

Biosurfactants – Trends and Perspectives

HiPerIn 2.0 White paper

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Cluster Industrial Biotechnology e.V.

CLIB (Cluster Industrial Biotechnology) is an international open innovation cluster of large companies, SMEs, academic institutes and universities, as well as other stakeholders active in biotechnology and bioeconomy as a whole. The cluster comprises over 100 members with a share of about 25 % international members. The overall goal of CLIB is to network stakeholders along and across value chains and to identify new opportunities for innovation, projects, and business. Through this, the cluster develops cross-sectoral biotechnological solutions for sustainable processes and products. CLIB is a non-profit association, with its members shaping the cluster's interests and activities. The cluster coordinates several associated programs which cover different aspects of bioeconomy, and invites members to become involved. To this end, CLIB organises a number of events throughout the year: the annual CLIB international conference (CIC), the CLIB networking day (CND), forum events, topic-specific workshops, dedicated small partnering meetings and visits to partners, sites or meetings in Germany and abroad.

HiPerIn 2.0

HiPerIn 2.0 is a project funded by the Ministry of Economic Affairs Innovation, Digitalization and Energy of the State of North Rhine-Westphalia (MWIDE). HiPerIn 2.0 reflects the rapid change in biotechnology and includes cross-cutting issues which had been identified and validated by CLIB and in an exploratory phase. The increasing digitalisation of biotechnology, the renewed concept of a circular economy, the end-of-life debate, the public perception of biotechnology, and increased regulatory requirements are cross-cutting topics which are of interest to many stakeholders. CLIB pursues the topics of biosurfactants, textiles, flavours and fragrances, and food / alternative proteins. Another focus in the HiPerIn 2.0 project is the support for project consortia and the identification of potential funding lines.

Zusammenfassung

Derzeit werden die meisten synthetischen Tenside aus fossilen Rohstoffen hergestellt. Dieses White Paper behandelt den aktuellen Stand und die Perspektive von Biotensiden, derartige synthetische Tenside zu ersetzen. Mit speziellem Augenmerk auf die nordrhein-westfälische Industrie wurden Schlüsselakteure aus Industrie, KMU und Wissenschaft für diesen Bericht befragt. Neben wirtschaftlichen Aspekten spielen Chancen und Hemmnisse bei der Prozess- und Stammentwicklung eine große Rolle. Ohne öffentliche Unterstützung scheint das Risiko für Chemieunternehmen aktuell noch zu hoch zu sein, neben den etablierten Verfahren einen Produktionsprozess für Biotenside zu skalieren. Um eine nachhaltige Chemieindustrie am Standort NRW zu erhalten und im Sinne einer Biologisierung der Industrie zu fördern, sollte der Staat daher Anreize schaffen, die die wirtschaftliche Produktion und damit den Markteintritt von Biotensiden fördern.

Summary

Currently, most synthetic surfactants are produced from fossil-based feedstocks. This white paper covers the current state and perspective of biosurfactants to replace such synthetic surfactants. Focussing on the industry in North-Rhine Westphalia, key players from industry, SME and academia were interviewed leading to this report. Beside economic aspects, chances and obstacles of process development and strain engineering play a major role. Without public support, the risk seems too high for chemical companies to develop an economical production process for biosurfactants alongside established processes. For ensuring the state's industrial competitiveness in green chemistry, the state should create incentives promoting the economic production and thus the market entry of biosurfactants.

Introduction

Surfactants are amphiphilic molecules composed of a hydrophilic “head” and one or more hydrophobic “tails” (Figure 1). This structure leads to the formation of micelles by multiple surfactant molecules when they are in aqueous solution. The word “surfactant” describes a **surface-active agent** having the ability to emulsify aqueous and non-aqueous liquids. This property makes surfactants attractive for application in detergents, wetting agents, emulsifiers, foaming agents, or dispersants.

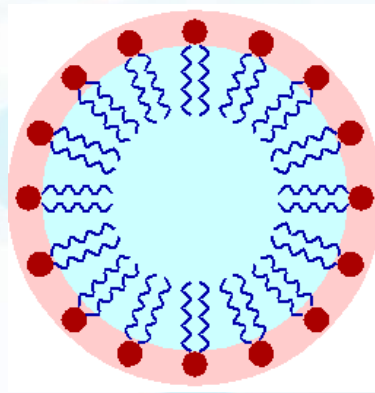


Figure 1: Schematic diagram of a micelle of oil in aqueous suspension, such as might occur in an emulsion of oil in water. In this example, the surfactant molecules' oil-soluble tails project into the oil (blue), while the water-soluble ends remain in contact with the water phase (red) (Gilbert, 2020).

The hydrophobic tail of surfactants is mostly composed of hydrocarbon chains that can be linear, branched, or aromatic. Based on the polarity of the hydrophilic heads, surfactants can be classified into four groups (anionic, cationic, zwitterionic and non-ionic).

Currently, most surfactants are produced synthetically by chemical processing of fossil-based feedstocks. Some bio-based processes are already established at industrial scale. These use renewable feedstocks like coconut and palm kernel oil for chemical synthesis. Exemplary surfactants produced from bio-based feedstocks are Methyl Ester Ketone (MES) or Alkyl Polyglucoside (APG) (Grand View Research, 2015). However, the produced surfactants are not necessarily biodegradable and compete in their substrates with the food industry. In contrast,

biodegradable surfactants can be consumed by microorganisms once they enter the environment. This property becomes especially relevant for applications where surfactants can accumulate in the ground water, rivers, or the sea.

Some microorganisms are known to produce specific surfactants. Those surfactants are produced by biological entities from natural resources why they have been determined as *biosurfactants*. The EU regulation No 648/2004 on detergents defines the required biodegradability of surfactants to reach 60%. Beyond these requirements, biosurfactants are mostly fully biodegradable. Even though bio-based feedstocks are important to reduce CO₂ emissions of the chemical synthesis of surfactants and many synthesis routes incorporate enzymatically catalysed reaction steps, the term “biosurfactant” will be used only for microbially / biotechnologically produced surfactants within this white paper.

Biosurfactants are comparable in their characteristics to non-ionic surfactants. Among other classes (phosphorolipids, lipopeptides and polymeric biosurfactants), glycolipids are the most relevant class of biosurfactants (Fakruddin, 2012). These molecules consist of a mono- or oligosaccharide bound to a lipid molecule. Three groups of glycolipids will be discussed within this paper: mannosylerythritol lipids (MEL), rhamnolipids (RL), and sophorolipids (SL). Their structure is show in Figure 2 A-C.

Most biosurfactants are produced naturally by microorganisms of different genera such as bacteria (e.g. *Pseudomonas aeruginosa*, *Bacillus subtilis*, or *Acinetobacter calcoaceticus*), yeast (e.g. *Pseudozyma rugulosa*), or fungi (e.g. *Ustilago maydis*, or *Candida bombicola*). While the evolutive advantage of producing biosurfactants is not yet fully understood, experts discuss their role in the competition for resources and the protection of the microorganism under harsh conditions (Soberón-Chávez & Maier, 2011).

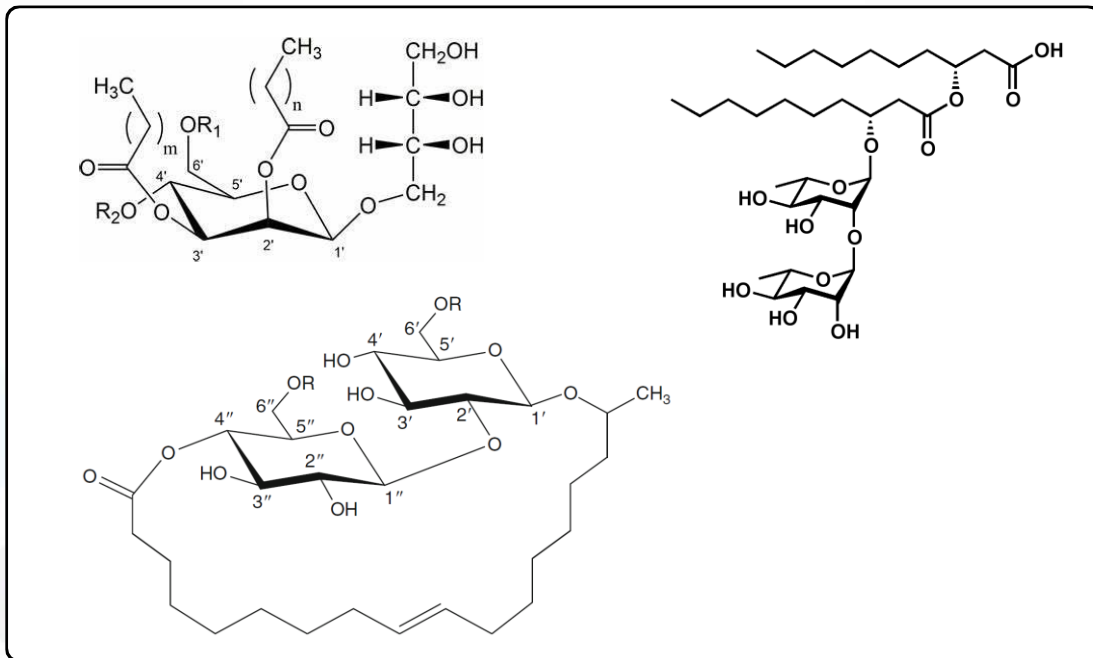


Figure 2: Chemical structure of different biosurfactants. (A) mannosylerythritol lipids (MELs) (Beck & Zibek, 2020); (B) rhamnolipids (RLs) (Wikipedia, 2020); (C) sophorolipids (SLs), shown here: lactonic form (Van Bogaert & Soetart, 2011).

The global surfactant market

The annual worldwide production of non-ionic surfactants has reached 11.5 million tons in the last years. These surfactants could, theoretically, be replaced totally by biosurfactants. A selling price of 1 to 3 US\$ / kg for the conventional surfactant alkyl polyglycoside leads to an estimated annual market volume of about 23 billion US\$ (Santos, Rufino, Luna, Santos, & Sarubbo, 2016). Although biosurfactants have been discovered long ago, their production at industrial scale has just started. Biobased surfactants produced by chemical processing of biogenic resources accounted for 7% of the surfactants used in personal and home care products in Germany in 2015 (FNR, 2021). In emerging regions like Asia, biobased surfactants have with < 2% an even lower share of the total surfactant market (Morgan Stanley, 2019). Given these numbers, the global market for microbial biosurfactants is negligible compared to both, the conventional surfactant and the biobased surfactant market. The two major reasons for that are I. a high production cost (and selling price) of biosurfactants and II. the low

production capacity leading to a missing security of supply for large production volumes.

Compared to conventional surfactants, biosurfactants show a high production cost and a low production capacity. With the highest current production volume, SL is produced at 500 tons per year and sold at 10 to 15 € / kg. RL costs 15 to 20 € / kg. Currently, MEL can be produced only at a cost of 100 to 150 € / kg. For all biosurfactants, production cost must be reduced significantly to compete with conventional surfactants. High cost for substrate and downstream processing as well as low productivities of the applied microbial strains are key hurdles that must be overcome to increase the annual turnover for biosurfactants.

Most formulators of personal care and home care products purchase their ingredients from fine chemical producers. The producers have established processes for the economic production of petrol-based ingredients such as surfactants. Thus, transforming these established structures into a production of bio-based fine chemicals requires active cooperation and a common strategy between ingredient producers and formulators. A strong demand for bio-based and sustainable cleaning agents by the end consumer could foster this transformation.

The surfactant industry in NRW and Germany

Conventional surfactants are produced by the chemical industry worldwide. The large chemical sector in Germany and more detailed in North-Rhine Westphalia is also active in this field. Several push and pull factors such as an increased demand of the end consumer for eco-friendly and sustainable personal care and home care products or the implementation of sustainability goals in public contracts could help to develop the industrial landscape towards the increased application of biosurfactants. Due to its excellent academic landscape and innovative industry, NRW has the chance to step forward as a pioneer of the sustainable production

and application of eco-friendly biosurfactants at large scale. However, the lack of capital and the high risk of investment for scaling up production processes currently hinder the reduction of production cost and the increase of production capacities for biosurfactants. An independent scale-up facility, supported by the state to strengthen the local bioindustry and to attract new companies, could help to overcome these current hurdles in the process development and process scale-up of biosurfactants.

Some examples already show innovative approaches in the application of biosurfactants. As a pioneer in the industrial production of SL and RL, Evonik Industries has shown how to overcome challenges during process development. Evonik successfully transferred the metabolic pathway for RL production into a non-pathogenic production host and decoupled it from oil as second substrate. Furthermore, their in-house expertise in the production of antifoam agents helped to solve issues with foaming control and product recovery at large scale. Evonik has partnered with Unilever to introduce the first rhamnolipid-based dishwashing agent into the Chilean market in 2019 (Bettenhausen, 2020). Moreover, Evonik and Henkel together developed the first RL-containing toothpaste that entered the consumer market in France, Spain, and Italy in 2020. These examples demonstrate the need for extensive exchange and cooperation between fine chemical producers and formulators.

Industry vision and perspectives

Many companies have announced ambitious sustainability goals in the past. Exemplary, Unilever intends to reduce the greenhouse gas emissions caused by the life cycle of their products by half until 2030 (Unilever, 2021). Henkel aims to reduce the carbon footprint of their production by 75% until 2030 (Henkel AG & Co. KGaA, 2021).

The chemical industry is increasingly aware that the development and production of biosurfactants replacing conventional surfactants is one key to lower their carbon footprint. Accordingly, a significant increase of published patents on biosurfactants can be observed in the last decades (Figure 3).

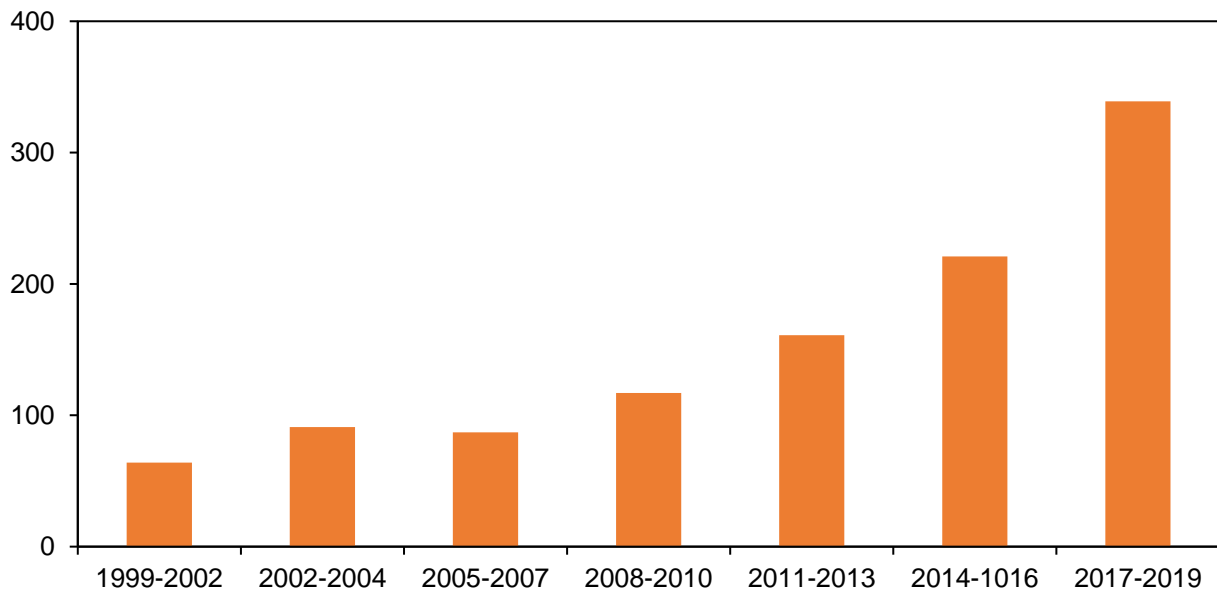


Figure 3: Number of global patents on biosurfactants, published in periods of 3 years [Data from Google patents].

Up to date, important surfactant characteristics, such as foam formation and stability, surface activity or cleaning efficiency, are comparable between biosurfactants and conventional surfactants. Disregarding their production cost, they are even preferable due to several aspects. Biosurfactants are nontoxic and biodegradable making them ideal for applications in the environment, such as agriculture or water treatment. The huge number of biosurfactant variants provides a pool for specific modifications that can be used in future to tailor the functionality of the final product by rational design of the surfactant molecule.

Market segments and potential fields of application

Surfactants play an important role in our everyday life. Most surfactants are used in the home care sector as detergents or cleaning agents. But the range of applications also covers the personal care sector. Among products of these sectors, surfactants account for 32% of the ingredients (FNR, 2021). Toothpastes,

cosmetics, creams, shower gels, hair conditioners and many other products would not be possible without the use of surfactants as emulsifiers. Especially in this sector, mildness to the skin plays a major role. Biosurfactants cause less skin irritation compared to conventional surfactants, which justifies their use in the personal care sector (Liebig & Schwab, 2020).

Surfactants are also used in the food and feed industry. Many industrially processed foods contain emulsifiers that enable homogenous mixing of hydrophilic and hydrophobic ingredients, e.g., in bakery products, milk products or sweets. Developing towards a responsible nutrition, customers care more about the origin of ingredients in their diet and demand for a transparent labelling of the used ingredients. A developing demand for biologically produced food additives will help biosurfactants to establish in the food sector.

In pharmaceutical applications, surfactants are used as emulsifiers for active pharmaceutical ingredients but also as adjuvants in vaccines. Some biosurfactants show antimicrobial or antiviral activities making them promising candidates for future use in therapeutics acting as emulsifier and active ingredient at the same time.

The biodegradability of biosurfactants also opens a broad field of applications in the environment. They can be used in agriculture to stabilize herbicides, pesticides and insecticides that are applied on the field. The use of biosurfactants helps to minimise the required amount of active ingredients and therefore provides an environmental benefit as less substances with potential risk to the environment end up in the ground water. In a different area of application, biosurfactants can also be used to counteract human-made pollution. As large oil spills put marine ecosystems at great risk, the use of biosurfactants to emulsify and remove contaminated water can even help to protect the natural ecosystem from man-made threats.

Beside the mentioned fields of applications, biosurfactants can be used in many industries where water is used as interactive medium, such as, e.g., the petroleum industry, bioprocessing, textiles, paint, leather, papermaking, and others (Fenibo, Ijoma, Selvarajan, & Chikere, 2019). These applications take advantage of the multifunctionality of biosurfactants including stabilising, wetting, antimicrobial, moisturising, emulsifying and anti-adhesive properties (Akbari, Abdurahman, Yunus, Fayaz, & Alara, 2018).

Many biosurfactants can be engineered and modified further. This makes them adaptable to future industry needs and opens a door towards the tailored production based on known linkages between structure and functionality of the molecule. These novel functionalities are not limited by the reaction routes of synthetic surfactants as microorganisms can efficiently build up biosurfactants of higher complexity. However, the linkage of structure and functionality is not yet fully understood, why further research is required in this field.

Current hurdles of biosurfactants' market entry

To meet current market needs, synthetic surfactants are produced at low cost and high quantities. As the characteristics of biosurfactants currently do not differ enough from synthetic surfactants to justify a significantly higher selling price, the overall production costs of biosurfactants must be competitive. A reduction of the production cost can be achieved by the adaption of multiple determining factors. One factor is the cost for the raw material. Many current processes are based on sugar as main carbon source which accounts for about 50% of the process cost (Chong & Li, 2017). Alternative feedstocks which are not competing with the food sector, like molasses, used cooking oil, or cellulosic biomass could reduce the substrate cost. However, this requires the development and engineering of the production organisms to expand their substrate portfolio towards novel substances.

Downstream processing is another cost driver for most biotechnological processes. To address this issue, the recycling of solvents used in extraction and the development of integrated production and product recovery processes was tackled already in academic research and seems to be promising (Tiso, et al., 2020).

Coming along with process scale-up and increasing annual production of biosurfactants, the price will decrease and enable entrance into the mass market. A proper scale-up of most biosurfactant fermentation processes requires a reliable system for foam control. Strongly foaming biosurfactants are desirable for manual cleaning applications like, e.g., hand dish washers, glass cleaners, shower gels and others, but foam formation is also hindering the upstream production of biosurfactants at large scale. Research in bioprocess engineering has identified some initial results that should be developed further, e.g., foam fractionation, separation, the implementation of foam traps, or the addition of anti-foam agents.

Some manufacturers report a bad odour and colour of biosurfactants that prevents them from being utilised in cosmetics and other personal care products. With respect to the later field of application, process development can be intensified in this context to remove undesired side-products which show a disturbing influence on the sensory perception.

Beside pull effects by an increased demand for sustainable ingredients in daily products from the consumer perspective, framework conditions and regulations could act as push factors to establish the biosurfactant industry in the market. Many patents have been published in the last decade (see Figure 3) making it challenging for new companies to enter the biosurfactant market. Especially the large-scale production of biosurfactants with an uncertain sales market for major customers poses a high risk for potential investors. Defining public procurement criteria can help solve this chicken-and-egg problem. A requirement to use sustainably produced personal and home care products (e.g. hand soap, cleaning

agents) in public facilities can help create a secure sales market for the biosurfactant manufacturing industry.

The academic landscape in NRW is at the forefront of the research on novel biosurfactants. However, the capacity and investment for the required technology transfer to larger scales is currently missing why promising results from academia remain unused. At this point, the targeted funding of application-related projects and supporting the start-up scene to covering these intermediate scales could help to increase the TRL of current processes and to fill the gap between research and production scale.

Sustainability goals and consumer perspective

From a consumer perspective, biosurfactants possess some favourable properties. The demand for sustainable ingredients in products of the daily life is increasing constantly (Rosenberg, 2018). In personal care products like shower gels or shampoos, synthetic surfactants could irritate the skin or cause allergies. Proven by field tests of novel formulations for personal care products, the application of biosurfactants in personal care products could be beneficial for every individual person as biosurfactants are milder and gentler to the skin than synthetic surfactants (Liebig & Schwab, 2020). However, the end consumer needs to understand the benefit of a natural ingredient replacing a petrol-based chemical in everyday products. Terms like 'bioeconomy', 'biosurfactant' or 'sustainable production' lead to totally different associations among individuals. This can be tackled by educational awareness campaigns explaining the need for a responsible use of natural resources.

Once established in the market, environmental-friendly biosurfactants show further fields of application in agriculture or in remediation. Hydrophobic organic compounds or heavy metal contaminants could be removed from soil by biosurfactants demonstrating their broad applicability in different sectors.

From the industrial point of view, several aspects support the change from petrol-based to biosurfactants. Most companies welcome to diversify their resource suppliers. Using alternative feedstocks makes them more independent from a single oil supplier.

Governmental regulations and their own sense of responsibility push the industry towards a reduction of greenhouse gas emissions. Companies often translate these regulations into sustainability goals for their long-term strategy. One of the requirements for new production processes is often to reduce the overall carbon footprint for the desired product. Even though biosurfactants are low-volume and high-performance ingredients, the transformation of petrol-based towards bio-based feedstock materials in the surfactant production helps to reduce the overall carbon footprint of corresponding end products like cosmetics or household cleaning agents.

Stakeholders in NRW and abroad

Many companies and academic institutions in NRW are active in the field of biosurfactants. In individual cases, large companies, e.g., Evonik Industries or Henkel, are producing SL and RL at economic scale or formulating them in their end products, respectively. These examples demonstrate the possibility of innovative developments and products, designed in NRW. The research on new functionalities and process design promotes the start-up scene in NRW as the winner of the G-BiB competition 2019, Engineering Biosurfactants (EBS), has demonstrated.

With excellent applied research at the universities in NRW, researchers from all over the globe have recognised the professional scene. Exemplary, the BioSC research project Bio² successfully demonstrated a process design for the integrated production and recovery of RL and MEL in an interdisciplinary consortium of seven research institutes. But also, the university of applied

sciences in Cologne is collaborating with industrial partners to look for alternative synthesis routes of bio-based and bio-catalysed surfactants.

Out of NRW, the Innovation Alliance Biosurfactants provides a network of universities, independent research organisations, SME, and industry to collaborate and develop new biosurfactants with tailored functionalities. Werner & Mertz as large formulator of home care products supports by an initiative the use of plant-derived biobased surfactants from European fields instead of tropical feedstocks (Werner & Mertz GmbH, 2018).

Out of Germany, other consortia of industry and academic institutions are also involved in innovative research and process design. E.g., Holiform, a British company, developed a continuous process for the production and recovery of SLs. Ecover is a Belgian formulator of biosurfactants in laundry detergents, cleaning agents and other products. The Bio-Based Europe Pilot Plant in Ghent is involved in several scale-up processes and projects on the production and economisation of various biosurfactants.

All these activities demonstrate the willingness of the local, national, and European industry to replace conventional surfactants with biosurfactants. However, this can only be one little step towards a sustainable and environmental-friendly production of products in our daily life.

Future perspectives

Biosurfactants show the potential to replace fossil-based chemicals in a billion-dollar market. This will help to reduce the carbon footprint and greenhouse gas emissions during the production of surfactant-rich products in the home care and personal care sector. However, for achieving this goal, the production cost of biosurfactants needs to be reduced strongly to compete with established synthetic surfactants on the market. Once, the price and the cleaning properties of biosurfactants are competitive to synthetic surfactants, the mass market for

personal care and home care products will open towards these new ingredients. The demand on the consumer side can be stimulated by activities or campaigns to strengthen the awareness of sustainable production among the population. Sustainability goals in public procurement can be a tool to boost the initial demand for cleaning products produced from renewable substrates. These levers can help to convince the chemical industry to invest in the process design and development of a new type of surfactants.

Research and development

Research in the field of biosurfactants develops in two different ways. First, chemical and strain engineering aim to design new variants of biosurfactant molecules providing additional functionalities that add a higher value to the product. Some biosurfactants already show antimicrobial or antiviral effects. For example, Lipopeptides are relatively easy to modify by altering the peptide chain of the molecule. This offers a huge potential for the rational design of variants leading to targeted functionalities of the produced biosurfactant. However, lipopeptides are not yet produced beyond laboratory scale why they are not discussed further in this paper.

Other research approaches focus on engineering the production strains towards increased productivities or a broader substrate portfolio. The reduction of side-products and the utilisation of alternative feedstocks both affect the overall economy of the process, leading to the second large field of process development, the biochemical process engineering. For funding an efficient screening of potential alternative production strains, short projects of up to 3 years and a low TRL could help getting the broad microbial expertise in NRW to deal with this topic. Several process limitations must be overcome before biosurfactants can replace fossil-based surfactants completely. Three major issues in the fermentative production of biosurfactants are foam formation, heterogeneity leading to complicated process control, and small reactor volumes. Once these limitations

are tackled, a continuous large-scale production process with an integrated product separation could be key to bring biosurfactants on the market at a competitive price. With the targeted development of anti-foam agents, Evonik has successfully demonstrated how the interdisciplinary work of bioprocess engineers and chemical engineers solved a fundamental issue during process scale-up. Even though SLs and RLs are already placed and applied in niche markets, the production capacities must be increased significantly to – one day - fully replace petrol-based surfactants. With its academia, start-ups, and industry, NRW shows a high potential to develop into a hot spot for research and production of biosurfactants.

Entry into target markets

As many processes based on renewable feedstocks, the economy of the biosurfactant production is strongly linked to the global oil price. As long as fossil-based feedstocks are available at large volume and low cost, renewable feedstocks will hardly be an economic alternative. Incentives for the use of renewable feedstocks or additional charges on petrol-based feedstocks (like an extended European Emissions Trading System) could promote the transformation of the chemical industry. From the application point of view, the most promising target markets are those where biosurfactants can be sold at a higher price such as the personal care and cosmetics sector – and, more specifically, the niche markets of natural and eco-friendly cosmetics. Entering these niche market could help to build-up larger production facilities for biosurfactants providing the capacity to enter larger surfactant applications like detergents or cleaning agents. However, the dependence on the global oil price poses a risk of investment to the companies. If this risk is assessed as too high, most companies will probably stick to fossil-based surfactants for the coming years.

Conclusion

Surfactants are currently produced by chemical conversion of fossil-based feedstocks. These surfactants can be produced at low cost and high quantities. They are applied widely in industrial products. The general industrial development towards sustainable production processes and the structural change from fossil-based to regenerative carbon-sources implements the research on biosurfactants to replace synthetic surfactants. The already available biosurfactants show similar characteristics while being produced by fermentation from biological resources and being biodegradable.

Currently, the major hurdles for the broad industrial application of biosurfactants are the high production cost and the low production volume. Therefore, biosurfactants will not replace fossil-based surfactants in the short-term but in a more or less rapid process, depending on various circumstances. A holistic approach is required that covers the entire value chain. This requires active cooperation and a common strategy between ingredient producers and formulators. For a moderate market entry, key sectors should be identified that require low quantities of biosurfactants and that can afford their high current cost. A higher sales price of the final product can be put into perspective by the valuable, natural ingredients and added benefits by special properties of biosurfactants. First examples show the successful production of biosurfactants at industrial scale and their application in selected personal and home care products.

These applications in niche markets could set a starting point for increasing the production capacities and the fields of application by lowering the production cost further. Companies worldwide are looking at the potential of biosurfactants but hesitate to fully replace conventional surfactants in their formulations due to the mentioned reasons of production cost and availability. Several levers were identified to support the development of a biosurfactant industry in NRW and Germany in general. Regulations on the carbon footprint of ingredients applied in

everyday products could help as well as public procurement criteria to provide a safe market demand to producers. The chance for NRW is to act as a pioneer region in this field by supporting the local industry early on. This head start in development can create a sense of change in the start-up scene and lead to novel foundations of innovative process concepts. Securing jobs in the region, governmental support can ensure the state's industrial competitiveness for decades to come.

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