications Office of the European Union: Brussels, BE, 2019. https://op.europa.eu/en/publication-detail/-/publication/8eccea76-1ec7-11e9-8d04-01aa75ed71a1/language-en (accessed 2022-02-28)

20. Bettenhausen, C. Evonik invests in rhamnolipid biosurfactants - New capacity is Slovakia bolsters partnership with Unilever. *c&en - Chemical & Engineering News (Washington, DC)*, February 19, 2022. https://cen.acs.org/business/specialty-chemicals/Evonik-invests-rhamnolipid-biosurfactants/100/i3 (accessed 2022-02-28).

21. Roelants, S.; Everaert, B.; Redant, E.; Vanlerberghe, B.; Soetaert, W., *Microbial biosurfactants, from lab to market : hurdles and how to take them, AOCS Annual Meeting, Abstracts*, Orlando, USA, April 30 – May 3, 2017; Donna Elbon Ed.; https://www.aocs.org/attend-meetings/archives/2017-aocs-annual-meeting-and-short-courses (accessed 2022-02-2028)

22. Anastas, P. T.; Warner, J. C. *Green Chemistry: Theory and Practice*; Oxford University Press, 1998.

23. Adlercreutz, D.; Tufvesson, P.; Karlsson, A.; Hatti-Kaul, R. Alkanolamide biosurfactants: Techno-economic evaluation of biocatalytic versus chemical production. *Industrial Biotechnology* **2010,** 6 (4), 204-211. DOI: 10.1089/ind.2010.6.204

24. Kopsahelis, A.; Kourmentza, C.; Zafiri, C.; Kornaros, M. Gate-to-gate life cycle assessment of biosurfactants and bioplasticizers production via biotechnological exploitation of fats and waste oils. *Journal of Chemical Technology & Biotechnology* **2018,** 93 (10), 2833-2841. DOI: 10.1002/jctb.5633

25. Baccile, N.; Babonneau, F.; Banat, I. M.; Ciesielska, K.; Cuvier, A.-S.; Devreese, B.; Everaert, B.; Lydon, H.; Marchant, R.; Mitchell, C. A.; Roelants, S.; Six, L.; Theeuwes, E.; Tsatsos, G.; Tsotsou, G. E.; Vanlerberghe, B.; Van Bogaert, I. N. A.; Soetaert, W. Development of a Cradle-to-Grave Approach for Acetylated Acidic Sophorolipid Biosurfactants. *ACS Sustainable Chemistry & Engineering* **2017,** 5 (1), 1186-1198. DOI: 10.1021/acssuschemeng.6b02570

26. *ecoinvent Database 3.7*. ecoinvent Association. https://ecoinvent.org/the-ecoinvent-database (accessed 2022-02-28)

27. Zah, R.; Hischier, R. *Life Cycle Inventories of Detergents. Final report ecoinvent data v2.0, No. 12*; Swiss Centre for Life Cycle Inventories, Zurich, CH, 2007. https://ecoinvent.org/the-ecoinvent-database/data-releases/ecoinvent-version-2 (accessed 2022-02-28)

28. Guilbot, J.; Kerverdo, S.; Milius, A.; Escola, R.; Pomrehn, F. Life cycle assessment of surfactants: the case of an alkyl polyglucoside used as a self emulsifier in cosmetics. *Green Chemistry* **2013,** 15 (12), 3337-3354. DOI: 10.1039/C3GC41338A

29. Alkyl Polyglucosides (APG) from coconut oil. *CPM-LCA-Database LCI data*. Chalmers University of Technology Göteborg. http://cpmdatabase.cpm.chalmers.se/Scripts/sheet.asp?ActId=akzsrf002-1999-01-26-841 (accessed 2022-02-28). (CAS RN: N/A).

30. *ERASM Life cycle ecofootprinting (SLE)*. European Committee of Organic Surfactants and their Intermediates (CESIO). https://www.erasm.org/index.php/erasm-research/manufacturing/raw-material-sourcing/life-cycle-inventories (accessed 2022-02-28)

31. Tiso, T.; Ihling, N.; Kubicki, S.; Biselli, A.; Schonhoff, A.; Bator, I.; Thies, S.; Karmainski, T.; Kruth, S.; Willenbrink, A.-L.; Loeschcke, A.; Zapp, P.; Jupke, A.; Jaeger, K.-E.; Büchs, J.; Blank, L. M. Integration of Genetic and Process Engineering for Optimized Rhamnolipid Production Using Pseudomonas putida. *Frontiers in Bioengineering and Biotechnology* **2020,** 8 (976). DOI: 10.3389/fbioe.2020.00976

32. Kubicki, S.; Bator, I.; Jankowski, S.; Marius, T.; Schipper, K.; Feldbrügge, M.; Tiso, T.; Blank, L.; Jaeger, K.-E.; Thies, S. Colorimetric assay for high throughput quantification of biosurfactants in culture supernatants. In *Biosurfactants 2019 - Book Of Abstracts*, University of Hohenheim - Bioprocess Engineering: Hohenheim (DE), 2019. https://biosurfactants.uni-hohenheim.de/en/bookofabstracts2019 (accessed 2022-02-28)

33. Bator, I.; Wittgens, A.; Rosenau, F.; Tiso, T.; Blank, L. M., Comparison of Three Xylose Pathways in Pseudomonas putida KT2440 for the Synthesis of Valuable Products. *Frontiers in Bioengineering and Biotechnology* **2020,** 7 (480). DOI: 10.3389/fbioe.2019.00480

34. Bongartz, P.; Bator, I.; Blank, L.; Wessling, M. Foam-less fermentation for biosurfactant synthesis via advanced membrane aeration. *Chemie Ingenieur Technik* **2020,** 92 (9), 1204-1204. DOI: 10.1002/cite.202055036

35. Bongartz, P.; Keller, R.; Schelden, M.; Wandrey, G.; Büchs, J.; Wessling, M. Blasenfreie Membranbegasung zur Biotensidproduktion im NRW-Strategieprojekt BioSC Focus Lab Bio 2. *Chemie Ingenieur Technik* **2018,** 90, 1250-1250. DOI: 10.1002/cite.201855261

36. Biselli, A.; Willenbrink, A.-L.; Leipnitz, M.; Jupke, A. Development, evaluation, and optimisation of downstream process concepts for rhamnolipids and 3-(3-

hydroxyalkanoyloxy)alkanoic acids. *Separation and Purification Technology* **2020,** 250, 117031. DOI: 10.1016/j.seppur.2020.117031

37. International Organization for Standardization (ISO). *ISO 14040:2006 - Environmental management - Life cycle assessment - Principles and framework*; Geneva, CH, 2006. DOI: 10.31030/3179655

38. International Organization for Standardization (ISO). *ISO 14044:2006 - Environmental management - Life cycle assessment - Requirements and guidelines*; Geneva, CH, 2006. DOI: 10.31030/2761237

39. *LCA Software GaBi 10.5*; Sphera Solutions GmbH: Leinfelden-Echterdingen, 2021. https://gabi.sphera.com/international/index (accessed 2022-02-28)

40. Rodrigues, L. R., Microbial surfactants: Fundamentals and applicability in the formulation of nano-sized drug delivery vectors. *Journal of Colloid and Interface Science* **2015,** 449, 304-316. DOI: 10.1016/j.jcis.2015.01.022

41. Ghista, D. N., *Biomedical Science, Engineering and Technology*. London (UK): IntechOpen Limited, 2012.

42. Kaczorek, E., Effect of External Addition of Rhamnolipids Biosurfactant on the Modification of Gram Positive and Gram Negative Bacteria Cell Surfaces during Biodegradation of Hydrocarbon Fuel Contamination. *Polish Journal of Environmental Studies* **2012,** 21 (4), 901-909. DOI: 10.15244/pjoes

43. Sharma, D.; Saharan, B. S., *Microbial Cell Factories*. Boca Raton (US): CRC Press, 2018.

44. Rau, U.; Nguyen, A.; Schulz, S.; Wray, V.; Nimtz, M.; Roeper, H.; Koch, H.; Lang, S. Formation and analysis of mannosylerythritol lipids secreted by Pseudozyma aphidis. *Applied microbiology and biotechnology* **2005,** 66, 551-9. DOI: 10.1007/s00253-004-1672-9

45. Morita, T.; Konishi, M.; Fukuoka, T.; Imura, T.; Kitamoto, D. Production of glycolipid biosurfactants, mannosylerythritol lipids, by Pseudozyma siamensis CBS 9960 and their interfacial properties. *Journal of Bioscience and Bioengineering* **2008,** 105 (5), 493 - 502. DOI: 10.1263/jbb.105.493

46. LAS - Linear Alkylbenzene Sulphonate. *Human and Environmental Risk Assessment (HERA) Risk Assessments*: Human and Environmental Risk Assessment (HERA), 2013. https://www.heraproject.com/files/HERA-LAS%20revised%20April%202013%20Final1.pdf (accessed 2022-02-28). (CAS RN: 68411-30-3).

47. NINOL 11-CM. *Products and markets - product description*. Stepan Company 2021. https://wwwprd.stepan.com/content/stepan-dot-com/en/products-markets/product/NINOL11CM.html (accessed 2022-02-28). (CAS RN: 68603-42-9).

48. 1-Propanaminium, 3-amino-N-(carboxymethyl)-N,N-dimethyl-, N-C8-18 acyl derivs., inner salts. *ECHA Registration dossiers*. European Chemicals Agency 2021. https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15295/4/9 (accessed 2022-02-28). (CAS RN: 97862-59-4).

49. Bongartz, P.; Bator, I.; Baitalow, K.; Keller, R.; Tiso, T.; Blank, L.; Wessling, M., A scalable bubble-free membrane aerator for biosurfactant production. *Biotechnology and Bioengineering* **2021,** 118, 3545 - 3558. DOI:10.1002/bit.27822

50. Bator, I.; Karmainski, T.; Tiso, T.; Blank, L. M. Killing Two Birds With One Stone – Strain Engineering Facilitates the Development of a Unique Rhamnolipid Production Process. *Frontiers in Bioengineering and Biotechnology* **2020,** 8, 1 - 16. DOI: 10.3389/fbioe.2020.00899

51. Demling, P.; von Campenhausen, M.; Grütering, C.; Tiso, T.; Jupke, A.; Blank, L. M. Selection of a recyclable in situ liquid–liquid extraction solvent for foam-free synthesis of rhamnolipids in a two-phase fermentation. *Green Chemistry* **2020,** 22 (23), 8495-8510. DOI: 10.1039/D0GC02885A

52. *GaBi professional databases*. Sphera Solutions GmbH: Leinfelden-Echterdingen, 2021. https://gabi.sphera.com/international/databases/gabi-databases/professional (accessed 2022-02-28)

53. Spörri, A.; Kägi, T., LCA of EU beet sugar. Part I/II: Conducting a LCA of sugar production in the European Union. *Zuckerindustrie. Sugar industry* 2015, 140(8), 492-499 /529-592. DOI: DOI: 10.36961/si16693 /si16773

54. Knoll, A. J. Betrieb von Rührkesselbioreaktoren unter erhöhten Reaktordrücken. Ph.D. Thesis, RWTH Aachen, Aachen, DE, 2008.

55. *Wirtschaftliche Vereinigung Zucker (WVZ) Website*. http://www.zuckerverbaende.de/zuckermarkt/zahlen-und-fakten/zuckermarkt-deutschland/futtermittel-aus-zuckerrueben.html (accessed 2022-02-28)

56. Fazio, S.; Castellani, V.; Sala, S.; Schau, E.; Secchi, M.; Zampori, L.; Diaconu, E., *Supporting information to the characterisation factors of recommended EF Life Cycle Impact Assessment methods. New models and differences with ILCD*. European Commission, Ispra, 2018.

https://op.europa.eu/en/publication-detail/-/publication/de9a732b-130c-11e9-81b4-01aa75ed71a1 (accessed 2022-02-28)

57. European Union – Joint Research Center (JRC). https://eplca.jrc.ec.europa.eu/LCDN/developerEF.xhtml (accessed 2022-02-28).

58. European Commission. *Commission recommendation (...) on the use of the Environmental Footprint methods to measure and communicate the life cycle environmental performance of products and organisations*. Last revised 12/2021. https://ec.europa.eu/environment/publications/recommendation-use-environmental-footprint-methods_en (accessed 2022-02-28)

59. Flottweg SE, *FLOTTWEG SEDICANTER® - Erschließen Sie neue Anwendungsbereiche*. Flottweg SE, 2016. https://www.flottweg.com/fileadmin/user_upload/data/pdf-downloads/Sedicanter.pdf (accessed 2022-02-28)

60. Budsberg, E.; Morales-Vera, R.; Crawford, J.T.; Bura, R.; Gustafson, R. Production routes to bio-acetic acid: life cycle assessment. *Biotechnology for Biofuels and Bioproducts* **2020,** 13, 154-154. DOI: 10.1186/s13068-020-01784-y