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# Biosurfactants and bioamphiphiles, survey, perspectives and applicative potential from a colloid science point of view

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## Abstract

Biological surfactants are amphiphilic molecules obtained from biobased resources, like plants, sugars and oils, using a variety of physical, chemical, biochemical or biotechnological methods. More specifically, the word *biosurfactant*, or *microbial biosurfactants*, is classically used for those molecules, like sophorolipids, rhamnolipids or surfactin, produced by fermentation. Historically developed by microbiologists and originally used as natural emulsifiers, recent trends in fundamental and applied research depict a set of molecules with a rich, and somewhat unexpected, physicochemical behavior making it difficult to introduce them as such in existing formulations. A broad research activity is then developing worldwide both in academia and industry with the goal of better understanding this class of amphiphiles with the ultimate perspective of introducing them to the market in fields as varied as detergency, cosmetics, pest control, medicine.

## Introduction

Historically obtained from materials of natural origin, from animal fat (Marseille soap) or plant extracts (saponins), surfactants have become of considerable importance during the 20<sup>th</sup> century. In the field of public health, for example, detergents have significantly contributed to reducing the spread of infections, and therefore epidemics. Combined with other organic and/or mineral elements, they have also contributed to the development of fields such as cosmetics, pharmacopoeia, painting, the food industry and much more. Therefore, such success stimulated a change in the feedstock of surfactants, which switched from natural to petrol based; this allowed chemists to combine various building blocks

in order to tackle a wider range of more and more specific applications. Such a need led to environmental pollution on the one side and health problems to humans, like skin irritation, on the other.

Surfactants with improved biodegradability, like ester quats, fatty esters of quaternary ammonium mono alcohols, or molecules partially obtained from bio-based products and assembled through synthetic chemistry, like fatty alcohol ethoxylates or alkyl polyglucosides, have gradually replaced surfactants entirely derived from petrochemistry. However, much progress still remains to be made. In this regard, this trend has been accentuated over the past thirty years by the appearance, and production on a semi-industrial scale, of surfactant molecules that are not only bio-based, but exclusively produced through a biological process, excluding any synthetic (organic) chemical step. Such molecules are generally produced by fermentation by yeasts and bacteria and are known as *microbial biosurfactants*. In the literature, one can find various ways to address them, such as “*biosurfactants*” alone, “*biosurfactants of microbial origin*”, “*bioamphiphiles*” or “*microbial amphiphiles*”...

The first reports on the surface activity of microbial extracts with surface active properties can be traced back to the late 1940's. At that time, the discovery of penicillin in 1929 by A. Fleming motivated a strong research development in microbiology, which was most dedicated to the antibacterial activity of metabolic products of microbial cultures. Within this context, Jarvis and Johnson identified in 1949 a known antimicrobial compound produced from *P. aeruginosa*<sup>1</sup> as being a lipidic derivative of rhamnose. In 1951, following a similar path in the research of antibiotics from the fungus *U. maydis*, Haskins and coll. isolated ustilagic acid, today known as cellobioselipids.<sup>2</sup> Similar discoveries were done few years later on other microorganisms, *B. subtilis*, *T. apicola*, from which it was possible to extract surfactin and sophorolipids, respectively.<sup>3,4</sup>

Research in microbial biosurfactants continued all along the second half of the 20<sup>th</sup> century, strongly motivated by the oil crisis, which triggered an increase in the cost of petrochemicals, and also for environmental reasons. The possibility to ferment hydrocarbon substrates, including crude oil, by microorganisms had generated the hope that microorganisms could be useful in the bioremediation of oil spills and coastal pollution, typically through the *in situ* production of what were addressed at that time as bioemulsifiers first and biosurfactants afterwards.<sup>5</sup> Eventually, the *ex situ* production of biosurfactants took over the *in situ* use of microorganisms, essentially for the possibility to control and boost the biosurfactant production. The direct application of their emulsifying properties in treating oil spills, or simply cleaning industrial scale oil tanks, gave a boost to the research in this field from the

mid- 1980s.<sup>6,7</sup>

In the past 40 years, the research in biosurfactant science has evolved beyond the fields of ecology and microbiology. Genetic engineering now secures controlled production, including molecular variation and high titers, of biosurfactants.<sup>8</sup> The antimicrobial properties, which actually were at the focus in the original discovery of biosurfactants, are now specifically studied and strongly developed. Detergency has probably become the most obvious field of application, as one can observe by the massive recent investments by the chemical industry.<sup>9</sup> Studies on the interaction of biosurfactants with other systems (materials, membranes, macromolecules) as well as studies about their physicochemical properties are stimulating potential applications in the fields of chemistry, material science, engineering and even questioning the notion of the term biosurfactant itself.<sup>10–12</sup> In short, the field is simply exploding from both fundamental and industrial perspectives.

This opinion has the aim of presenting the current research trends on microbial biosurfactants under the prism of colloid and interface science, a point of view, which was seldom employed so far. The Current Opinion in Colloid and Interface Science (COCIS) was, to the best of our knowledge, the first forum, in 2002, to consider microbial biosurfactants as important amphiphiles for the colloids community<sup>13</sup> and it has renewed this engagement in a recent special issue on biosurfactants.<sup>a</sup>

## Production

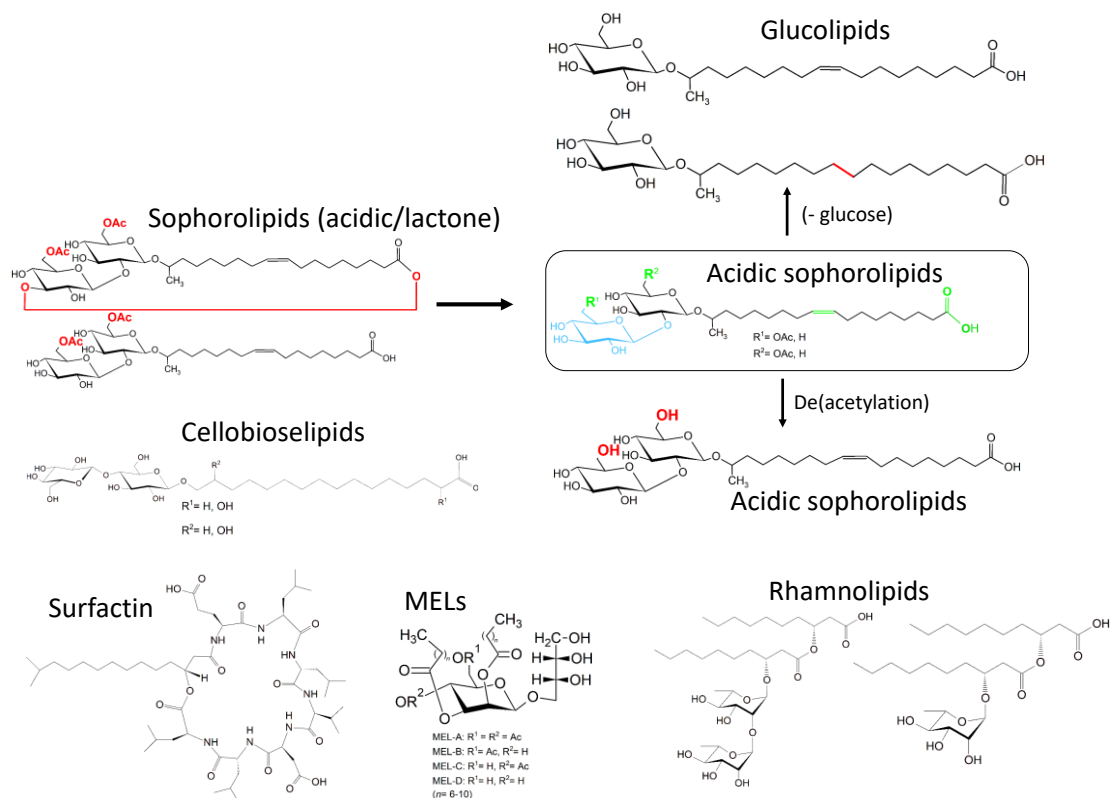
The molecules that are historically cited as being microbial biosurfactants are relatively few and known since at least four decades. In this respect, the reader will realize that the content proposed by review papers published between the mid 1970s and 1980s<sup>5,6,14</sup> has not drastically evolved in more recent reviews,<sup>15</sup> which however tend to be more and more dedicated to individual compounds.<sup>8,16</sup> In the category of glycolipids (Figure 1), one finds sophorolipids from *S. bombicola*, rhamnolipids from *P. aeruginosa*, mannosylerythritol lipids from *C. antarctica*, cellobioselipids from *U. maydis* and trehalolipids from the genus *Rhodococcus*. In the category of lipopeptides one finds surfactin, fengycin, mycosubtilin and iturin from *B. subtilis*, among others. Recent books will help the reader sailing through the most recent trends in microbial biosurfactants production and potential applications.<sup>16</sup>

At the present state of the art, only about two to three molecules are produced at a (semi-) industrial scale and potentially ready to use in commercial products. Historically, Soliance in France, now Givaudan, used to produce raw mixture of sophorolipids. As of today, sophorolipids are produced

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<sup>a</sup> <https://www.sciencedirect.com/special-issue/10DHHNGZQGB>

by Evonik (Germany), Locus (USA), Holiferm (UK) or Saraya (Japan). Rhamnolipids are produced by Evonik (Germany), AGAE technologies (USA) or Jeneil (USA). Surfactin and other lipopeptides are produced by Lipofabrik (Belgium). A more complete list of producers worldwide can be found in related review articles, which also provide the market prize for most compounds.<sup>17</sup>



**Figure 1 - Most important microbial biosurfactants found in the literature. MELs= mannosylerythritol lipids**

Evaluating the actual cost of biosurfactants is still quite a challenge.<sup>17</sup> Purified, mg-scale, amounts of biosurfactants can be bought for several hundred dollars, while the raw material purchased directly from the producer can be obtained at more contained costs (dozen(s) \$ per kg). However, as well-explained by several authors, the raw mixtures generally contain a complex cocktail of congeners,<sup>18</sup> with residual fatty acid content (used as carbon source for fermentation).<sup>8,19</sup> Salts, coming from the complex composition of fermentation broths, can also be present, as it is not uncommon that producers sell either a hydrolyzed batch, in the case of sophorolipids to reduce the molecular complexity (hydrolysis opens the lactone ring and it eliminates the acetylation), or the neutralized version to improve water solubility, as in the case of rhamnolipids. Genetic engineering and downstream separation processes also have a critical importance. Currently, compounds such as

deacetylated acidic sophorolipid can be obtained as a relatively pure batch, rather in the classical mixture with lactone sophorolipids contaminated with residual fatty acids. However, the drawback today is the limited, although improved with respect to the past, production process.

### **Intellectual property**

Another issue that still characterizes the field is the intellectual property. The development of fermentation processes, purification, characterization and registration is an enormous economic risk for companies and this needs to be protected by intellectual property. The biosurfactants are, however, known for decades and are present in nature since thousands of years, so claiming of the chemical structure of the molecules is not possible. Production processes and protocols during downstream processes are thus essential information, which will strongly influence the success of a development. These production parameters should, however, be kept secret and should not be published in a patent since it will be hard to control how different companies produce their biosurfactants. So, the only field left for protection by intellectual property are applications and description of combinations of biosurfactants with other compounds in order to obtain a given effect. In this respect, it is not uncommon that large amounts of a given biosurfactant compound require direct negotiation between the user and the supplier, not only in terms of cost but also in terms of the development (and hence protection) of potential applications. Often, the user collaborates directly with the supplier and, even if at the moment most research is still at a fundamental level, the potential for product development is quite high. For a broad overview of patents up to 2006 in the field, one can consult Shete et al.<sup>20</sup>

### **Self-assembly and lyotropic liquid crystalline behavior**

With the exclusion of lipopeptides, most biosurfactants share a bolaform molecular structure (Figure 1), with one of the two headgroups often being chemically reactive (generally, a carboxylic acid). From both a theoretical and experimental points of view, the bolaform shape of an amphiphile should follow the same structure-property relationship known for head-tail amphiphiles.<sup>21</sup> However, it is well-known that bolaform amphiphiles, topic of a broad, but also poorly organized, research activity in the past decades, display unexpected self-assembly properties, strongly dependent of the chemical group(s) at both ends of the molecule.<sup>22,23</sup> To the best of our knowledge, a general understanding of the self-assembly properties of synthetic bolaamphiphiles does not exist, yet, and an empirical approach to the study of their phase diagram is still necessary. In this regard, the unexpected self-

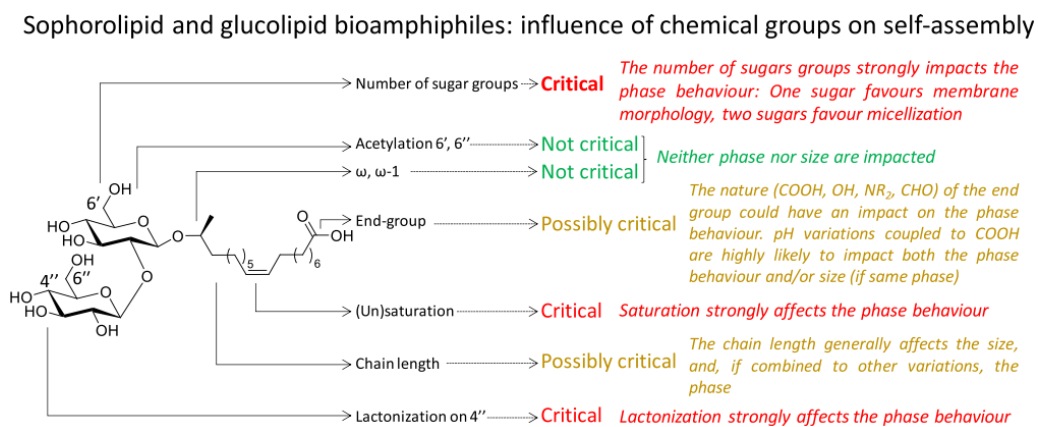
assembly properties of many biosurfactants can be precisely explained by the combination of the bolaform shape of most of them in association to least one chemically-reactive site. If the structure-property relationship is well established for head-tail surfactants, it has only recently been addressed for sophorolipids and sophorosides,<sup>24</sup> and much still needs to be understood.

The solubility of ionic surfactants depends on the temperature and above the so-called “Krafft temperature”, solubility is higher than the critical micelle concentration (cmc) and the surfactant dissolves in micelles. Knowing the Krafft temperature is of high relevance for applications, for instance in the development of “cold washing” conditions in laundry. Classically, the Krafft temperature is relevant for surfactants with strong ionic character (e.g., sulfates, sulfonates, quats), while for amphiphilic molecules containing pH-dependent chemical groups (e.g., carboxylic acids), as found for soaps and biosurfactants, consideration of the Krafft temperature is more cumbersome, as it depends on pH, counterion type and salt concentration.<sup>25-27</sup> The Krafft temperature also depends on the chain length and length distribution, the longer the chain the higher the Krafft temperature; however, odd-even dependency is observed, and branching and double bonds can have the effect of lowering the Krafft temperature.<sup>27,28</sup> Thus, Krafft temperatures are then expected to be below room temperature or even below 0°C for biosurfactants like sophorolipids and surfactin. Within the framework of studies related to laundry applications, it was shown that the surface activity of rhamnolipids hardly depend on temperature<sup>29</sup> and that the Krafft temperature of mixtures rich in di-rhamnolipids is below 0°C.<sup>30</sup> For comparison, sodium laurate has a Krafft temperature of 25°C.<sup>25</sup>

Another well-known phenomenon in surfactant science is the cloud point, referring to the lower solubility of, generally, non-ionic surfactants (with ethylene oxide or sugar headgroups) due the tendency to form temperature-dependent hydrogen bonds.<sup>31,32</sup> If the cloud point of non-ionic surfactant like fatty alcohol ethoxylates had been widely investigated in purified systems, it is well-known the impact of headgroup and hydrophobic chain polydispersity, inevitable for both ethoxylated<sup>33</sup> and alkylpolyglucosides.<sup>34</sup> If, to the best of our knowledge, no reports on cloud point exist for biosurfactants, some literature on the influence of mixture of non-ionic ethoxylates and biosurfactants on the cloud point does exist; sophorolipids were for instance described to increase the cloud point,<sup>35</sup> similarly for rhamnolipids at pH 7,<sup>34</sup> but not at pH 5, where the cloud point rather decreases due to the pH-induced change in polarity of the rhamnolipid. This aspect stresses again the importance of controlling pH in the formulation of biosurfactants. Overall, as found for ethoxylated and polyglucosidic surfactants, one can also expect a congener effect on the cloud point for the biosurfactant family, whereas by “congener effect” one refers to the fact that biosurfactants are

generally constituted by complex mixtures of molecules with well-defined structures.

Self-assembly of microbial biosurfactants is now established, and in particular the critical dependency of self-assembly on pH and other structural molecular features (Figure 2). By controlling the degree of protonation, pH is a lever on the non-ionic or ionic state of biosurfactants, and consequently, on the presence of electrostatic repulsive forces, which generally contribute to stabilize micellar structures below about 10 wt% in water. Data published over the years on sophorolipids, rhamnolipids and surfactin strongly corroborate the statement. In the ionic form, the impact of the fatty acid chemistry (saturated, monounsaturated, hydroxylated) on the biosurfactant assembly is relatively limited, with few exceptions, one being represented by sophorolipids containing a *trans* C18 fatty acid.<sup>36</sup> This compound, unique and formerly studied for the purpose of fundamental understanding, has shown a slow but progressive transition from micelles to fibers even in its anionic form. Interestingly, it must be noted that the nature of the charge, negative ( $COO^-$ ) or positive ( $NH_3^+$ ), does not seem to have a remarkable impact on the morphology of the aggregates, at least in sophorolipids.<sup>37</sup>



**Figure 2 – Tentative understanding of the chemical groups in sophorolipids and sophorosides on their self-assembly properties. Reproduced with permission from Ref. <sup>24</sup>.**

In the absence of repulsive interactions, hence in the non-ionic form of biosurfactants, the self-assembly is simply unpredictable and it must be studied case by case. The chemical nature of the fatty acid becomes very important as well as the type of headgroup, mono-saccharide (e.g., as in glucolipids or mono-rhamnolipids), di-saccharide (as in sophorolipids or di-rhamnolipids) or peptide. In the case of a monounsaturated *cis* fatty acid (typically, oleic acid), one can expect to find self-assembled structures with a finite curvature (micelles, vesicles). This is also the case for short chain fatty acids, as in the case of rhamnolipids.<sup>38</sup> For saturated fatty acids, one finds more rigid structures, like fibers



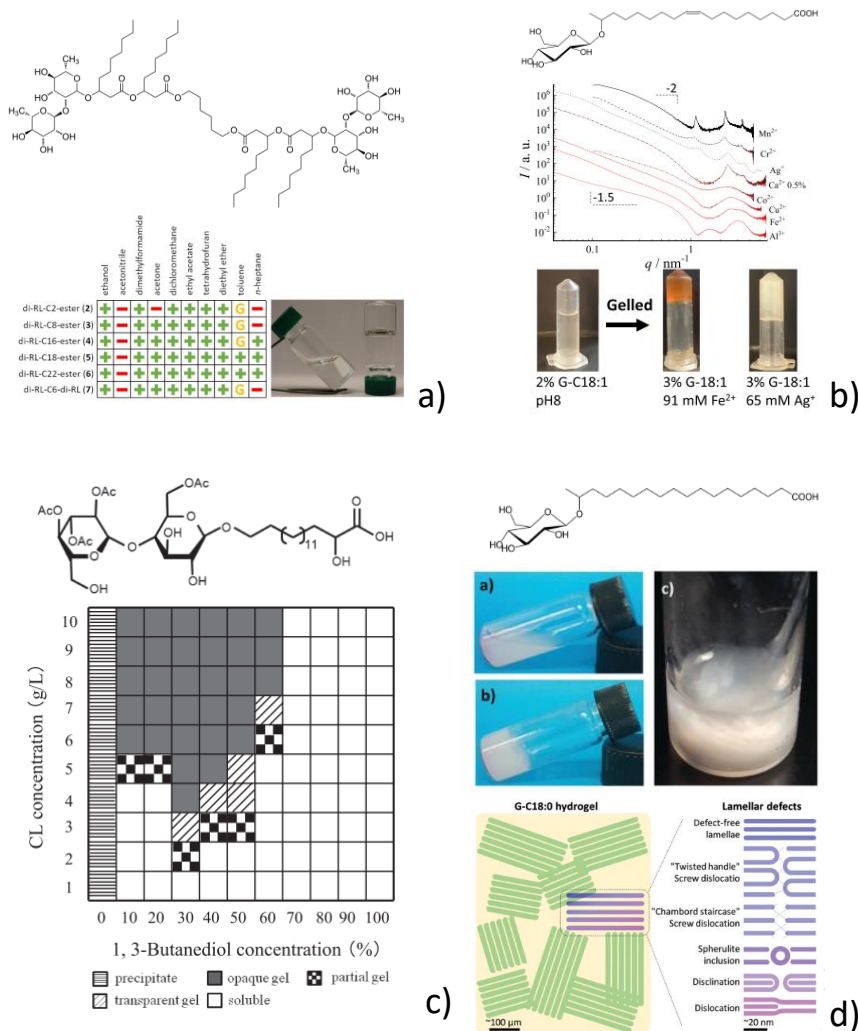
or lamellae.<sup>11</sup> However, the nature of the headgroup also plays a critical role at the same time: mono-saccharides (e.g., glucose, rhamnose), characterized by a relatively small area-per-headgroup ( $\sim 50 \text{ \AA}^2$ ), tend to drive the assembly towards low curvature structures (e.g., vesicles), while disaccharides tend to drive towards high-curvature structures, e.g. micelles.

This being said, di-saccharides seem to play an important, underestimated, role. As underlined by Sanchez-Fernandez,<sup>39</sup> the conformation of the sugar headgroup in sugar-based surfactants is very important and still poorly understood. Authors nicely pinpoint the strong differences in phase behavior of alpha and beta isomers of the same amphiphile (e.g., maltosides). At the moment, very little is known on the impact of isomerization in the case of microbial biosurfactants, but both data and simulation suggest that this issue is critical. If no data exists in the use of the alpha or beta isomer, few publications seem to underline that the higher conformational freedom of disaccharides can have a strong impact on the self-assembly properties. For instance, di-rhamnolipids in their protonated form are expected to assemble into a micellar phase but they rather precipitate in a lamellar phase.<sup>40</sup> It was made the hypothesis that the conformation of the outer rhamnose group plays a key role. This seems to be confirmed by numerical simulations, showing intra-molecular hydrogen bonding between the outer rhamnose and the COOH group.<sup>41</sup> Sophorose and other relevant carbohydrates are known to have several energetically favorable conformational minima,<sup>42</sup> which could explain the kinetically-trapped micellar phase of sophorosides, of which the thermodynamic structure seems to be a semi-crystalline fiber.<sup>43</sup> Conformational issues also seem to play a role in surfactin,<sup>44</sup> although one must say that this is not uncommon for peptides.

Finally, besides pH, the effect of temperature, ionic strength and type of metal ions on self-assembly were only partially investigated. As of current knowledge, it seems to be important to pursue these studies in a thorough manner, as the impact on self-assembly seems to be important and unpredictable.<sup>11,38,45</sup>

Concerning more precise details on the recent advances in the current literature on solution self-assembly, lyotropic liquid crystalline phase behavior and molecular modelling can be found in recent review papers.<sup>11,38,39,46-49</sup> Control and understanding of molecular self-assembly, especially in relationship to the molecular features (Figure 2), paves the way to unexpected discoveries, such as the ability of specific molecules to trap water in their fiber or wormlike micellar phase and stabilize complex viscoelastic fluids.<sup>50</sup> To date, cellobioselipids, sophorolipids, sophorosides and glucolipids were reported to form hydrogels (Figure 3), of which the structure strongly depends on the nature of the self-assembled state. This aspect was recently reviewed, as well,<sup>50</sup> and shortly commented on

below. In most cases, these glycolipids behave as low molecular weight gelators (LMWG) by crystallizing into twisted ribbons entangled in a network. This is the case of disaccharide-based biosurfactants with a saturated fatty acid tail, like cellobioselipids<sup>51</sup> and sophorolipids.<sup>52</sup> However, unexpected gelation can also occur from molecules containing an oleyl backbone, as observed for sophorosides<sup>43</sup> and single-glucose lipids,<sup>45</sup> however requiring different physicochemical conditions. For saturated molecules, pH generally triggers the fiber phase and gelation, while in the case of monounsaturated molecules, temperature or complexation were shown to be the main driving force. Additional factors can play an important role, like the conformation of the disaccharide, a phenomenon which is neither controlled nor fully understood at the moment. The chemical nature of the counterion also seems to be important: ions existing as free aqueous species at pH below 10, like  $\text{Ca}^{2+}$  or  $\text{Ag}^+$ , are suspected to form strong complexes with carboxylate groups, while ions with a more pronounced acidic character are suspected to neutralize the negative charge of carboxylate groups and promote wormlike micelles, instead of fibers.<sup>45</sup> A similar behavior has not yet been reported for sophorolipids and rhamnolipids, of which the assembly, probably due to the bulkier carbohydrate headgroup, seems to be less sensitive to the type of counterions. Both the gel formation properties and structure of single-glucose lipids with a saturated backbone are also unexpected. One finds swollen lamellar phases instead of fibrillar gels.<sup>53</sup> Finally, chemical derivatization can sometimes generate hydrogels with wormlike structure, as found for dirhamnolipid esters.<sup>54</sup>



**Figure 3 – Self-assembled gels obtain from various microbial amphiphiles. a) Wormlike organogels from dirhamnolipids esters (reproduced with permission from Ref. <sup>54</sup>); b) fibrillar ( $\text{Mn}^{2+}$ ,  $\text{Cr}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Ca}^{2+}$ ) and wormlike ( $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Al}^{3+}$ ) gels from single-glucose lipid (C18:1) (reproduced with permission from Ref. <sup>45</sup>); c) fibrillar organogel from cellobioselipid (reproduced with permission from Ref. <sup>51</sup>); d) lamellar hydrogel from single-glucose lipid (C18:0) (reproduced with permission from Ref. <sup>53</sup>).**

### Towards the formulation of biosurfactants

At the present state of the art, studying the interactions with other molecular components is a key step in the formulation development based on microbial biosurfactants, because the application properties of biosurfactants are not optimized, yet, and their cost is still too high. At the moment, no exhaustive data exists in this field. However, one can find pieces of information concerning the mixing behavior with both biosurfactants<sup>55</sup> and synthetic surfactants,<sup>47,55–57</sup> with macromolecules,<sup>11,58</sup> with lipid membranes,<sup>59</sup> with enzyme and proteins.<sup>12,60</sup>

### *Interactions with surfactants*

Formulation developers want to have a choice in order to fine-tune their formulations according to a variety of parameters like foam, viscosity, detergency, mildness and price. Due to low availability of biosurfactants, their prices are higher than for conventional surfactants and mixing of conventional surfactants and biosurfactants is inevitable. Thus, most studies with binary or ternary surfactant systems contain classical surfactants with straight alkyl chain and one polar headgroup and biosurfactants with short / bulky (as in case of rhamnolipids) or kinked shaped (as in case of sophorolipids) hydrophobic moieties and two polar head groups (sugar and COOH), with few exceptions.<sup>55,56</sup> The interaction between both anionic and cationic surfactant with mono-rhamnolipid at pH 7.1 showed that packing is less dense as expected, probably due to the mismatch of the chain length and geometry.<sup>61</sup> Surprisingly, mixing of deprotonated mono-RL with cationic surfactant did also not yield precipitate at equimolar stoichiometry.<sup>61</sup> This weaker interaction also hinders well-known synergisms like interaction of anionic surfactant with sulfate groups and zwitterionic / amphoteric surfactants,<sup>62</sup> which is often utilized to build viscosity via wormlike micelles. The interaction between biosurfactants and both cationic and zwitterionic surfactant depends on the pH-value, the degree of protonation, respectively.<sup>48</sup> The pKa-value of the biosurfactants is becoming as important as, e.g., the Krafft temperature or cloud point for ionic and non-ionic surfactant, respectively. The pKa-value of biosurfactants depends, however, on other formulation components, as can be expected from the behavior of fatty acids or other COOH containing surfactants.

### *Formulation with macromolecules*

The use of macromolecules in the context of biosurfactant science has so far concerned the study of hydrogels, nanoparticles, complex coacervates and more complex multicomponent formulations.<sup>58</sup> Polymers like silk fibroin, gelatin, alginate or chitosan were used to demonstrate the possibility to integrate biosurfactants, which could have, or not, the ability to change the properties of the macromolecular scaffold. It was for instance shown that sophorolipid micelles accelerate the gelation of silk fibroin, like other anionic surfactants.<sup>63</sup> On the other hand, biosurfactant fibrous gel networks can nicely integrate a macromolecular network to improve its elastic properties, which can in turn be controlled by triggering phase transitions in the biosurfactant phase.<sup>64</sup> Triggering phase transitions of a biosurfactant associated to an oppositely-charged polyelectrolyte can also generate new phases<sup>65</sup> which might also find their way into applications like hair-conditioning.<sup>66</sup> An increasing

number of studies shows some interest towards the development of biosurfactant-macromolecular nanoparticle systems based on gellan gum, zein, polylactic acid and polyethylene glycol for the encapsulation and delivery of active compounds,<sup>67</sup> possibly stimulated by pH.<sup>68</sup>

#### *Formulation with membrane phospholipids*

Biosurfactants are very well-known for their antimicrobial properties, for which they were historically discovered. A number of studies hence deals with the interaction between biosurfactants and phospholipid membranes, with the goal of better understanding the impact of such amphiphiles on the membrane stability.<sup>59</sup> At the present state of the art, rhamnolipids are the most studied biosurfactant in interaction with 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (PLPC), 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (DMPS), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and various other more complex lipid mixtures, some used as bacterial model membranes. Other biosurfactants include trehalolipids, sophorolipids, mannosylerythritol lipids, lipopeptides and aescin. Despite the effort, data are still lacking so that drawing a general trend is not possible yet. One can safely state that biosurfactants, and rhamnolipids in particular (the most studied system so far) have preferred interactions with lipid membranes. Impact on the liquid-to-gel transition temperature seems common, although the direction of the shift (lower or higher temperatures) seems to depend on the membrane composition and can thus not be generalized.<sup>69</sup> Membrane insertion and pore formation has been reported several times for several biosurfactants.<sup>70</sup> Membrane fluidity, an important biophysical property, has been supposed to increase upon adding rhamnolipids or surfactin, but the amount of studies is very limited<sup>59</sup> and the mechanism not yet understood. pH is also important, as shown for the enhanced membrane activity of rhamnolipids and sophorolipids at acidic pH, but again, the amount of studies is limited.<sup>71</sup>

#### *Formulation with oil (emulsions)*

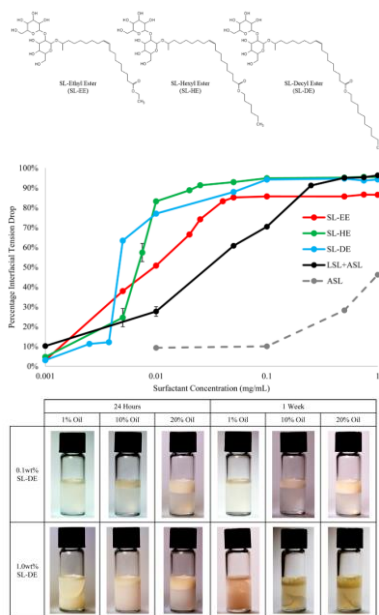
The quest for biological emulsifiers is a relatively old task and the development of biological surfactants from microbial origin is tightly linked to the need of recovering crude oil,<sup>5-7</sup> as mentioned earlier in the manuscript. So the number of publications related to biosurfactants and emulsification of oil (may it be mineral or biobased oil) is large. The underlying mechanism is, however, different. In enhanced oil recovery (EOR), it is less the emulsification of the oil itself but rather the effect of the surfactant on the wettability of the rock, which determines interaction between oil and rock and can thus lead to a release of the oil from the rock.<sup>72</sup> Of course, interfacial tension between water and oil

will also be reduced in the presence of biosurfactant and the use of crude mixtures might eventually yield better results due to several effects which are needed in oil field applications. A strong stabilization of an emulsion is, however, not intended in oil field applications since the crude oil needs to be separated from the water in order to be concentrated and further processed. For other fields of application, like food science and cosmetics, stability is important and purified material / individual congeners are commonly used.<sup>73</sup> In this respect, it is not surprising that modification of selected microbial surfactant give improvements above the natural occurring molecules if emulsification with low surfactant-to-oil ratios are investigated, since these kind of emulsions are not naturally occurring. Thus chemical modification as e.g. by sophorolipid esters, with tail of different length, have shown an improved emulsifying potential towards various natural and crude oils (Figure 4).<sup>74</sup> Recent reviews point out those studies specifically exploring the emulsifying properties of biological surfactants and the reader is encouraged to explore them for more information.<sup>72,73,75</sup>

The research on the emulsifying properties of biological surfactants is far from being completed over the years. At the beginning, the initial research activity involved crude surfactant formulations, while the trend today is to study the emulsifying properties of selected congeners. On the other hand, the widening of the applications leads towards the study of the conditions to form stable microemulsions<sup>76</sup> and nanoemulsions<sup>77</sup> as well as emulsification with additives, like macromolecules<sup>78</sup> or particles<sup>79</sup> or even to prepare pH-switchable nanoemulsions<sup>80</sup> and Pickering emulsions.<sup>78</sup> Ternary (oil/water/biosurfactant) phase diagram studies are the key to design stable microemulsions and these are still rare in the field.<sup>47</sup> Several, although quite few, attempts exist in calculating the hydrophilic-lipophilic balance (HLB) for biosurfactants. The HLB scale was developed to create an empirical relationship between the surfactant properties (e.g., oil-in-water or water-in-oil emulsifier, for instance) and the balance between the hydrophilic and hydrophobic portions of the molecule. Initially developed for polyoxyethylene-type surfactants, the HLB has been widened to a much broader class of molecules by including the contribution of specific chemical groups, which may have an unexpected influence on the properties. The first version of the HLB considered a numerical index between 0 and 20, whereas an HLB between 0 and 10 identifies antifoaming agents, emulsifiers (for w/o emulsions) and wetting agents while HLB between 10 and 20 identifies emulsifiers (o/w), detergents and solubilizers. recent reviews<sup>11,81</sup> have reported typical values of HLB for biosurfactants. Values between 9 and 13 (wetting agent, o/w emulsifier, detergent) were estimated for mono-rhamnolipids and di-rhamnolipids, while values around between 12 and 15 for sophorolipids. Sophorolipids with a modified, more hydrophobic, tail were reported to have values around 8, while mannosylerythritol

lipids have values ranging from 6 to 12, depending on the degree of acetylation. Despite these efforts, it seems to the authors that calculation of HLB for biosurfactants is not systematic and its reliability unclear, probably due to the atypical chemical structure (bolaform, divalent) of most compounds and responsivity to pH. More systematic work, but also consideration of more complex models, like the hydrophilic-lipophilic difference (HLD),<sup>82</sup> are needed to reliably associate this approach to biosurfactants.

The association of biosurfactants to other substances to prepare emulsions in view of more complex formulations also constitutes hot topic of research, namely for application in cosmetics.<sup>83</sup> Besides natural polyelectrolytes,<sup>78</sup> work has been performed on whey protein<sup>84</sup> or zein.<sup>85</sup> Finally, biosurfactants were also successfully tested as demulsifier for crude oil water emulsions.<sup>86</sup> These examples show that the suitability of biosurfactants per se in certain applications needs to be carefully evaluated.



**Figure 4 - Structures of sophorolipid esters and their use in emulsifying almond oil. The percentage interfacial tension is shown against the surfactant concentration (LSL: lactonic sophorolipids; ASL: acidic sophorolipids) while the appearance of the emulsion is shown against the surfactant and oil concentrations in time Reprinted from Ref. <sup>74</sup>. with permission from the American Chemical Society.**

### Potential applications and future development

First association of most people being asked about applications of (bio)surfactants are the obvious ones that everyone experiences in daily life, when it comes to cleaning of surfaces (kitchen), fabric / clothes (laundry) or body (skin, hair). These applications are meaningful if consumers are

willing to pay higher prices for formulations containing biosurfactants, with obvious advantages like mildness and biobased origin. Consumer brands are thus also the drivers in developing formulations based on biosurfactants.<sup>87</sup> These applications aim to partly substitute conventional surfactants with biosurfactants in optimized and established formulations / applications. However, this replacement might not leverage the full potential of biosurfactants,<sup>87</sup> since their self-aggregation might be different to conventional straight chain surfactants.<sup>10,88</sup> The most prominent evidence for the difference of biosurfactants like sophorolipids (SL) and rhamnolipids (RL) to conventional surfactant can be found in their double hydrophilic structure. The polar carboxylic group might bind to inorganic surfaces of metal oxides, which might be slightly positively charged whereas the hydrophilic sugar groups provide compatibility with water. Thus, first examples for tentative applications of SL and RL were in stabilization agents for nanoparticles.<sup>89</sup> Following this application in which small amounts of biosurfactants are used in lab-scale experiments, the field of metal flotation in ore with SL and RL gained significant interest in the past years.<sup>90</sup> Finally, not to forget the original interest of the scientific community of these molecules as bioemulsifiers in the bioremediation of oil, and this since the 1970s.<sup>5</sup> The organisms can cope with both high concentrations of hydrocarbons and eventually use them as substrate to grow thus cleaning soil by metabolization of hazards. Applications in food science and cosmetics<sup>73,75,83,91-93</sup> were also mentioned, but in all cases, at the present state of the art, we are not aware of actual commercial goods fully based on biosurfactants, even if very few examples of formulations can be found.

Future developments not only concern applications but also the molecules themselves, as one cannot ignore the recent advances in generating new amphiphilic molecules of microbial origin, which may have interesting properties but also serve as standards for quantification and analysis.<sup>94</sup> Chemical engineering of existing biosurfactants is a classical, obviously the easiest and most reliable way. Hydrolysis, hydrogenation, ozonolysis, amidation, esterification, polymerization and more<sup>95</sup> are classical paths since long employed with sophorolipids<sup>95</sup> and which successfully lead towards a wide family of compounds, like saturated SL, short-chain SL, aminyl SL, divalent SL, ammonium SL, etc... In comparison, chemical derivatization of other biosurfactants has been a much less explored route,<sup>54</sup> so far, although chemically-modified rhamnolipids were produced long ago already.<sup>96</sup> Enzymatic catalysis has also been employed since long time,<sup>97</sup> but at a much less extent. This route is certainly very promising because it does contribute to degrade the environmentally-friendly image of the biosurfactants.

Microbial engineering is the other obvious route to generate variation in the biosurfactant



portfolio. Expanding the type of microorganisms can certainly provide a broad molecular variety, as known for rhamnolipids,<sup>98</sup> mannosylerythritol lipids<sup>99</sup> and actually almost all biosurfactants. The main drawback of this approach are the selectivity and low production titers. Feeding the microorganisms with a desired substrate can sometimes generate new compounds, although purification can be cumbersome due to the tendency of fungi and bacteria to produce *de novo* intermediates. One can outline a certain success in the production of sophorolipids derivatives, like petroselinic SL,<sup>100</sup> among others (saturated, elaidic, linoleyl). As far as the authors (who are not trained microbiologists) understand, this is a trial and error approach, where failure can come from low titers and high molecular diversity.

Genetic engineering is the third method and it has proved highly reliable to produce new compounds. Most work has been done so far in the field of sophorolipids since when the gene of *S. bombicola* were described.<sup>101</sup> Single-glucose lipids<sup>102</sup> among other compounds (acetylated sophorolipids, lactonic sophorolipids, tetrasophorose bolaform sophorosides or sophorolipid alcohol) were reported and studied in the past years.<sup>8</sup> Putting aside the large amount of preliminary work needed to develop engineered strains, the main advantages are the high selectivity and uniformity<sup>103</sup> and, in some cases, high titers. However, consumers' negative perception of genetically-modified organisms could be a non-negligible factor to limit this route in the long run. Moreover, chemical post-modification of biosurfactants obtained by fermentation might also reduce attractiveness for consumer and also the footprint and environmental gentleness would suffer from additional steps.

Finally, an interesting approach combines both chemical and microbial engineering strategies for the development of new families of biosurfactants with interesting structures, self-assembly, antimicrobial and gene-transfection properties.<sup>24,104</sup> Downstream processing is also a key factor towards the synthesis, production and commercialization of biosurfactant in view of generating formulations and products with reproducible properties.<sup>105</sup>

### **Understanding structure-property relationship**

As mentioned above biosurfactants had been known in the 1970s when all the relevant theories and models on aggregation, surface activity and interaction between different surfactant had been illuminated. And although the behaviour of alkylpolyglucosides and fatty acids / soaps had also been extensively studied, there is still little understanding of the double hydrophilic surfactants carrying both sugar groups and carboxylic acid groups. Studying soaps can be time-consuming due to (kinetically trapped) multiple co-existing phases and because of the experimentally hard-to-study

stimulus of degree of protonation and susceptibility of certain counterions as e.g. magnesium and calcium. Surfactants carrying sugar molecules as hydrophilic headgroups are made by chemical reaction with no specific prevalence to potential stereocenters. Several studies on sugar surfactants and on biosurfactants such as e.g. rhamnolipid with chiral carbon atoms show differences between the enantiopure components and also to diastereomeric mixtures.<sup>94</sup>

A forecast of the properties either if it is surface tension, foaming or wetting is thus hard to obtain without having the molecules actually in ones hand. And all of that will depend on the degree of protonation which on one hand will add ionic character to the molecule / micelles which might impart interaction with oppositely charged molecules as well as that the protonated carboxylic acid will interact differently to sugar molecules of other surfactants as compared to the ionic soap. The understanding of all these complex interaction on molecular level is more than challenging and can only be tackled by proper analysis of the surfactant, control of the aqueous solution including electrolytes and pH-value as well as by sophisticated methods like small angle neutron scattering,<sup>46,106</sup> and needs to be accompanied by simulation.<sup>107</sup> However, also systemic understanding of interaction between different (bio-)surfactants, e.g. by beta-parameter describing the interaction between two surfactants by studying the critical micelle concentration of the pure (bio-)surfactants and their mixtures would help to foster the understanding. The beta-parameter would not discriminate if the interaction is related to headgroups or hydrophobic part of the molecules (or both) but would at least a sum parameter. Same is true for other methods like isothermal calorimetric titration which measures the interaction via dissipated heat upon mixing of different surfactants.<sup>108</sup>

## **Conclusion**

This review, and in particular the special issues on biosurfactants in COCIS as well as numerous articles and review recently released might create the impression that a lot of progress in the field of biosurfactants had been obtained in the last years. The gain in knowledge seems to be, however, limited to certain representatives like sophorolipids and rhamnolipids. The research on these two types of molecules is reasoned in the collaboration between fermentation experts and physical chemists, which were able to reveal some of the intriguing properties of these compounds.

But there is definitely more molecules available, which are needed to be made accessible by experts in synthesis (either biological or chemical). Proper characterization of the molecules is the first step, finding more and more formulations, synergistic effects and applications are the prerequisite for further potential commercial success of biosurfactants. Scale-up of fermentation processes is already

a difficult task to do; implementing of new surfactants even adds complexity on top of that.

The mildness towards enzymes make all biosurfactants interesting in application in which intended (cosmetics) or non-intended (cleaning) contact with the molecules exist. Further strong and close collaboration is needed between universities, academia and private sector as well as between companies. The multiple inter- and intra-molecular interactions, hydrogen bonds, stereochemistry and degree of protonation give biosurfactants innumerable ways to interact with each other which makes the study of their behaviour difficult, yet interesting.

The application potential of biosurfactants in a number of fields, cosmetics, home and personal care, agriculture, pisciculture, materials science, pharmaceuticals and medicine, just to cite the most discussed in the literature, will only be unlocked when key questions will be solved, namely the production costs, the purity and homogeneity, the phase diagram, the importance of molecular conformation and structure-properties relationship in general, formulation with other surfactants, just to cite the most important aspects.

### **Declaration of competing financial interests**

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Jochen Kleinen reports a relationship with Evonik Industries AG that includes: employment. Niki Baccile declares no competing financial interests or personal relationships that influence the work reported in this paper

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