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Innovation Alliance Biosurfactants

Accelerating the biologization of surfactant production – from lab to market

www.allianz-biotenside.de/en

Imprint

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Innovation Alliance Biosurfactants

Overall aim of the Innovation Alliance Biosurfactants

The aim of the Innovation Alliance Biosurfactants was, for the first time in Germany, to establish a strategic alliance between renowned companies and research institutions in order to jointly establish a scalable production process for biosurfactants using biotechnological methods from domestic renewable raw and residual materials, and to systematically investigate their application potentials.

To this end, the production and purification of biosurfactants from various renewable raw materials were to be optimized within the consortium so that they could be used in the application areas of laundry and cleaning products, dishwashing detergents, cosmetics, personal care, agronomy, food, lubricant additives, and firefighting agents as alternatives to chemically synthesized surfactants. The Alliance Biosurfactants also involved companies that previously had little or no exposure to biotechnological processes and products. A greater biologization of surfactant production is only possible through key innovations and strategic cooperation among raw material producers, process engineers, surfactant manufacturers and users, and research institutions.

ALLIANZ BIOTENSIDE

German logo of the Alliance Biosurfactants

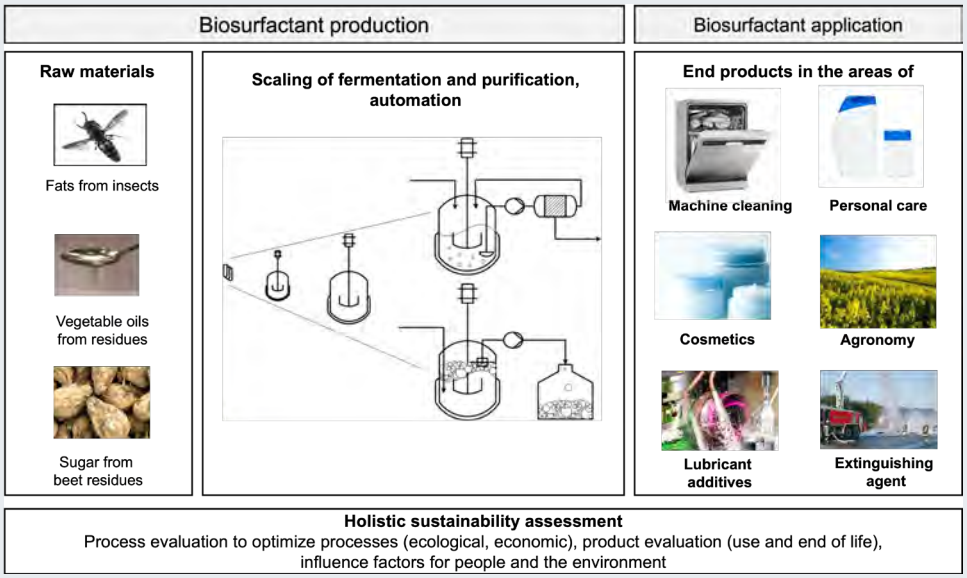
300-L bioreactor

In the first project phase of the Innovation Alliance, various biosurfactants – rhamnolipids, mannosylerythritol lipids, cellobiose lipids, surfactin, and novel microbial biosurfactants – were produced fermentatively from renewable raw materials and investigated with regard to their specific application properties. By using different microorganisms and feedstocks, such as fatty acids of varying chain length, the properties of the biosurfactants could be varied over a wide range, thereby optimizing their functional properties. In addition to fermentative production, the Alliance Biosurfactants also investigated the enzymatic synthesis of new glycolipids. By combining different sugars and fatty alcohols of varying chain lengths, new biosurfactant molecules with application-tailored functional properties were defined and produced (structure-based development). In parallel, the entire value chain was holistically assessed with regard to ecological and economic aspects, and recommendations for action were derived for the respective partners.

For the second project phase, the biosurfactants to be investigated were narrowed to the most promising combinations from the first phase, focusing on the production of mannosylerythritol lipids (MEL; 3 structural variants), cellobiose lipids (CL; 3 variants), surfactin (1 variant), and enzymatically produced biosurfactants (2 variants). The production processes were further supported by technical advancements and automation by various companies, as well as by holistic process evaluation. In terms of applications, the second project phase built on the most promising approaches from the first phase and gained new partners and application areas.

Collaboration along the value chain

Different sugar- and oil-containing raw and residual materials were selected and supplied by Hermetia and various associated partners. Conversion into the respective biosurfactants was carried out using optimized fermentative and enzymatic processes with subsequent purification at the academic institutions Fraunhofer IGB, KIT, the University of Hohenheim,



Value chain from domestic renewable raw materials to biosurfactant-containing end products for various application areas within the Innovation Alliance Biosurfactants (Phase 2).

and the University of Stuttgart IGVP. Process development was also supported by technical enhancement and automation of processes at the industrial partners FESTO, ifm, Metrohm, MyBiotech, and the Steinbeis Transfer Center Innovative Process Analytics (IPAT) under subcontract to Fraunhofer IGB. Biosurfactant formulation and application addressed the creation of formulations with the produced biosurfactants in the areas of laundry and cleaning products, cosmetics, bioremediation, crop protection, lubricants, and firefighting agents. Drawing on the expertise of the industrial partners Henkel, Dalli, BASF, Festo, Oelheld, and Dr. Sthamer, various application areas were opened up, thereby increasing the likelihood that a marketable application can be found for the produced biosurfactants. Across the entire process chain, a comprehensive sustainability assessment of biosurfactant production was carried out with respect to ecological, economic, and social aspects. This included life cycle assessment (LCA) from raw material to biosurfactant to end product by the University of Stuttgart IABP, as well as techno-economic evaluation (TEE) and assessment of technology readiness levels (TRL) of processes by the respective biosurfactant producers.

Acknowledgements

After seven years of intensive research and development in the Alliance Biosurfactants we look back with gratitude and pride. Together we have succeeded in blurring the boundaries between the fields of biotechnology, chemistry, and engineering and thereby achieving outstanding results. Personally, I am especially thankful for the high level of commitment, dedication, and passion shown by everyone involved. We are all looking forward with great excitement to the next steps toward industrial scale-up and market launch.

We would like to express our special thanks to the German Federal Ministry of Research, Technology and Space – formerly Ministry of Education and Research – for funding the work of our alliance. Without this crucial support we would not have been able to perform this level of excellent research.

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From larvae to biosurfactant: establishing insect lipids as a sustainable source of raw materials

Economical biosurfactant production from domestic, renewable feedstocks and residues using insect lipids as an alternative substrate to boost end-product value, enabled by *Hermetia illucens* larvae cultivated on residues.

Black soldier fly larvae

In 2023, an estimated 58.2 million tons of food were discarded in the European Union due to non-consumption. This equates to approximately 130 kilograms of food waste per inhabitant each year. As natural recyclers, insects can successfully convert organic materials (including former meat and fish products or kitchen waste) that would otherwise be thrown away or sent for recycling. While such materials are not necessarily suitable for other animals used for food production, insect larvae have the unique ability to convert these residues into proteins, lipids and insect residue substrate, which has proven to be an effective soil fertilizer. The circularity of the European insect sector would increase by closing the loop on unused edible resources. However, former foodstuffs of animal origin may not currently be used. According to Article 10(f) of Regulation (EC) No 1069/2009, products of animal origin or foodstuffs containing products of animal origin, which are no longer intended for human consumption for commercial reasons or due to manufacturing problems or packaging defects or other defects and which do not indicate a risk to human or animal health, may not be used as feed substrates, even if they would be used in technical products after the raw material has been extracted. Expanding the range of substrates permitted in insect breeding to include former meat- and fish-containing foodstuffs and subsequent kitchen waste will further improve the footprint of the insect sector while enabling the efficient conversion of such inputs into valuable and sustainable products, allowing the safe reintroduction of valuable nutrients into the feed chain after bioconversion by insects.

The production of insect fat is an innovation that still requires research and development work. As part of the work packages, the entire production cycle for Hermetia raw materials protein and fat was presented. The fatty acid composition and total fat content of *Hermetia illucens* change during the different larval stages. This can be used to control harvest periods in larval breeding and thus the extraction of certain fatty acids. Additional influencing factors are the nutrient components in the substrates, which are decisive for the fatty acid spectrum. Since medium-chain fatty acids are of interest for the formulation of biosurfactants, the larvae should be provided with a balanced supply of nutrients during breeding to ensure optimal growth and development. Within the Innovation Alliance Biosurfactants, the supply of raw materials and scale-up to produce insect fat have been optimized. One of the goals was to identify a cheap, sustainable substrate source with a reproduceable fatty acid spectrum. In addition to substrate analysis, turnover rate, food conversion rate (FCR) and the resulting fatty acid spectrum were evaluated.

The work package focused on optimizing separation for fat extraction. After the larvae have been cultivated, the product is processed from dried larval material. The subsequent mechanical separation into protein meal and insect fat is carried out by means of pressing and heat treatment. Various filtration systems were evaluated for further purification, with pre-qualification using different filter media on a laboratory scale and trials with a bag filter system on a pilot scale. The implementation of fat extraction using a chamber filter

Black soldier fly

> Nature knows no waste.“

Heinrich Katz,
Managing director
Hermetia Baruth GmbH

press for large-scale production was successfully realized within the project period. The produced fat contained mainly (> 50 percent) lauric acid, hence very similar to tropical oils like palm kernel or coconut oil.

In addition, the breeding of insect larvae is being continuously developed. In large-scale breeding processes, it is essential to precisely tailor atmospheric conditions to the specific requirements of each substrate. The aim is to implement additional automation processes that enable precise control depending on emissions and temperature within the breeding facilities without compromising breeding duration or mass growth. Previous trials in the bioreactor have shown that the optimal temperature ranges for maximizing biomass growth are very defined.

In an integrated production plant with modern factory planning methods, process monitoring with the recording of production parameters and their automatic control and regulation plays a decisive role. The mass flows must be controlled and the climate control designed in accordance with the findings from previous projects, both in terms of hardware (fans, supply and exhaust air treatment) and software (parameter control). Product quality, e.g. fatty acid composition, must be determined in real time and incorporated into the process control system as a control variable. Inline control is preferable, as its results are fed directly into the process control system. The determination and definition of target parameters is a prerequisite for this control loop. This requires the process control system to be designed as a learning system.

Conclusion and recommendations

The use of insect lipids from *Hermetia illucens* as a raw material for biosurfactants is technologically feasible and promising both ecologically and economically. Successful industrial implementation however requires modernization of the legal framework, automated, scalable production, and data-driven process management along the entire production chain.

The Innovation Alliance Biosurfactants has laid the major groundwork. Now it is time to translate this into actionable measures that are compatible with policy and industry.

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MEL biosurfactants: fermentation scale-up and optimized purification

Robust fermentation of mannosylerythritol lipids (MEL) from regional lipid substrates with optimized downstream processing and integrated process analytics highlights scalable and sustainability-assessed biosurfactant production routes.

Objective

In the first project phase, several microbial strains and regional lipid substrates were evaluated and the resulting MEL structures determined by advanced analytical techniques like chromatography and mass spectrometry at the Fraunhofer IGB. It was found that strains and substrates influenced the MEL structures in terms of fatty acid pattern and acetylation degree. For the second phase, we focused on two selected microbial strains and aimed to establish robust, efficient and scalable processes for producing the respective MEL structures. Medium composition and growth decoupled fed batch strategies were optimized, and clear scale up criteria were derived and validated in stirred tank reactors up to 75 L. Downstream processing was streamlined, covering dewatering, liquid-liquid extraction and final purification, with reduced solvent demand and simplified routes where feasible. Process analytical technologies from our partners should be integrated for the process, including in situ Raman spectroscopy and soft sensing, to enable real time monitoring and prepare future closed loop control. Continuously updated life cycle and techno economic models aimed to identify environmental hotspots, cost drivers and robust optimization paths, so that the MEL value chain minimizes complexity and resource use while maintaining product quality and scale up readiness.

MEL offers a very broad spectrum of applications due to its structural diversity. In this regard, we have achieved major milestones in process development for robust production."

Dr.-Ing. Susanne Zibek,
Group leader Bioprocess Development, Fraunhofer IGB

Results

Medium optimization by a design of experiments approach identified a growth inhibiting trace element regime while confirming a positive effect of nitrate on growth. Implementing different fed batch strategies increased the cell density and supported an efficient conversion of lipids to MEL during the production phase. Across different reactor scales, the core performance indicators were achieved with a MEL concentration up to 48 g/L and a productivity up to 0.5 g/L·h, depending on the strain and feedstock. Processes were established with rapeseed oil and an insect derived lipid stream.

Downstream processing (DSP) was simplified and intensified. Crossflow microfiltration was implemented as the primary dewatering method, while salt induced phase separation was employed for highly viscous broths to achieve reliable volume reduction. Liquid-liquid extraction conditions were defined to obtain spontaneous phase separation at lowest possible solvent ratios, enabling scalable batch extraction without centrifugation. Adsorptive purification on silica was scaled to higher loadings with markedly lower solvent use per gram of product. Depending on the route, the purity of MEL after DSP reached 94–99 percent. Respective samples of these purified MELs up to a total amount of > 5 kg were supplied to our partners for application testing.

Process analytical technologies and automation delivered by our partners increased the stability and transparency of fermentation. In situ Raman spectroscopy was able to track substrates and products, although harmonized measurement parameters were identified as key for a reliable model transfer. Foam level and headspace pressure sensors supported control in foaming regimes. A soft sensor for real time estimation of biomass and substrates during growth was implemented, and its extension to the production phase is underway to enable model assisted feed control. A life cycle assessment framework by our partner, coupled to our kinetic models, identified environmental hotspots across fermentation and DSP and guided the choices on solvent loops and energy input. In parallel, a techno economic model with a 10 m³ reference case – including solvent recycling and staggered fermenter operation – mapped the dominant cost drivers and evaluated future scale up scenarios.

Left: Sampling of MEL fermentation broth from a 75-L-bioreactor. Right: Purified mannosylerythritol lipid for application testing

Conclusion and recommendations

By combining fermentation scale up, intensified downstream processing and integrated analytics, a viable pathway to high purity MEL from regional feedstocks has been established. The derived performance indicators together with the sustainability and economic models, demonstrate clear priorities for the next development stages.

For the next scale up steps, targeted funding is required to realize integrated demonstration runs in the 100–1000 L range with complete downstream processing, solvent recovery loops and energy integration. These experiments should validate scale dependent control strategies for foaming and mass transfer, confirm the established KPIs under industrially relevant conditions, and generate high quality datasets that continuously feed back into the life cycle and techno economic models. Process intensification should prioritize chromatography free routes wherever specifications allow, implement efficient solvent recycling, and define acceptance criteria for crude MEL to minimize unit operations and bring down costs. LCA and TEE models should be continuously updated with process changes so that equipment, utilities and operating strategies are guided by quantified environmental and cost impacts.

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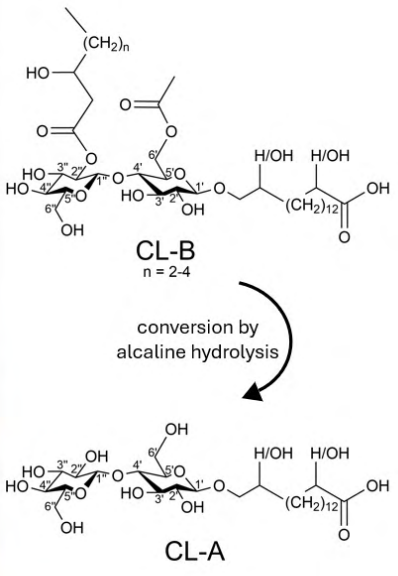
Integrated foam fractionation for efficient cellobiose lipid (CL) production

An integrated strategy coupling robust fermentation with engineered foam fractionation turns foam formation into an in situ separation lever and accelerates a pathway for scalable cellobiose lipids production processes.

Objective

The main objective of the IGVP (University of Stuttgart) was to establish a robust, high productivity production process for cellobiose lipids (CL) using two fungal producers (one strain yielding only CL and another co producing minor parts of MEL) that were selected based on results from the first project phase. We optimized culture medium and feeding, quantified oxygen uptake rates, and performed scale up from 10 to 1000 L. Another central goal was in situ product removal via engineered foam fractionation to maximize CL recovery, supported by automation and sensor integration. The downstream process – which consists of several solid-liquid separation steps: precipitation, washing and solvent extraction – should be optimized by defining minimum solvent ratios for extraction and suitable pH windows. Moreover, we compared different scalable separation technologies. In collaboration with our partner IABP, life-cycle analysis (LCA) and techno economic evaluation (TEE) guided choices to lower energy and material use.

Purified and dried CL as a white powder, presented together with the structural formulas of the CL variants: CL-A can be synthesized from CL-B via alkaline hydrolysis.

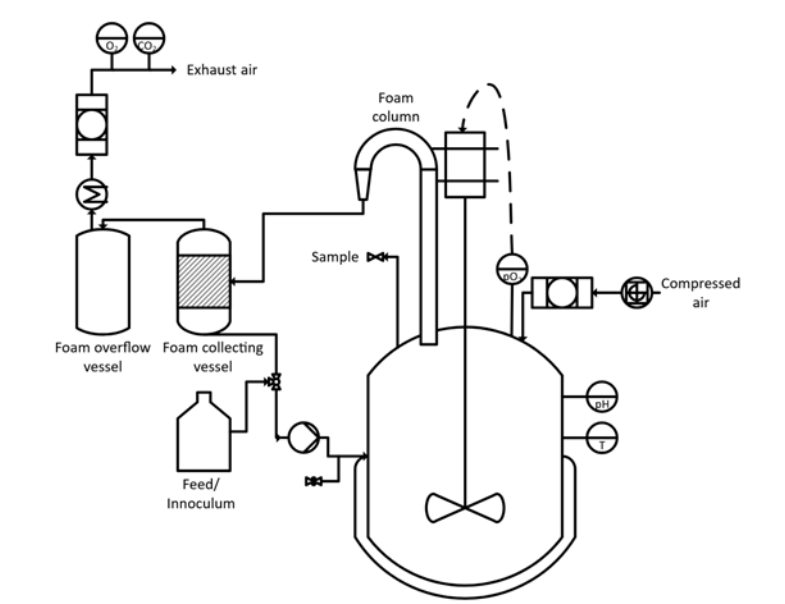


Results

We successfully established a stable fermentation process with an optimized mineral salt medium, where an additional feeding of carbon and nitrogen sources increased both biomass and CL concentration. Oxygen transfer analysis defined a practicable operating window for stirring and aeration, and we found out that short term O_2 and pH shifts were tolerated without performance loss. Scale up was demonstrated at 10, 42 and 1000 L. Lab and pilot runs achieved CL titers above 30 g/L, while a 1000 L campaign highlighted morphology control as a key factor for maintaining substrate uptake and production rates.

Automated aeration with modular ceramic spargers from our project partner maintained dissolved oxygen at markedly lower gas flows than ring spargers, directly targeting a major energy hotspot. Foam fractionation turned excessive foam formation from a previous problem into an advantage: Integration of a foam column enabled very high enrichment and essentially complete recovery of CL from broth and deposits, with only minimal biomass loss. Sensor assisted foam handling (impedance probes with online back flush via foamate recycle) provided reliable foam level signals for adaptive aeration and safe bioreactor operation. Raman models were able to track key media components. A short "burst foaming" step at the end of the fermentation further reduced residual CL in broth, highlighting a simple method to facilitate downstream processing.

In downstream processing, we established robust purification routes for production of two different CL structures. For the native structure CL B, a single acidic wash removed residual sugars effectively, and extraction was completed in one step when the ethanol fraction in the extractant was kept above 60 percent. This aligned well with pre-concentrating the broth using a disc-stack centrifuge or a filter dryer so that the residual water content before extraction was low enough. For the modified structure CL A, alkaline hydrolysis at pH 12 proved effective either directly from the broth or via post modification of purified CL B. Delivering CL A as alkaline solution or wet acidic suspension simplified formulation by our application partners and eliminated multiple unit operations. Respective samples of both CL-B and CL-A were supplied to our partners for application testing.



Schematic representation of a system for foam fractionation in CL processes with an integrated foam column and the recirculation of the foamate into the bioreactor.

Conclusion and recommendations

Our results demonstrate a coherent pathway in which engineered foam fractionation is key for a stable process. The next development phase should be enabled by targeted public co funding for pressure stable, hard piped foam fractionation lines at pilot scale with automated aeration and respective sensors, to validate recovery/enrichment targets and generate robust and reliable datasets. Funding should also prioritize energy and solvent efficient downstream processes as well as digital twins that couple inline Raman and soft sensors with live LCA/TEE to guide bioreactor operations. Finally, standardization/regulatory work for both production strains, including quality specifications for alkaline CL A solutions and wet acidic suspensions needs to be addressed.

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From microbes to markets: surfactin for a sustainable future

Bioprocess engineering enabled a record surfactin titer delivering high-quality biosurfactants for industrial application studies in the project.

Objective

At the University of Hohenheim, the objective within the Innovation Alliance Biosurfactants project was to develop robust processes for the production of surfactin, a cyclic lipopeptide biosurfactant naturally produced by *Bacillus subtilis*. This included the targeted adaption of medium composition and feeding strategies, combined with the integration of new sensor and automation technologies. In parallel, biological performance indicators such as yields, productivity and space-time-yield were systematically determined to provide a solid basis for process evaluation and to ensure the production of larger sample quantities for downstream studies. While the fermentation focused on achieving high titers and space-time yields, the downstream processing aimed at developing efficient separation and purification methods for surfactin from complex fermentation broths. Various approaches were investigated to identify scalable methods capable of supplying project partners with high-quality sample material. Additionally, different drying techniques were compared to provide surfactin in a stable, powder form suitable for application testing.

Fermentation setup at the University of Hohenheim (Department of Bioprocess Engineering) showing three 42 L and one 16 L bioreactor. Taken on December 4, 2015, by M. Zentsch.

Results

The process optimization study at the University of Hohenheim focused on identifying the most efficient feeding strategy for producing surfactin in *Bacillus subtilis*. A series of fed-batch cultivations were performed using the bioreactor pipeline (see photo below), in which the bioprocess was systematically varied. The aim was to investigate the relationship between biomass formation, substrate conversion and surfactin productivity. The results showed a balanced metabolism with minimal overflow effects and led to very high product titers. Lower and higher feeding growth rates resulted in reduced productivity, due to nutrient limitation and acetate accumulation respectively. These fermentation data formed the basis for developing a mechanistic process model. A kinetic fed-batch model was implemented to describe the dynamic interaction between biomass, glucose, and surfactin. This model was subsequently expanded to incorporate key inhibitory by-products, enabling accurate prediction of metabolic behavior across various feeding regimes. A new process design was then simulated using this validated model, in which the conventional batch phase was omitted and feeding began immediately after inoculation. This model-based approach enabled more consistent process control, reduced cultivation time, and achieved a surfactin concentration of 46 g/L triplicating the space-time yield. This represents a significant improvement compared

to previous setups. Overall, the combination of experimental optimization and model-based process design established a robust and scalable strategy for industrial surfactin production within the Innovation Alliance Biosurfactants framework.

Conclusion and recommendations

Public funding for the development of surfactin and biosurfactants in general has proven to be essential. The fundamental problem that applies to all biosurfactants is that, in the initial phase, only relatively expensive and small sample quantities are available, meaning that application research does not appear to be economically viable. As a result, demand is minimal and it is uneconomical to establish the production of larger sample quantities. However, since biotechnological development and sample provision are relatively expensive, this negative feedback loop must be broken through public funding if environmentally friendly and climate-neutral biosurfactants are to be economically successful. Two positive examples where this has been achieved with the help of public funding are sophorolipids and rhamnolipids.

Purified surfactin obtained from the cultivation of Bacillus subtilis. Taken on July 10, 2024, by A. Hermann.

Specifically, for surfactin, the results show how important it is to combine genetic strain development, which was not funded here, with process engineering data and digital process development in order to achieve robust and scalable production of surfactin. The next step for industrial implementation is to transfer the optimized surfactin process to pilot scale, together with advanced sensor integration and automated control for real-time decision-making. Improving data connectivity between fermentation and downstream processes will further increase process reliability and shorten development time.

Working on biosurfactants today feels like building the internet in 1995.”

Prof. Dr.-Ing. Rudolf Hausmann,
Head of the Department of Bioprocess Engineering, University of Hohenheim

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Optimization and scale-up of the enzymatic synthesis of xylitol and sorbitol lipids

Objective

The KIT subproject aimed to establish new sustainable and scalable processes for the enzymatic synthesis of tailor-made glycolipid biosurfactants. Products and reaction media should be non-toxic, mild, and biodegradable. The enzymatic syntheses should be carried out both in "green" organic solvents and in deep eutectic solvents (DES) previously prepared easily from inexpensive plant-based raw materials.

Results

After extensive preliminary investigations, KIT focused on the scale-up of xylitol and sorbitol lipid synthesis reactions with C12 and C18 fatty acids using the immobilized lipase Novozym 435, up to batch sizes of 100 g, including subsequent product processing. Significant progress was made in syntheses in organic solvents using various novel reactor concepts, such as a rotating bed reactor (RBR) and a controllable microwave reactor (MWR).

In various DES, rapid and virtually by-product-free syntheses were achieved both by establishing a "2-in-1 concept" with the simultaneous use of the sugar alcohol building blocks xylitol and sorbitol as a substrate and component of the DES reaction medium, and also by using a controllable microwave reactor. Rapid preparation of DES in a microwave reactor and the execution of enzymatic syntheses in these media were achieved, alongside the development of downstream processing and purification workflows for the resulting products.

Reproducible and semi-automated protocols were established for the isolation of the products from the reaction media and their subsequent chromatographic purification using medium-pressure chromatography. These protocols enable high product yields and allow the reuse of solvents, chromatography materials, and columns, including extraction with "green" solvents and their subsequent successful recycling.

Conclusion and recommendations

The scalable synthesis processes developed at KIT – all published in detail – provide potential users in a wide variety of application areas with a "toolbox" for the enzyme-catalyzed synthesis of glycolipids in both organic solvents and DES. For future industrial applications, this provides a new, broadly applicable and environmentally friendly process for the sustainable production of a wide spectrum of novel surface- and interface-active glycolipid biosurfactants based on renewable raw materials for commercial use in the detergent, cosmetics and pharmaceutical sectors.

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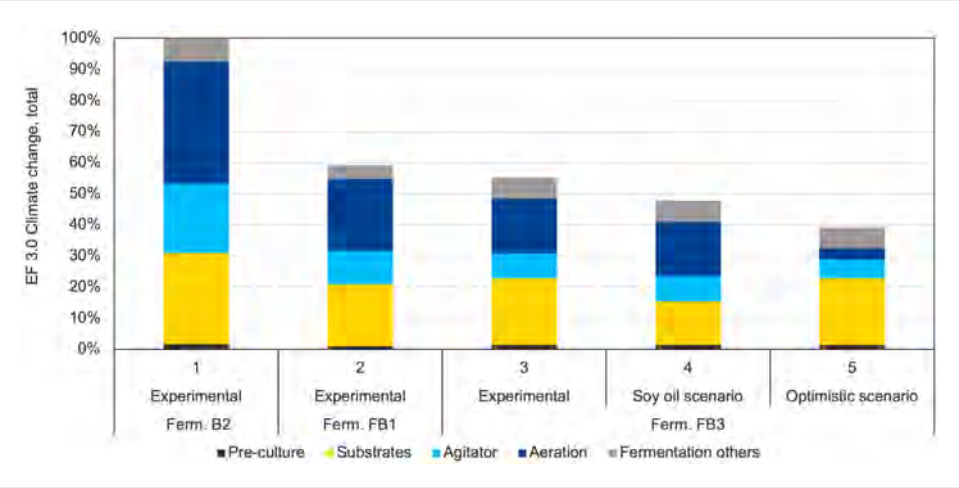
Integrating life cycle assessment in sustainable bioprocess development

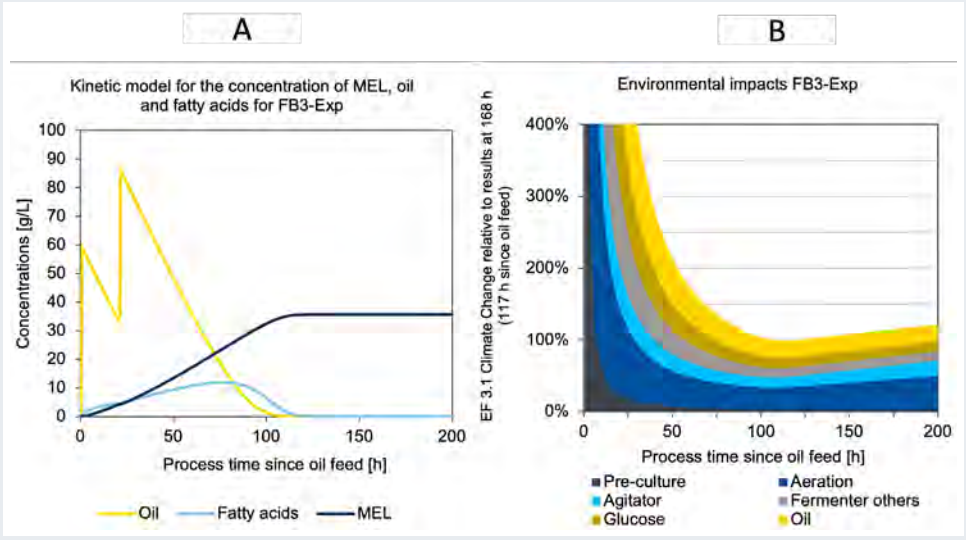
Combining life cycle assessment (LCA) with bioprocess modelling demonstrates significant potential for environmental improvements in the production and potential application of biosurfactants and their substrates.

Objective

Within the Alliance Biosurfactants, the Institute of Acoustics and Building Physics (IABP) at the University of Stuttgart aims to advance the sustainability of biobased products. Drawing on over 35 years of experience in the field of life cycle assessment (LCA), IABP evaluated the environmental impacts of various biosurfactants, particularly mannosylerythritol lipids (MEL) and cellobiose lipids (CL) as well as surfactin and enzymatic produced glycolipids. By integrating LCA into the development process, the project identified key areas for improvement and optimization across different substrates and production methods.



Results of scenario analyses for MEL fermentation in the impact category climate change. The results are normalised to scenario 1, representing the fermentation of MEL in a batch process at the beginning of the project. Scenarios 2 and 3 represent follow-up experimental fermentations (FB1 and FB3). They are characterised by improved fed-batch process control and achieve higher product concentrations. Furthermore, various substrate sources were investigated, one of which is represented in scenario 4, where rapeseed oil used for the experiments is substituted with soybean oil. Scenario 5 describes an optimistic outlook with yet realistic assumptions for future process optimisation based on experiment FB3. B = batch, FB = fed-batch.



Monod kinetics for the concentration of MEL, oil and fatty acids (A) and the potential environmental impact in category climate change, total (B) over the process time of the production phase of the MEL fermentation

Results

The project involved close collaboration with various research institutions and industry partners to utilize experimental data and expert knowledge regarding scale-up assumptions for the LCA models.

The LCA results for MEL and CL revealed that key contributors to the environmental impacts of biosurfactants include substrate provision and energy demand, particularly during fermentation and purification processes. Significant reduction potentials were identified – over 40 percent – through various optimizations in these areas, including the use of renewable energy sources and alternative substrates, some of which have already been achieved during the project's duration. Additionally, dynamic analyses were conducted by combining LCA and bioprocess modelling in a new approach, providing deeper insights into process optimization and sustainability. Furthermore, various purification routes for MEL and CL were evaluated. This way, levers to improve the environmental impacts from solvent and energy use were identified.

LCA screenings for surfactin production identified comparable hotspots to MEL and CL, specifically substrates, electricity used in fermentation, and purification. For enzymatically produced glycolipids the substrate provision was the primary impact factor, while the synthesis and purification steps showed a comparably lower contribution.

Building on the LCA of biosurfactant production, three potential applications for biosurfactants were analysed to assess the influence on the overall application formulation, as well as covering the life cycle stages for use and end of life. The assessment covers a laundry detergent, a cosmetic cream and a lubricant emulsion, providing insights into the application-specific hotspots and links them to the biosurfactant use in the products.

Figure 1 (page 15) is from the following publication (translated): Bippus, L.; Briem, A.-K.; Beck, A.; Zibek, S.; Albrecht, S. (2024): Ökobilanz für die Bioprozessoptimierung – Herstellung des Biotensids MEL. Biospektrum 30, 714–717 (2024). https://doi.org/10.1007/s12268-024-2305-8

Figure 2 (page 16) is from the following publication: Bippus, L.; Briem, A.-K.; Beck, A.; Zibek, S.; Albrecht, S. (2024) Life cycle assessment for early-stage process optimization of microbial biosurfactant production using kinetic models - a case study on mannosylerythritol lipids (MEL). Front. Bioeng. Biotechnol. 12:1347452. https://doi.org/10.3389/fbioe.2024.1347452

Next to the biosurfactant production and potential application, possible substrates were studied, with a special focus on insect oil from *Hermetia illucens* serving as an alternative oil source. The LCA for Hermetia oil demonstrated significant environmental benefits when utilizing organic waste as a substrate. This approach not only reduces greenhouse gas emissions but also capitalizes on waste valorisation, highlighting the potential for sustainable bioprocessing in the production of insect-based oils.

Scientific dissemination was advanced through multiple publications detailing the LCA results, reinforcing the critical importance of continuous updates to LCA models based on new experimental data and eventually industry data. The findings emphasize the necessity of integrating sustainability metrics in product and process development to achieve ecological optimization.

Conclusions and recommendations

The project results highlight the essential role of life cycle assessment as a guiding tool in sustainable bioprocess development. By identifying environmental hotspots and optimization potentials, LCA enables informed decision-making among researchers and industry partners. The collaboration between various institutions fosters innovation and enhances the ecological sustainability of biotechnological products. Future efforts will focus on refining LCA models and exploring additional biobased materials to further mitigate environmental impacts. Methodological work will continue, incorporating dynamic approaches and investigating more process-integrated, digitized methods.

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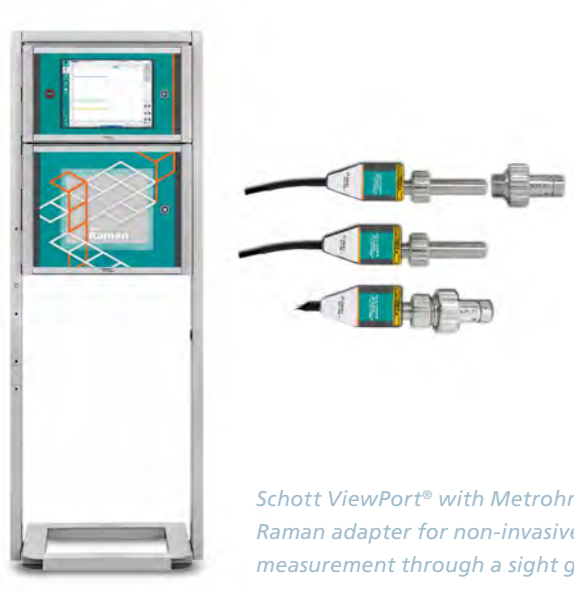
Inline Raman spectroscopy for monitoring multiple bioprocess reactors

Metrohm developed an inline Raman analytics for real-time monitoring of the productivity and yield of the biosurfactants MEL and CL – from raw material control to large-scale production.

Objective

As part of the project, an inline Raman analytics system is being developed and integrated into biotechnological processes to enable real-time monitoring of the fermentation of the biosurfactants mannosylerythritol lipid (MEL) and cellobiose lipid (CL). The focus is on precise monitoring of the fermentation process to determine biomass concentration. This enables targeted process optimization, such as the development of suitable feeding strategies during the growth phase and the increase of product formation rates during the production phase. Furthermore, Raman spectroscopy is used as a selective method to efficiently monitor, optimize, and accelerate scale-up processes. Another key aspect is the development of robust, process-stable sensor technology and the transfer of laboratory-based Raman insights into industrial applications.



Schott ViewPort® with Metrohm Raman adapter for non-invasive online measurement through a sight glass

Results

For the experiments, a Metrohm Raman Analyzer and various immersion probes – suitable for in-situ analyses in both challenging process environments and biological samples – were used. The probes were integrated into different bioreactors of varying volumes.

The results confirm the detectability of substrates and biosurfactants in real fermentation media. The spectral selectivity enables precise concentration tracking and forms the basis for developing robust real-time analytics.

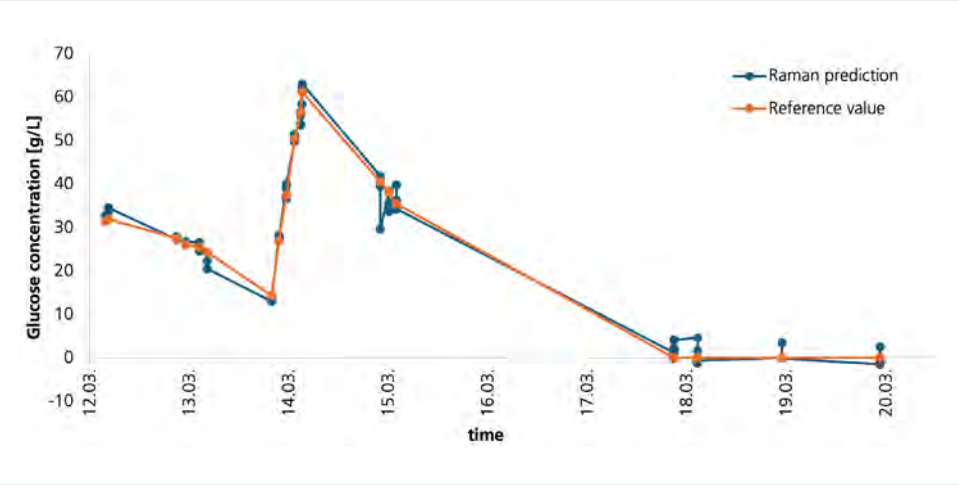
Furthermore, inline monitoring of biosurfactants in real fermentation media was implemented. The goal was to develop spectrally selective inline concentration tracking that eliminates the need for labor-intensive sampling and reference analytics.

Throughout the project, various fermentation batches have been successfully monitored in both laboratory reactors and at the 42 L scale with the partners Fraunhofer IGB and IGVP of the University of Stuttgart. This demonstrated the scalability from lab scale to pilot plant. A particular challenge was the fluorescence that overlapped relevant information in certain spectral regions. For each investigated process parameter, individual models with different data preprocessing methods were evaluated – including Metrohm's proprietary XTR fluorescence suppression.

To improve plug-and-play capability, a special probe adapter, the SCHOTT ViewPort® (see figure on the right) was developed alongside

an immersion probe, enabling fully non-invasive analytics. Using the adapter typically eliminates the need for re-certification of measurement points. It also ensures high flexibility regarding reactor size, wall thickness, and material.

Robust calibration models for predicting all process-relevant parameters were created for both CL and MEL fermentations. The figure below shows an example trend for the parameter glucose over the entire fermentation period compared to the reference values.



Concentration profile of the parameter glucose over the entire fermentation period

Conclusion and recommendations

Within the framework of the project, precise monitoring of the fermentation process for determining biomass concentration was successfully established, confirming the potential of Raman-based real-time analytics for process monitoring in fermenters. Raman spectroscopy, combined with robust and process-ready sensor technology, proved to be a powerful and selective technique for efficiently monitoring, optimizing, and accelerating scale-up processes. Despite varying process conditions – such as different shear forces, flow rates, fermentation parameters, and hardware configurations – robust and reliable calibration models were successfully developed for various fermentation batches.

Offline techniques, such as classical laboratory analytics, require time-consuming and labor-intensive manual sampling and preparation. Modern methods like Raman spectroscopy enable chemical-free and non-destructive real-time monitoring of fermenters. Future development aims to capture as many fermentation processes as possible to expand the models and increase their robustness. This will make automated process control based on model-driven predictions increasingly feasible.

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Innovative automation of bioprocesses

Soft sensing, aeration, and process control: New solutions for increased productivity in biotechnological production

Process Control

The goal of the sub-project BioBasics was to transform and improve the automation of a 30-liter stainless steel bioreactor at the faculty of Bioprocess Engineering of the University of Hohenheim in its entirety. This involved eliminating software bugs, extending functionalities, simplifying the human-machine interface, and making process parameters and data more accessible to operators.

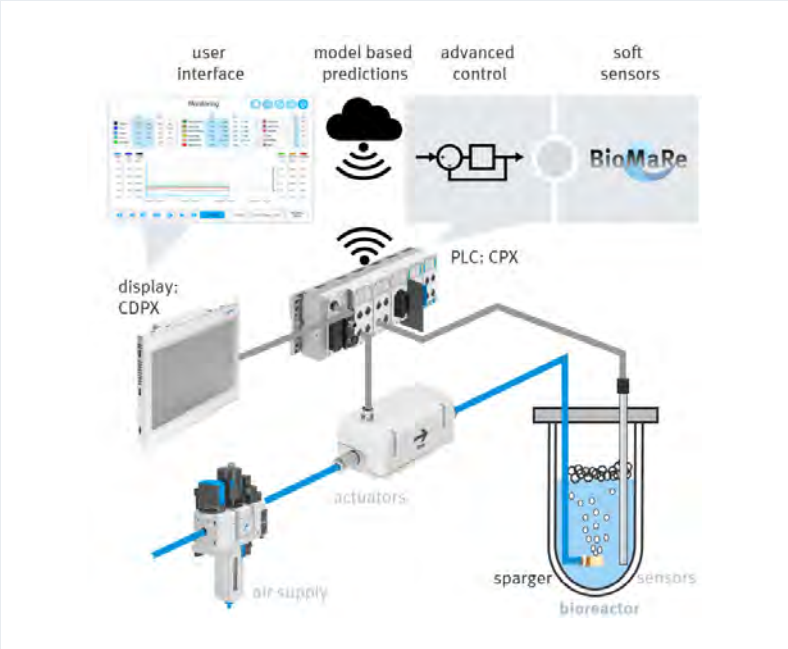
To achieve these software improvements, numerous hardware components within the control cabinet were replaced with Festo components. This upgrade enabled access to all sensor data and control over all actuators. Key replacements included the central programmable logic controller (PLC), the main user interface display, motor controllers, and remote input units for sensor connections (Remote I/O).

With hardware access established, a new software stack was implemented. Significant improvements included:

- Visualization and logging of sensor and actuator data
- Cascaded control of dissolved oxygen
- Linear and exponential feeding intervals
- Access to all variables via OPC UA

Thanks to the BioBasics automation system, cultivation processes can now be executed with greater efficiency and reliability. Usability has improved, and process understanding has significantly advanced.

Bioprocess automation domains addressed by Festo in scope of the Alliance Biosurfactants.



Soft Sensing

The BioMaRe sub-project focused on advancing model-based soft-sensing to estimate biomass concentration during cultivation, emphasizing non-substrate-limited phases and integrating an enhanced mechanistic model of the MEL production strain. Building on prior work (Beck et al., 2022), the model was extended and adapted for soft-sensor use. A key goal was automating a pulse-wise oil feed triggered by exceeding a biomass threshold to induce product formation.

The soft-sensor estimates biomass from online data including gas flow, feed rates, reactor volume, and exhaust gas composition. Feed rates were calculated from weight signals using numerical differentiation with Kalman filtering. The model uses differential balances

Coarse bubbles produced by conventional stainless-steel tubes (left) versus fine bubbles produced by porous materials (right) for the aeration of culture broth.

New porous materials offer a huge efficiency boost in aeration!”

Dr. Daniel Wibbling

for biomass, substrate, nitrogen, fatty acids, oil, product (MEL), and biomass subtypes.

Validation against offline data showed accurate biomass and substrate tracking, with deviations mainly due to oil affecting biomass dry weight measurements. Product concentration prediction performed well in some cases but had higher errors due to the model’s open-loop design lacking real-time correction.

Overall, the BioMaRe soft-sensor enables effective online biomass estimation and process automation, supporting improved control of fed-batch fermentations for MEL production.

Aeration

The aim of this sub-project was to evaluate various porous aeration modules to achieve optimized gas input in the bioreactor, a crucial step in the cultivation of microorganisms. A total of 15 elements made of three different materials ceramic, stainless steel, and polyethylene were tested.

The results showed that the porous elements with the highest overall efficiency of gas-liquid transfer (kLa) values, compared to the standard elements used in conventional bioreactors, resulted in 63–121 percent higher kLa values at low stirrer speeds below 300 rpm. This clearly improved performance was confirmed in field trials with *Escherichia coli* K12. After a cultivation period of seven hours, the three porous elements showed three times the biomass of the non-porous standard element. While the standard element contributed to oxygen limitation after just two hours, the porous elements never showed a dissolved oxygen (DO) value below 15 percent. One of the elements even maintained a DO value of over 70 percent throughout the entire cultivation period.

The cultivation data were subsequently evaluated within the framework of Environmental Footprint 3.1, which resulted in a CO_2 footprint being calculated over a service life of 500 cultivations. Here, too, the CO_2 consumption for the standard element was found to be more than twice as high regarding the energy consumption of the bioreactor, the stirrer, and the compressed air supply.

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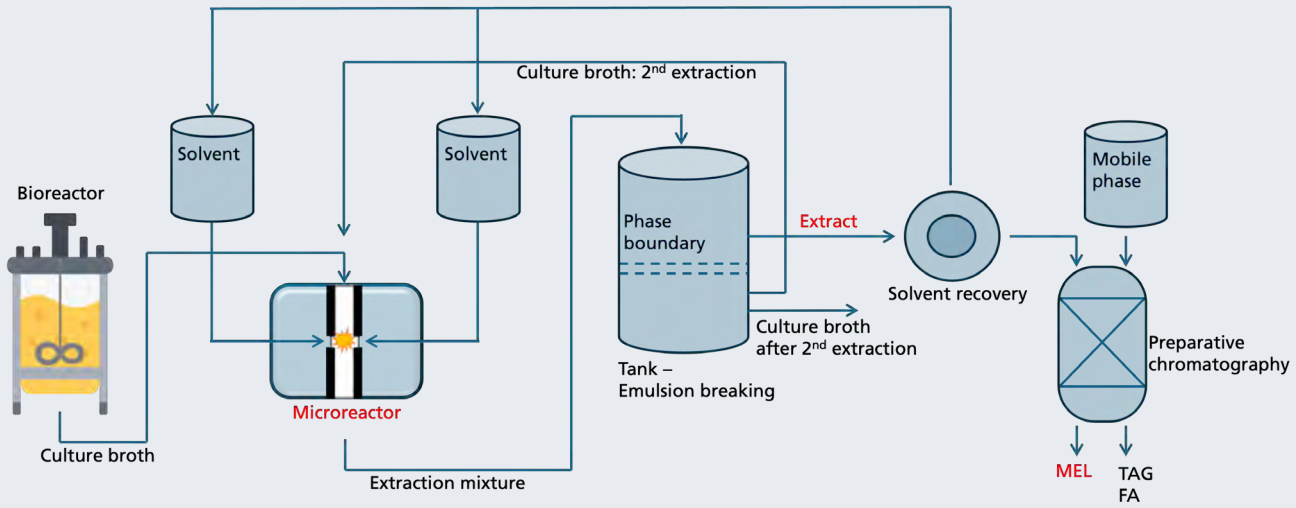
Efficient extraction using microreactor technology

A novel tailored downstream process for extracting mannosylerythritol lipids (MELs) from fermentation broth was developed. Therefore the scalable continuous microreactor technology was implemented and adapted for extraction.

Objective

The surfactant market is currently dominated by petrochemically and oleochemically produced products. Biosurfactants offer an environmentally friendly alternative, but scalable processes enabling cost-effective production beyond lab scale have been lacking.

Within the Innovation Alliance Biosurfactants, MyBiotech GmbH aimed to further develop and integrate its patented microjet reactor technology into the downstream processing of biosurfactants – particularly MELs – from fermentation broths. Continuous extraction was intended to improve yield, sustainability, and cost-efficiency of MEL production and enable industrial scalability.



Process flow diagram of the developed procedure for processing MELs from fermentation broth. TAG = "Triacylglycerides", FA = "Fatty acid"

> **Continuous microreactor extraction combines efficiency with sustainability and scalability – one step toward industrial bioproduction."**
>
> **Dr. Daniela Becher,**
> Head of R&D Biotech, MyBiotech GmbH

Results

Initial trials extracting MELs from fermentation broths supplied by project partner Fraunhofer IGB showed that microjet reactor technology enabled markedly more efficient mixing and thus higher extract quantities compared to previously used extraction methods. The yield of extracts obtained via microjet reactor was about ten percent higher than the reference extraction, while the proportion of desired MELs remained constant.

One of the greatest challenges was extensive emulsion formation during extraction, which initially required numerous centrifugation steps. Targeted process analyses and a systematic Design of Experiments (DoE) approach enabled identification and optimization of the decisive parameters. This initially made it possible to reduce the number of necessary centrifugations from twenty to four. A subsequent optimization round ultimately established a centrifugation-free process. In parallel, the influence of biomass composition on downstream processing was investigated. Tailored fine-tuning of the final downstream parameters following finalization of the optimized bioprocess can thus significantly reduce raw material consumption.

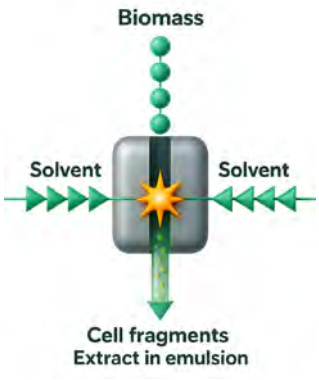
Evaluating different microjet reactor setups further increased yield, simplified handling, and improved tolerance towards precipitates present in the fermentation medium.

In a final demonstration, the process chain for the downstream processing of five liters of fermentation broth was successfully implemented. The expected productivity and process stability were confirmed at the scaled-up level. A sustainability assessment conducted by the Institute for Acoustics and Building Physics (IABP) at the University of Stuttgart showed an improved environmental footprint per kilogram of product, driven by the increased yield.

Conclusion and recommendations

The results demonstrate the high potential of the microreactor technology for biotechnological extraction processes. The combination of increased efficiency, sustainability, and straightforward scalability makes it a future-ready solution for industrial biotechnological applications.

The interdisciplinary Innovation Alliance Biosurfactants has already taken a major step toward market readiness of biobased surfactants. For biosurfactants to reach their full market potential, however, targeted political and economic frameworks are needed. Funding programs should support industrial scaling and the construction of biobased production infrastructure. Clear market incentives – such as sustainability labels or green procurement criteria – can strengthen demand. Only coordinated action by all stakeholders will enable a successful transition to a biobased, resource-efficient economy.



Operating principle of extraction using continuous microreactor technology.

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Inline measurement of foam density for control of CL fractionation

In the cellobiose lipid (CL) process, foam formation is deliberately exploited for in situ product removal. The approach combines dielectric impedance spectroscopy with a cleaned LMT sensor tip to measure foam height and foam density inline and to control aeration for stable foam fractionation.

In industrial bioprocessing, foam is typically classified as a process disturbance. Standard foam control relies on chemical antifoams or mechanical foam breakers to prevent product loss and filter contamination. For microbial production of cellobiose lipids (CL), however, this is counterproductive, as CL accumulate at the gas-liquid interface. Targeted foam fractionation enables in situ product removal as a first purification step and requires active control of the foam phase.

Foam formation is primarily driven by the aeration rate, which must meet the oxygen demand of the biomass while simultaneously optimizing foam height and density for fractionation. Adaptive control therefore depends on real-time data. Optical measurement techniques are unsuitable because of the strong adhesion tendency of cellobiose lipids.

The concept uses dielectric characterization by impedance spectroscopy. The measurement

Bioreactor headplate after CL fermentation with persistent foam deposits Source: Amira Oraby

principle exploits the difference in relative permittivity εr between the gas phase (air: εr ≈ 1) and the liquid phase (medium: εr > 50). The effective permittivity of the foam correlates with the liquid volume fraction and thus with the foam density.

Technically, an ifm limit level sensor of the LMT series is employed. The sensor operates with a frequency sweep (50–200 MHz) and extends up to 25 cm into the reactor, depending on design. The sensor tip (sensitive zone approx. 11 mm) couples an electromagnetic field into the medium. Evaluation of the impedance change enables detection of the phase transition and determination of foam density. The system reliably distinguishes between gas phase and wet foam but reaches its physical limit with very dry foams of low liquid content.

A key challenge is the formation of static, highly viscous foam deposits on reactor surfaces. These deposits cause fouling of the sensor tip and attenuate the signal, which hampers detection of the process foam. To counter this, an integrated cleaning strategy was implemented: the fractionated foam is collected externally, and the liquid recovered by phase separation is pumped back into the reactor.

The return stream is guided through a pipe oriented towards the sensor tip. The volumetric flow continuously rinses the surface; despite limited pressure, this is sufficient to suppress deposits. During phases of high foam production, the increased recirculation volume further enhances self-cleaning.

Validation runs under variable aeration confirmed a consistent correlation between aeration rate, foam density, and sensor signal. The combination of dielectric measurement and hydrodynamic cleaning enables robust inline monitoring and provides the basis for automated control of foam fractionation.

LMT105 sensor during test run (sensitive zone in beige polymer), flushed with clear water to simulate inline cleaning
Source: Amira Oraby

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Biosurfactants in laundry detergents – sustainable innovation at Henkel

Henkel investigated the use of Biosurfactants in laundry detergents. MEL demonstrated promising cleaning performance when combined with fast-adsorbing surfactants. The results highlight the potential of sustainable alternatives to fossil-based surfactants such as LAS.

Objective

As part of the second phase of the Innovation Alliance Biosurfactants, Henkel aimed to systematically evaluate the application potential of biotechnologically produced biosurfactants in laundry detergent formulations. The focus was placed on mannosylerythritol lipid (MEL), which, due to its hydrophobic properties, appeared particularly promising for the removal of fat-based stains.

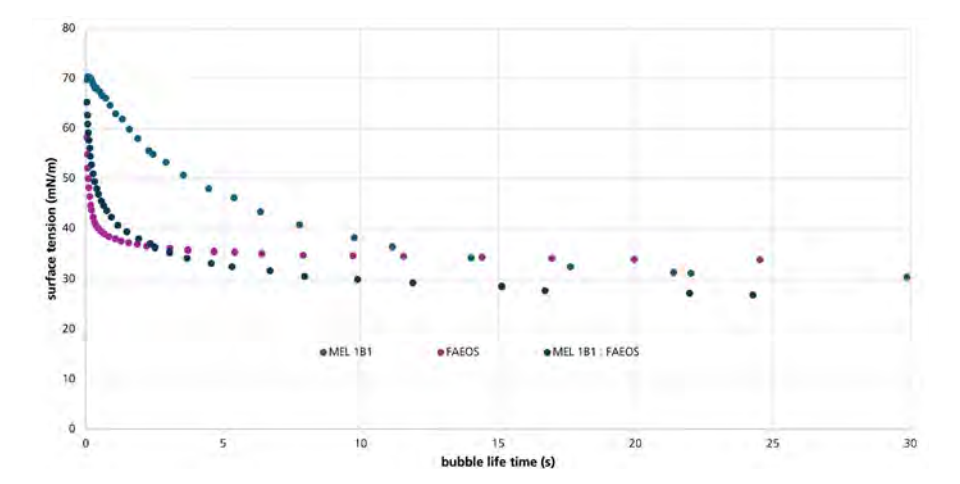
A key aspect of the study was the comprehensive physicochemical characterization of MEL variants. The goal was to understand the structural and surface-active properties of MEL through analyses such as interfacial tension, phase behavior, and adsorption kinetics, and to identify suitable surfactant partners that could generate synergistic effects when combined with MEL.

The identified surfactant combinations were initially screened in miniature wash tests to assess their cleaning performance. Once larger sample quantities became available, the most promising formulations were further validated in real wash trials using standardized stain sets. Additionally, the storage stability of the developed formulations was examined under various temperature conditions.

Henkel's overarching goal was to develop innovative surfactant systems that deliver both high cleaning performance and improved sustainability compared to conventional, fossil-based surfactants like LAS (linear alkylbenzene sulfonates).

MEL is not a drop-in surfactant – but with the right combination, it nearly matches the performance of conventional systems and opens new pathways for sustainable product development."

Peter Schmiedel,
Head of R&D Material Science at Henkel Consumer Brands



Dynamic surface tension versus bubble life time for MEL, FAEOS and a mixture of both.

Results

Henkel demonstrated that MEL has strong potential for use in laundry detergents, especially when combined with suitable surfactant partners. Physicochemical analyses revealed that MEL is more hydrophobic than conventional surfactants, making it well-suited for removing fatty soils. However, MEL also showed slow adsorption kinetics at interfaces, which limits its standalone performance.

Consequently, MEL alone did not achieve sufficient cleaning results. Only through targeted combinations with fast-adsorbing anionic surfactants such as FAEOS (fatty alcohol ethoxy sulfates) or SMCT (sodium methyl cocoyl taurate) synergistic effects could be achieved, accelerating adsorption and significantly improving cleaning performance on greasy stains.

These findings were first obtained in miniature wash tests (50 mL wash liquor) and later confirmed in full-scale wash trials using larger sample volumes. Notably, the surfactant system composed of 33 percent MEL, 33 percent FAEOS, and 33 percent FAEO (fatty alcohol ethoxylate) delivered cleaning performance comparable to the LAS-based market reference – a major step forward in the development of sustainable alternatives.

Storage tests under various temperature conditions revealed thermal instabilities, including pH shifts and phase separation. These results emphasize the need for further optimization of the detergent formulations to ensure long-term product stability.

Conclusion and Recommendations

The findings from the Innovation Alliance Biosurfactants confirm that MEL-based surfactant systems hold significant promise for use in sustainable laundry detergent formulations. In particular, combinations with conventional, fast-adsorbing surfactants such as FAEOS and FAEO achieved cleaning performance on par with LAS-based systems. It is important to note, however, that MEL is not suitable as a direct drop-in replacement and requires tailored formulation strategies.

To enable the future use of MEL in household products, the economic viability of its production must be improved. This includes increasing fermentation yields, selecting cost-effective and sustainable raw materials, and simplifying downstream processing. Furthermore, clear consumer benefits must be demonstrated – such as a significantly improved environmental footprint or enhanced skin compatibility. Only if MEL proves to be ecologically, economically, and functionally competitive, it can be established as a viable alternative in mainstream formulations.

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Evaluation of biosurfactants for household cleaners

Dalli assessed biosurfactants for household cleaning, highlighting their potential and limitations compared to conventional petrochemical surfactants.

Objective

Within the project, dalli aimed to evaluate the application potential of biotechnologically produced biosurfactants for use in household cleaning products. Since different cleaner types place different functional demands on surfactants, such as low-foaming behavior in machine dishwashing, strong grease removal in alkaline cleaners, and clarity with low streaking in glass cleaners, the investigation covered a broad range of product categories, including machine dishwashing detergents, manual dishwashing liquids, glass cleaners, degreasers, bathroom cleaners, and all-purpose cleaners.

To address this diversity the evaluation mainly focused on four biosurfactant classes, mannosylerythritollipids (MEL), cellobioselipids (CL), rhamnolipids, and surfactin, selected for their differing solubility, hydrophilic-lipophilic balance and foaming behavior, which are critical for performance across the various cleaner types.

The primary objective was to develop a deeper understanding of the behavior and performance of the biosurfactants in comparison to established petrochemical surfactants. To achieve this, dalli conducted formulation studies to examine physicochemical properties such as incorporation, compatibility, storage stability, and phase behavior. Additionally, application-oriented cleaning tests specific to each product type, commonly used in industrial benchmarking were carried out. These investigations were designed to identify both the potential and the limitations of the biosurfactants in "real-world" cleaning formulations.

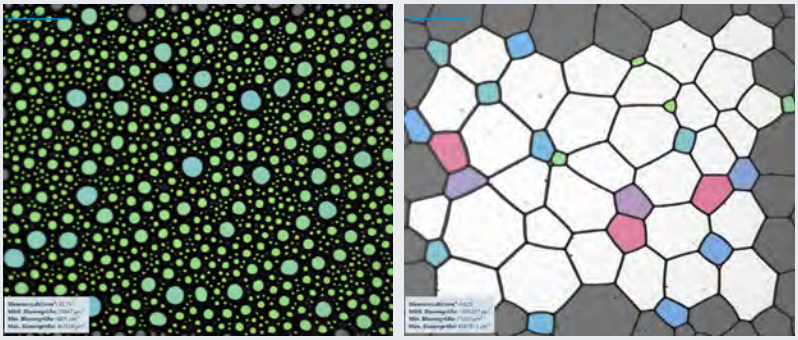
> **"Biosurfactants show functional potential – but meaningful adoption will depend on cost reductions and application-specific optimization."**
>
> **Dr. Stefan Müller**
> Head of R&D Home Care, DALLI-WERKE GmbH & Co. KG

Results

The evaluation demonstrated that MEL, rhamnolipids, CL, and surfactin exhibit distinct behaviors depending on the cleaning application. MEL and rhamnolipids generally showed good incorporation into various formulations and maintained overall stability, whereas surfactin and CL faced challenges with solubility and phase separation in several product types.

Cleaning performance tests revealed application-specific strengths: rhamnolipids performed well in dishwashing applications and glass cleaners, while MEL provided balanced cleaning and foaming properties suitable for multiple cleaner types and proved particularly effective in machine dishwashing applications. CL showed advantages in low-foaming applications, but limited compatibility restricted its broader use. Surfactin exhibited strong foaming potential, but faced challenges with incorporation into certain formulations, limiting its practical applicability.

The results provide a detailed mapping of the strengths and limitations of each biosurfactant, demonstrating that while no single biosurfactant can fully replace conventional surfactants across all household cleaner types, targeted selection and formulation strategies enable competitive performance in specific applications.



MEL: 1 min MEL: 60 min

KRÜSS Foam Analyzer images showing the foam structure of a MEL-containing dishwashing liquid formulation after 1 min and 60 min.

Conclusion and Recommendations

Overall, the results indicate that biosurfactants can complement but not replace conventional petrochemical surfactants in various household cleaners. Their effective application requires careful formulation to ensure incorporation, stability, and performance.

For wider industrial adoption, it is crucial to reduce production costs. This includes optimizing fermentation processes, increasing product yields, and streamlining downstream processing to make biosurfactants economically competitive with conventional surfactants. Scaling up production while maintaining consistent quality will be essential to enable the use of these sustainable surfactants in household cleaning formulations.

TQC Sheen multi-track wiper employed for standardized cleaning performance testing of all-purpose cleaners.

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