Advanced Materials from Microbial Fermentation: the Case of Glycolipids and Nanocellulose

Niki Baccile, Sophie Roelants, Eero Kontturi

Advanced Materials from Microbial Fermentation: the Case of Glycolipids and Nanocellulose

Niki Baccile,[a] Sophie Roelants,[b,c] Eero Kontturi[d]

[a] Laboratoire de Chimie de la Matière Condensée de Paris, Université Pierre et Marie Curie, 75252 Paris Cedex 05, France
[b] Bio Base Europe Pilot Plant, Rodenhuizekaai 1, 9042 Gent, Belgium
[c] Laboratory for Industrial Biotechnolgy and Biocatalysis, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000, Gent, Belgium
[d] Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University, P.O. Box 16300, 00076 Aalto, Finland

Abstract français

La chimie verte est une discipline relativement récente régie par douze principes fondateurs, incluant notamment l'économie d'atomes, la prévention de la pollution via des méthodes de synthèse chimique respectueuses de l'environnement, comme par exemple celles privilégiant le milieu aqueux aux solvants organiques, mais aussi le développement de produits chimiques et matériaux issus de la biomasse végétale. Dans ce contexte, la synthèse microbienne est un outil de choix pour supplanter, dans certains cas notables, les approches classiques basés sur la chimie organique de synthèse. Par exemple, les polyhydroxyalcanoates, des polyesters biodégradables, sont produits par voie bactérienne à partir de lipides et sucres et ils ont été identifiés comme des remplaçants potentiels au polypropylène dès les années '70.

Plus récemment, on commence à s'intéresser à la synthèse microbienne de composés sucrés polymériques, comme le dextrane ou la cellulose, ou lipidiques, comme les glycolipides amphiphiles. Bien que les pratiques de production microbienne de composés glycosylés datent de plusieurs décennies, l'essor de la chimie verte en tant que discipline au niveau international encourage des équipes de chercheurs pluridisciplinaires à se pencher sur la production, la diversification, l'étude des propriétés et les applications de cette classe de composés, dépassant ainsi la communauté des chercheurs en microbiologie, historiquement intéressés au développement des produits de fermentation de microorganismes.

Dans cet article nous souhaitons développer le thème mentionné ci-dessus en se focalisant sur la nanocellulose bactérienne, pour ce qui concerne les polymères glycosylés, et les biotensioactifs, pour ce qui concerne les systèmes lipidiques glycosylés. Le choix de ces deux systèmes est justifié par le fort développement des matériaux à base de nanocellulose mais aussi par le besoin de remplacer en partie les tensioactifs « classiques », sources non négligeable d'émission de CO_2 au niveau mondial. Nous illustrerons les principales classes de molécules, les méthodes classiques de synthèse, les propriétés ainsi que quelques exemples d'application notoire. Nous terminerons par quelques perspectives sur le sujet.

Abstract anglais

Green chemistry is a recent discipline ruled by twelve founding principles, which include, among others, atom economy, the prevention of pollution via environmentally friendly chemical synthesis methods, such as, for example, the choice of an aqueous medium over organic solvents, but also the development of chemicals and materials derived from plant biomass. In this context, microbial synthesis is a tool to supplant, in some notable cases, syntheses by a standard organic chemistry approach. For example, polyhydroxyalkanoates, biodegradable polyesters produced by bacteria from lipids and sugars, were identified as potential substituents of polypropylene since the 1970s.

More recently, attention has begun to be given to the microbial synthesis of polymeric sugars, such as dextran or cellulose, or lipids, such as amphiphilic glycolipids. Although the microbial production of glycosylated compounds can be traced back by several decades, the development of green chemistry as an international discipline is encouraging teams of multidisciplinary researchers to

focus on production, diversification, and applications of this class of compounds, thus going beyond the community of researchers in microbiology, historically interested in the development of fermentation products from microorganisms.

In this article, we wish to develop the above mentioned theme by focusing on nanocellulose, representing an important glycosylated polymer, and on biosurfactants, in regards of the glycosylated lipids. The choice of these two systems is justified by the strong development of nanocellulose-based materials but also by the need to replace in part the "conventional" surfactants, a significant source of CO_2 emissions worldwide. We will illustrate the main classes of molecules, the classical methods of synthesis, the properties as well as some examples of notorious application. We shall end with a few perspectives on the subject.

Introduction

The present work has the goal of illustrating two notorious examples of a sustainable chemistry approach in the field of molecular and material science. For the former, we have chosen the field of glycolipids, while for the latter, we have chosen the field of nanocellulosic materials. Glycolipids are generally considered as standard “green” surfactants in their alkyl polyglycoside (APG) form, obtained by standard glycosylation reactions from sugars and fatty oils.[1] These products are commercially available in many detergent formulations. On the other side, nanocellulose has now became a standard “green” material derived from cellulose after a strong acid hydrolysis. To overcome these standard approaches, microbial fermemtation techniques have been developed since decades to prepare biobased glycolipids and nanocellulose. An overview of each domain, including standard synthesis, properties and up-to-date applications are presented below.

Bacterial nanocellulose

*Nanocellulose: an introduction***.** Nanocellulose as a term originated only in the first decade of the 2000s[2] but many of the current nanocellulose species had been in existence at a much earlier date.[3] At present, one of the most researched aspects of nanocellulose concentrates on their use as a reinforcing phase in bio-based and/or biodegradable composites that may step in to replace plastics or appear as completely new materials in their own right.[4] In essence, nanocellulose is generally categorized under three discrete groups: cellulose nanocrystals (CNCs), cellulose nanofibers (CNFs) and bacterial cellulose (BC).[2] CNCs and CNFs are usually extracted from plant sources and the basis of their production involves disintegration of the plant cell wall. CNFs are in fact isolated cellulose microfibrils, the basic supramolecular units of native cellulose. Microfibrils are longitudinal threads of very high aspect ratio, just a few nanometers in width yet micrometers in length.[5] CNCs, in turn, are usually prepared by acid hydrolysis of native microfibrils. Controlled hydrolysis is able to degrade the disordered segments in the semi-crystalline cellulose microfibrils, leaving the crystallites intact.[6,7] The end result is CNCs: short, rigid rods of crystalline cellulose that spontaneously self-assemble into chiral nematic liquid crystals beyond a certain threshold concentration. In this short review, however, we will focus only on BC.

Bacterial cellulose: origin, preparation, morphology, general issues. BC is the odd one out of the nanocellulose family. It is usually treated as a separate category but essentially, BC could be considered a type of CNFs. BC consists of cellulose microfibrils synthesized by certain bacteria to an extracellular matrix.[8] BC microfibrils are in a completely isotropic arrangement, lacking the tight hierarchical morphology of plant cellulose confined in the cell wall (Figure 1a). Therefore, one can say that BC is directly synthesized as a nanocellulose species without the need for specific isolation from a growth matrix. BC is also the only chemically pure form of nanocellulose as the other species are always embedded in a matrix of hemicellulose and – with land plants – lignin which are next to impossible to remove completely during nanocellulose preparation.[9] It is only certain types of bacteria that are able to synthesize BC from glucose. The most popular species for actual BC production is called *Acetobacter xylinum* or in modern nomenclature, *Gluconacetobacter xylinus*. The BC synthesis was first reported in 1886,[10] although the product was only much later identified as cellulose.[11] Culturing with *G. xylinus* is an aerobic process where BC grows at air/water surface in order to utilize the oxygen from air.[8,12] The

most used monosaccharide sources for BC production are glucose and sucrose. Glucose is converted via several steps into uridine diphosphoglucose (UDP-glucose) which is then polymerized into cellulose, i.e., a homopolymer consisting only of anhydroglucose units (Figure 1d).[8,12] BC producing microbes are also able to utilize other monosaccharides to produce UDP-glucose and further to cellulose. A case in point is fructose – the other monosaccharide in the sucrose dimer – but several other monosaccharides have been trialed as a cellulose source as well.[8] Commonly, the method of growing BC in static conditions at the air/water surface is relatively slow but nevertheless suitable for laboratory work. A few semi-continuous bioreactor designs for static culturing have been proposed throughout the past years.[13] Moreover, submerged fermentation has been investigated but the techniques face intrinsic problems, above all the supply of oxygen is usually too low for sufficient production.[14] BC consists chemically from exactly the same polysaccharide that makes up the cellulose microfibrils in plant cell walls. BC strands are also called microfibrils but their morphology is somewhat different to those in plants. Cellulose microfibrils in plants are monodisperse in width, generally 3-20 nm depending on the botanical source, and micrometers in length, that is, they are somewhat reminiscent of spaghetti in nanosized form. Instead of a more or less square cross sectional shape prevalent in plants, BC microfibrils possess a rectangular cross section with sides of 7 × 70-145 nm, thus rendering the appearance of the microfibrils in the shape of flat ribbons, somewhat reminiscent to tagliatelle in nanosized form. Fink et al. proposed that the flat microfibrils consist of elementary fibrils of 7 × 13 nm cross section. Indeed, when preparing CNCs from BC, the flat structure is broken also at the crystallite level and the cross sections of the CNCs bear roughly the dimensions of 7 × 7 nm.

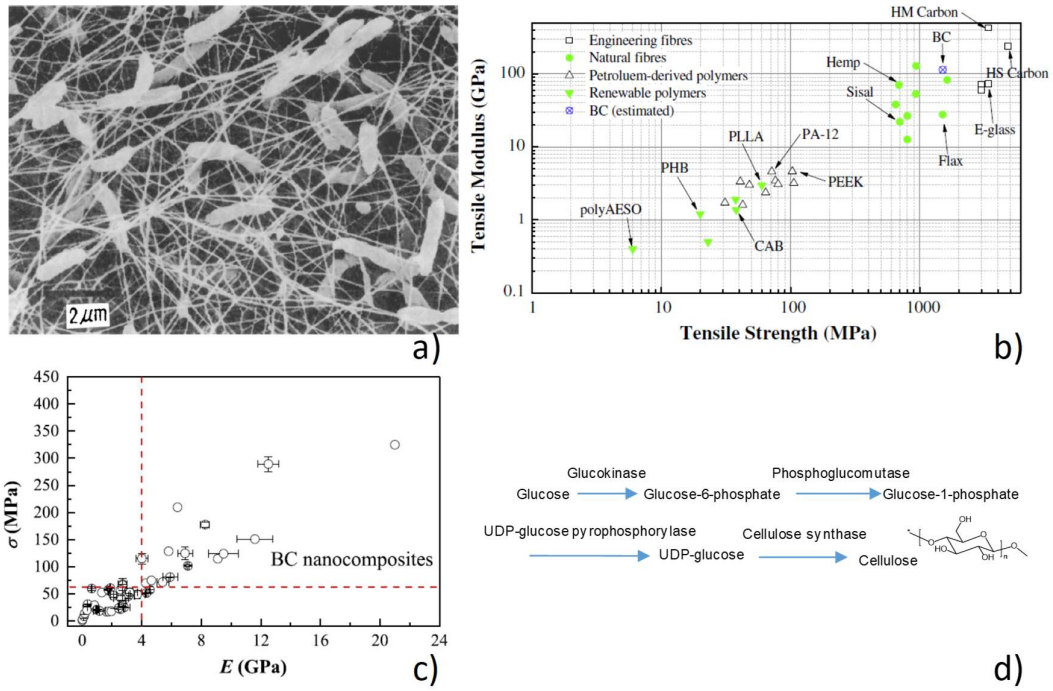

Figure 1 – a) Scanning electron micrograph of bacterial cellulose gel. The gel still contains the bacteria which are generally removed after culturing by a simple washing step. Reproduced from ref. 8. Copyright Springer 2000; b) Average tensile properties of BC in comparison to some commonly used synthetic and renewable fibers. c) Tensile strength versus tensile modulus of selected BC composites. The dashed lines denote tensile properties of neat PLA. Reproduced from ref. 15. Copyright Elsevier 2014. d) Simplified pathway from glucose to bacterial cellulose

Applications of bacterial cellulose. The peculiar feature of BC is that it forms a hydrogel pellicle after the synthesis. The pellicle consists of isotropic BC microfibrils. Additionally, BC is exceptionally effortless to purify from other components that cellulose; a mild alkali washing is sufficient to remove the bacteria and additional sugars. In other words, BC is a nanocellulose hydrogel in a very pure form,

which is something that many nanocellulose procedures are attempting to achieve from plant materials with much effort. It is, therefore, quite an obvious development that BC has been subjected to similar investigations as the rest of the nanocellulose family, composite materials being among the most popular targets for applications. Furthermore, biomedical applications, particularly wound dressing materials, have been fashionable with BC because of its chemical purity and non-toxicity. The rationale for using BC as a reinforcing phase in composites stems from its exceptionally high mechanical strength. Figure 1b shows the tensile strength and modulus of BC nanofibers in the context of a variety of other natural and synthetic fibers.[15] In general, the values reported for individual BC nanofibers as well as other nanocellulosic species vary somewhat, depending on the measurement technique.[15] It is obvious that BC works well as a composite reinforce: Figure 1c shows a collection of data from BC/polymer composites where the tensile strength has been plotted as a function of modulus.[15] The dashed lines denote the strength and modulus of poly(lactic acid) (PLA) because of its status as one of the highest performing non-petroleum derived polymers. The performance of BC and other nanocellulose grades has not lived up to their theoretical potential in polymer composite materials.[15] One of the major problems is the incompatibility between cellulose and the continuous composite matrix. Polymer matrices are often hydrophobic, resulting in aggregation and phase separation of the cellulose when mixed together.[16] Another seminal challenge is the susceptibility of BC to water and humidity: as with ordinary paper products, the strength of nanocellulose-containing products is usually impaired when in contact with water or vapor.[17] Multiple ways of surface modifying all kinds of nanocellulosic grades for better compatibility or water resistance exist[18] but the methods are usually not suited for large scale production required for composite preparation. For comprehensive reviews on polymer/nanocellulose composites, the reader is referred to articles by Siró and Plackett,[19] Siqueira et al.[20] and Lee et al.[15]

Biomedical applications are another strong research trend with BC-containing materials. Wound dressing, in particular, has received a lot of attention with even commercial or pre-commercial products in the line.[21] Here, the high mechanical strength of BC, its hygroscopicity, non-toxicity, antimicrobial nature, and ability to geometrically conform in various shapes are seen as the principal assets. The similarity of BC nanofibers with collagen fibers of the skin improves the biocompatibility. Other biomedical applications of BC include tissue-engineering[22] and drug delivery.[23]

Microbial glycolipid biosurfactants

The surfactant market. Surfactants are performance molecules that intervene in nearly every product and aspect of human daily life and find applications in/as very broad array of markets and applications: chemical, pharmaceutical, cosmetic & personal care, packaging, carpets & textiles, detergents, paper, adhesives, (3D printing) inks, mining and leaching, healthcare, polymer, food and feed, paints, surface and industrial coating, etc. The global turnover of surfactants was worth USD 30.64 Billion in 2016 and is expected to reach USD 39.86 Billion by 2021, registering a CAGR of 5.4% between 2016 and 2021[24] which shows that they are bulk products with a significant economic and environmental impact. Despite the efforts to move towards a more environmental friendly economy, only 3 % of the surfactant market share consists out of molecules, which are 100 % biobased and even a smaller part < 0,5 % of entirely biologically produced biobased surfactants, such as microbial, plant based or enzymatically produced biosurfactants. There are myriad pathways to process crude oil or gas towards the hydrophobic building blocks of synthetic surfactants, e.g. Fischer-Tropsch synthesis, oxo process, olefin oligomerization or Friedel-Crafts alkylation. The constant consumer and hence market demand for sustainable, biobased and green solutions, has resulted in a substantial increase of the development and use of partly biobased and even wholly biobased surfactants (WBS) (100 % derived from renewable (non-fossil) biomass in such applications.[25] With respect to this terminology: the European Commission has issued a mandate to the European Commission, for Standardization (CEN) to generate general standards providing a common basis with respect to terminology, bio based content determination, sustainability aspects, life cycle analysis and declaration tools for Business to Business (B2B) and Business to Consumer (B2C), which was done within the CEN/TC-276 technical committee in cooperation with the ERASM Biosurfactant Taskforce (www.erasm.org). Shortly said, the

four categories are shown in Figure 2a and range from > 95 % (wholly bio-based); > 50 % and < 95 % (majority bio-based); > 5 % and < 50 % (minority bio-based) and < 5% (non bio-based). The wholly bio-based surfactants (WBS) according to ISO 16128 norm, represent about 3 % of the European surfactant market and have the best image. Subsequently, there is a strong drive of the industry to apply this type of surfactants in their products, of course without giving in performance or cost. Most WBS are based on sugars coupled to fatty acids and/or alcohols i.e. methylester sulphonates, alkylpolyglucosides, sorbitan esters, anionic APG derivatives, sucrose esters, methyl glucoside esters, fatty acid N-methylglucamides, alkylpolypentosides, etc. However, some limitations/drawbacks are associated with the current WBS portfolio:

- the largest part of the wholly biobased surfactants on the market are produced through chemical processes, which negatively impacts their environmental profile
- functionality/variety is limited
- they do not offer an easy possibility for further derivatization/functionalization
- they show good performance, but do not boost performance by e.g. combining properties justifying their higher price
- can only be produced from first generation substrates

Several new technologies are currently being developed, that can alleviate these issues. Related to the first hurdle, full biological production processes are associated with an even better environmental profile and additionally offer better marketing opportunities to the companies, which is a big driver.

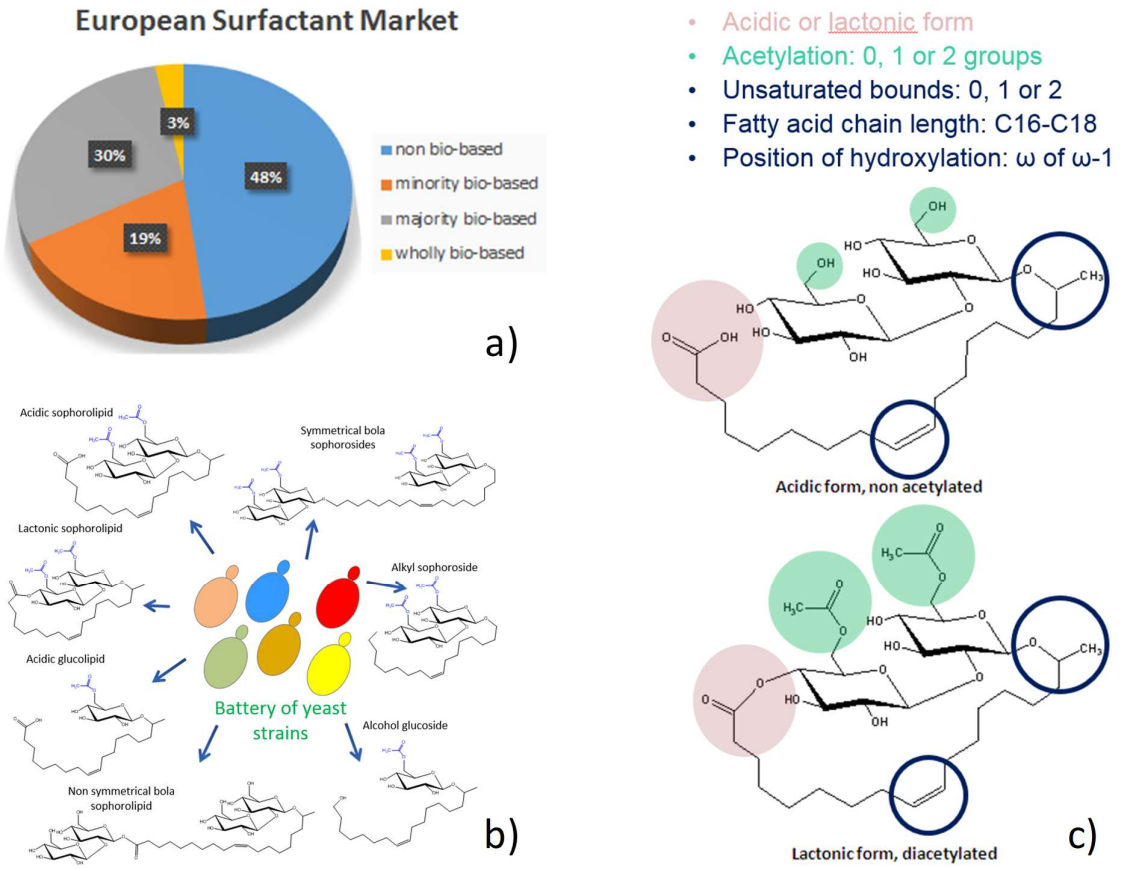

Figure 2 – a) Use of bio-based and non bio-based surfactants in the European Union, Norway, Switzerland and Iceland (2015).[25] b) Glycolipid portfolio developed by InBio.be based upon the yeast *Starmerella bombicola.* c) Representation of variation in wild type sophorolipids

Biosurfactants. The current state of the art for biologically produced surfactants (estimated by the authors to currently only account to a few thousand tonnes per annum thus representing only a very small amount of the surfactant market share) can be defined as:

- plant derived molecules like e.g. saponins or cardolite commercialized by e.g. Foodchem, Dr. H. Schmittmann GmbH and Cardolite respectively
- enzymatically produced molecules like e.g. enzymatic sugar esters, which are not on the market yet, but a lot of research is conducted in this field
- microbially produced molecules like sophorolipids (SLs) launched on the B2B market by Soliance, Evonik and planned by Croda and applied by Ecover, Saraya, Henkel, Soliance, etc.; rhamnolipids (RLs) commercialized by Logos, Jeneil, Urumqi Unite Biotechnology, Biotensidon, Agae and Rhamnolipid.inc; Mannosylerythritol lipids (MELs) commercialized by Toyobo, Kanebo, Damy Chemicals, Biotopia co. and investigated by many more; Lipopeptides (LPs) commercialized by Lipofabrik, Kanebo and Kaneka and Xylolipids (XLs) to be commercialized by INS.

Microbial surfactants already offer a solution to the first drawback mentioned above. They have been very extensively investigated as they offer some opportunities, e.g they can be produced from second generation (2G) substrates and waste streams like molasses, animal fats, dairy industry whey and other waste or side streams[26,27]. Recently, efficient sophorolipid production (wild type) was shown on 100 % 2G substrates (both hydrophilic as hydrophobic part) inside the framework of the BBI Carbosurf project, which will be published elsewhere. Derivatization efforts for sophorolipids -the best represented microbial biosurfactant on the market- have been investigated quite extensively[28]. In terms of chemical derivatization of wild type SLs, too many hurdles, like low yield and low uniformity related to the original complexity of the wild type SLs, are associated with these processes. Enzymatic derivatization strategies have also been investigated[28] and even commercialization efforts were done by the American company Synthezyme.

Three main reasons can be defined to explain low commercialization levels of biosurfactants. *Cost considerations* are to date the prime hindrance to market penetration of (microbial) biosurfactants. The maturity of the petrochemical industry makes purely cost-based competition unrealistic for most biosurfactants. Moreover, even the availability of biosurfactants with equivalent performance and at the same cost would not per se be sufficient to drive acceptance and utilization by consumers and brand owners. A better performance at an acceptable premium price would increase their marketability for mass consumption products as consumers anno 2017 demand environmentally friendly and benign products/processes, without wanting to give away functionality/performance. This situation requires the development of innovative and new solutions as the industry is currently increasingly driven by consumer awareness and opinion. A second hurdle is the *lack of diversity*. Formulation experts are like artists, demanding a diversified *palette* of molecules to shake and stir to get to a desired endpoint. Whereas the chemical industry has been very successful in developing an extraordinary wide variety of surfactant structures for a multitude of applications/functions, the biobased counterparts have not been able to offer the same diversity. To give an example, the chemically produced biosurfactants APG's (alkyl polyglucosides), mainly consist of mono-glucosylated (degree of polymerisation (DP) = 1) molecules and a small amount of di-glucosylated (DP = 2) molecules. Consequently, the structural diversity of these biosurfactants is rather low with an average DP ranging between 1,2 and 1,6. Although APGs are one of the most prominent biosurfactants on the market, their narrow product spectrum limits further growth. Moreover, they do not offer a possibility for further derivatization/functionalization. Several new technologies are currently being developed. The large chemical company Evonik for example launched sophorolipids, a microbial biosurfactant, as an ingredient on the European B2B market in 2015, while they are currently taking actions to do the same for the microbial surfactant rhamnolipids. Also other big companies like Croda and Henkel are highly active in this field. Although these observations are already a step in the right direction, the biosurfactant diversity can still by far not compete with the portfolio of fossil derived surfactants. A third reason is the fact that (microbial) biosurfactants typically are defined by the occurrence of very similar congeners i.e. *production of mixtures*. For the microbial biosurfactants sophorolipids, these are mainly divided into a mixture of lactonic and acidic sophorolipids (Figure 2c).

Additional variation within the congeners in the hydrophobic tail length, saturation degree and positioning of the sophorose head group (terminal (ω) and subterminal (ω-1) (Figure 2c). Easily over 30 congeners can as such be identified in 'sophorolipids', which are thus not defined by one single molecule. Mixtures might do the job and could thus be perfectly marketed, but these biological systems are sensitive to small variations in medium/cultures conditions, which will influence the composition of the mixture, which in turn gives rise to very different properties. This in-product-variation is one of the reasons of the very varying reports in the literature on their properties. The latter is simply unacceptable from an industrial point of view. Combined with the reasons mentioned above, this is one of the main reasons why microbial biosurfactants currently only represent a marginal part of the surfactant market.

A number of solutions can be defined for the mentioned bottlenecks. Variation can be increased by applying chemical and/or enzymatic derivatization possibly in combination with an expansion of the molecular variety through genetic engineering. The latter can also increase productivity (decrease of price) and uniformity (decrease of mixtures). The efforts spread over the past 15 years,[29] especially at the University of Ghent (Belgium),[30,31,32] have resulted in the generation of a proprietary platform technology for the production of new types of glycolipid molecules (Figure 2b). This has been accomplished by the development of molecular techniques and constant expansion of the molecular toolbox for this non-conventional yeast and through the development and use of several -omic strategies. Although these engineering efforts have thus been quite successful, none of the abovementioned efforts have yet resulted in the commercialization of a new-to-nature compound. This is due to a few reasons:

- new molecules translate/correspond with new processes, different properties and thus different applications.
- the non-optimized production processes and strains logically correspond with higher production costs
- the absence of the molecules on the market works inhibitory for the economy of scale to kick in nor is regulatory approval granted (or data available for initiation of a regulatory dossier) although the related wild type SLs are.
- the lack of knowledge on the properties of these new molecules

These hurdles are thus the next steps to take to valorized this technology and this is done by applying an integrative process design approach combining strain engineering with process development and optimization.

Applications. The discussion above mainly concerns the surface active properties, by far the most important from an industrial development point of view, of microbial glycolipid biosurfactants. However, a series of new perspectives have been opened by research groups working mainly in Japan, India, France and USA. The biocompatibility of most glycolipids and the presence of the free carboxylic acid group made them interesting candidates for the water stabilization of metal and metal oxide nanoparticles. The first work in this field was proposed by the group of Prasad at the NCL in Pune, India. Cobalt,[33] silver,[34] gold[35] but also iron oxide[36] nanoparticles (Figure 3a) have been used as systems to be coated with the acidic form of sophorolipids. In all cases, the resulting sophorolipid-coated system is highly dispersible in water and it was shown that, in the case of metal systems, sophorolipids can also act as a reducing agent, thus excluding the use of strong, classical, reducing agents like $NaBH_4$. Cytotoxicity and genotoxicity tests performed on the gold and silver nanoparticles systems have shown no specific biological activity below 100 □g/mL,[35] thus making these systems interesting candidates for biomedical applications. The impact of the sophorolipid coating on the colloidal stability was shown for the iron oxide nanoparticles, which were shown to be dispersible and stable in time up to 2 M KCl solution, thus showing the strong stability of the COOH-FeOx complex.[36] Antimicrobial activity of glycolipid biosurfactants in solution is a field of research since a long time. However, more recent works have shown their interest as surface antimicrobial and/or antiadhesive coatings. Dispersion of rhamnolipids on both hydrophobic (octadecyltrichlorosilane-modified glass) and hydrophilic (hydroxyl-rich glass) surfaces were shown to have an interesting antiadhesive effect on Gram-negative *E. coli*, *P. putida* and *P. aeruginosa* and on Gram-positive *B. subtilis*. In particular, adhesion of *P. aeruginosa* (number of attached cells per mm^2) was reduced by a factor 15 on the hydrophilic and by a factor 20,

respectively, on the hydrophilic and hydrophobic glass substrates with respect to control.[37] (Figure 3d) This system, together with *B. subtilis*, were by far the most effective with respect to the other pathogens tested in the study. On the contrary, if the glycolipid (sophorolipid) is chemically grafted on the substrate via its pending COOH group (amidation reaction with a surface aminothiol primer deposited on gold), biocidal properties against both Gram-positive (*L. ivanovii*, *E. faecalis*, *S. epidermidis*, *S. pyogenes*) and Gram-negative (*E. coli*, P. aeruginosa, *S. typhymurium*) bacteria are observed instead.[38,39] If the efficiency is, in the best case, only of about 45% of the bacterial population present on the surface, these works show the first biocidal effect of a glucose derivative and join the debate concerning the interactions between carbohydrates and cell membranes.[40,41]

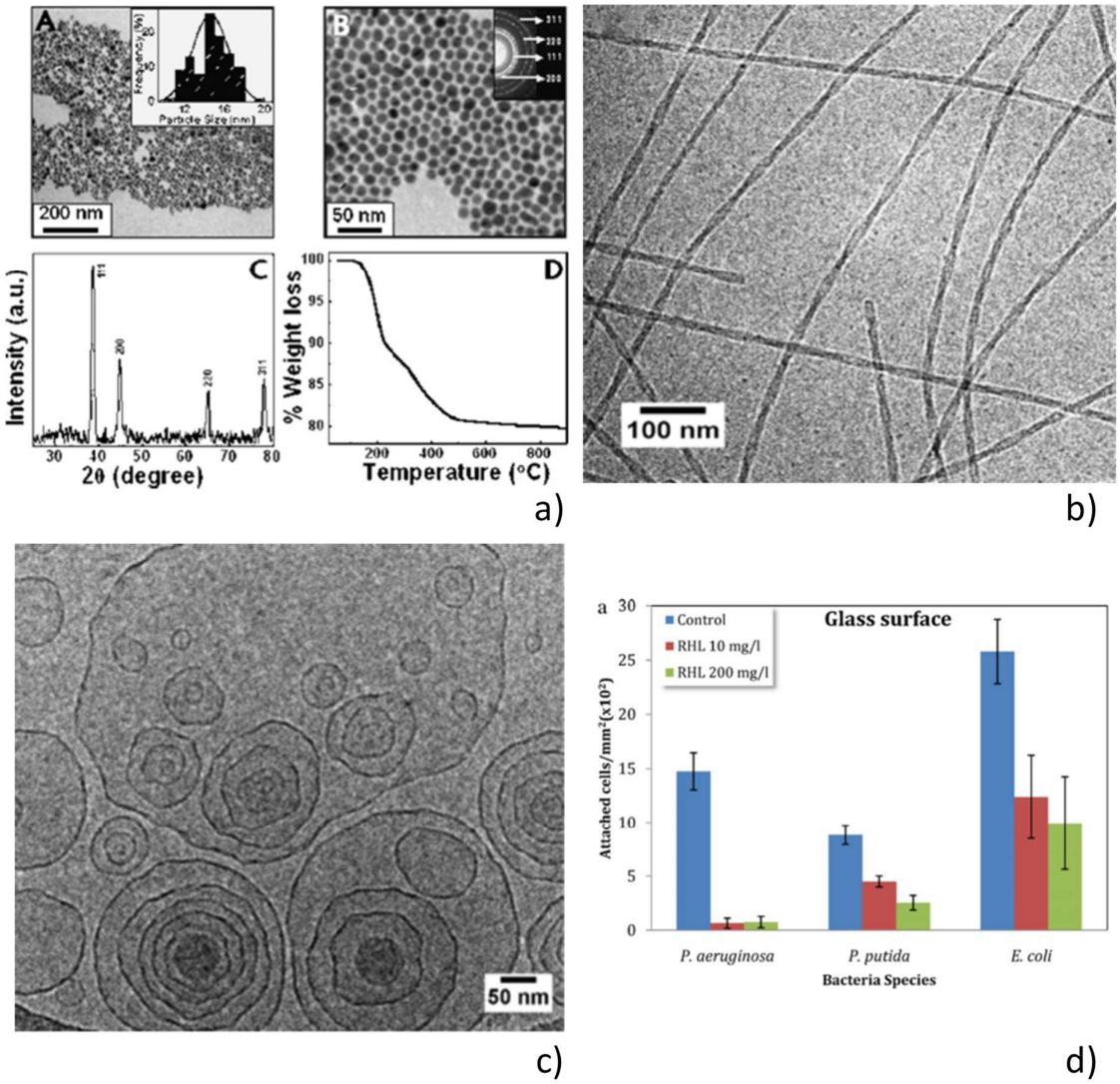

Figure 3 – a) Sophorolipid-capped silver nanoparticles (morphology, size, structure and organic content are respectively presented in panels A-B, C and D). Reproduced from ref. 42. Copyright Royal Society of Chemistry 2008. b) Self-assembled nanoscale twisted ribbons obtained from stearic acid sophorolipids. Reproduced from ref. 43. Copyright Wiley-VCH Verlag 2015. c) Glucosomes obtained from branched C22 sophorolipids. Reproduced from ref. 44. Copyright Wiley-VCH Verlag 2017. d) Antiadhesive effects of rhamnolipids-coated glass substrate. Reproduced from ref. 37. Copyright Wiley-VCH Verlag 2013

Surfactants are known to spontaneously self-assemble in water, and glycolipid biosurfactants do not derogate this rule. Self-assembly properties have been reported for mannosylerythritol lipids, rhamnolipids, sophorolipids, glucolipids, cellobioselipids, just to cite the most important ones.[45–50] If

the knowledge in this field is more or less advanced according to the effort that a specific research group has dedicated to a given family of molecules, one can summarize, without being exhaustive, the following morphologies: micelles,[45,50–52] vesicles,[45,48,49] fibers,[48,49] lamellar,[45,48] sponge[45] phases (Figure 3b-c). The nature of the molecule and concentration are the most obvious parameters that influence the self-assembled morphology, but pH[47,51,53] and temperature[45,47] have also been shown to have a strong influence on the self-assembly properties. This impressive load of work is still ongoing because the relationship between the structure of a given microbial glycolipid and the corresponding self-assembled phase is not obvious and cannot be based on the classical prediction based on packing parameter considerations, as recently shown for acidic oleic acid sophorolipids, which can form micelles or giant ribbons as a function of the purity but also, for a 100% pure compound, as a function of the preparation method.[54] One must here observe that microbial glycolipid systems are in fact never pure and in fact very hard to fully purify, because it is well known that microbial synthesis provide a system with a wide range of structurally-similar congeners where one or two are majoritarian.

Gene transfection, which consists in the delivery of genetic material across the cell membrane, is one of the applications in which control of self-assembly, and colloidal properties in general, is very important. In this field, mannosylerythritol lipids (MEL) have been employed as adjuvants in gene transfection of plasmid DNA using cationic liposomes as classical carriers and DNA binders.[45] It was shown that the presence of MEL increases the efficiency of gene transfection in NIH3, COS-7 and HeLa cells up to 50 to 70 times. The role of MEL seems to be the acceleration of membrane fusion between the cationic liposome and cell membrane, so that transfection efficiency is increased. More recently, quaternary ammonium-modified sophorolipids have been incorporated into negatively-charged DOPE liposomes and used as direct binders of negatively-charged plasmid DNA, to be transfected into A549, 16HBE and SKMEL28 cell lines.[55] Authors have found that two specific, long-chain, quaternary ammonium derivatives were highly efficient and with low cytotoxicity. The main difference between this work and the approach using MEL is the positively-charged sophorolipid obtained by chemical modification and its direct binding to plasmid DNA, thus giving it a direct role in terms of vectorization.

Conclusion

The present contribution shows two families of carbohydrate-rich compounds of microbial origin, one polymeric and the other molecular. Bacterial cellulose is an interesting polysaccharide challenging the use of plant cellulose for the easier approach in the purification and good water-dispersion properties. On the other side, microbial glycolipids are versatile compounds both in terms of lipid and carbohydrate structure that can self-assemble into a wide range of morphologies (micelles, vesicles, fibers…) and be used as antimicrobial and surface stabilizing agents, among others. Both families of compounds are biodegradable and non-toxic and have the goal of substituting, in the long run, petrochemical compounds. Nonetheless, in both cases, the industrial development of a fully microbial-based organic chemistry is far from being a present reality due to the high production costs and product variability.

References

1 W. Von Rybinski and K. Hill, *Angew. Chem. Int. Ed. Engl.*, 1998, **37**, 1328–1345.

2 D. Klemm, F. Kramer, S. Moritz, T. Lindström, M. Ankerfors, D. Gray and A. Dorris, *Angew. Chemie Int. Ed.*, 2011, **50**, 5438–5466.

3 A. F. Turbak, F. W. Snyder and K. R. Sandberg, United States, 1983.

4 K.-Y. Lee, Y. Aitomäki, L. A.Berglund, K. Oksman and A. Bismarck, *Compos. Sci. Technol.*, 2014, **105**, 15–27.

5 Y. Nishiyama, *J. Wood Sci.*, 2009, **55**, 241–249.

6 Y. Habibi, L. A. Lucia and O. J. Rojas, *Chem. Rev.*, 2010, **110**, 3479–3500.

7 E. Kontturi, in *Nanocellulose and Sustainability: Production, Properties, Applications, and Case Studies*, Taylor & Francis, 2017.

8 M. Iguchi, S. Yamanaka and A. Budhiono, *J. Mater. Sci.*, 2000, **35**, 261–270.

9 E. Kontturi, A. Meriluoto, P. A. Penttilä, N. Baccile, J.-M. Malho, A. Potthast, T. Rosenau, J. Ruokolainen, R. Serimaa, J. Laine and H. Sixta, *Angew. Chemie Int. Ed.*, 2016, **55**, 14455–14458.

10 M. Hakalahti, M. Faustini, C. Boissière, E. Kontturi and T. Tammelin, *Biomacromolecules*, 2017, **18**, 2951–2958.

11 H. Hibbert and J. Barsha, *J. Am. Chem. Soc.*, 1931, **53**, 3907.

12 R. E. Cannon and S. M. Anderson, *Crit. Rev. Microbiol.*, 1991, **17**, 435–447.
13 O. Shezad, S. Khan, T. Khan and J. KonPark, *Carbohydr. Polym.*, 2010, **82**, 173–180.
14 H.-J. Song, H. Li, J.-H. Seo, M.-J. Kim and S.-J. Kim, *Korean J. Chem. Eng.*, 2009, **26**, 141–146.
15 K.-Y. Lee, Y. Aitomäki, L. A. Berglund, K. Oksman and A. Bismarck, *Compos. Sci. Technol.*, 2014, **105**, 15–27.
16 A. K. Bledzki and J. Gassan, *Prog. Polym. Sci.*, 1999, **24**, 221–274.
17 N. Lavoine, I. Desloges, A. Dufresne and J. Bras, *Carbohydr. Polym.*, 2012, **90**, 735–764.
18 I. Filpponen, E. Kontturi, S. Nummelin, H. Rosilo, E. Kolehmainen, O. Ikkala and J. Laine, *Biomacromolecules*, 2012, **13**, 736–742.
19 I. Siró and D. Plackett, *Cellulose*, 2010, **17**, 459–494.
20 G. Siqueira, J. Bras and A. Dufresne, *Polym.* , 2010, 2.
21 W. K. Czaja, D. J. Young, M. Kawecki and R. M. Brown, *Biomacromolecules*, 2007, **8**, 1–12.
22 C. Zhijiang and Y. Guang, *J. Appl. Polym. Sci.*, 2011, **120**, 2938–2944.
23 M. C. I. M. Amin, N. Ahmad, N. Halib and I. Ahmad, *Carbohydr. Polym.*, 2012, **88**, 465–473.
24 *Biosurfactants Market*, 2018.
25 J. G. Tropsch, *INFORM*, 2017, **28**, 20–21.
26 H.-J. Daniel, R. T. Otto, M. Binder, M. Reuss and C. Syldatk, *Appl. Microbiol. Biotechnol.*, 1999, **51**, 40–45.
27 M. Takahashi, T. Morita, K. Wada, N. Hirose, T. Fukuoka, T. Imura and D. Kitamoto, *J. Oleo Sci.*, 2011, **60**, 267–273.
28 E. I. P. Delbeke, M. Movsisyan, K. M. Van Geem and C. V. Stevens, *Green Chem.*, 2016, **18**, 76–104.
29 F. Takahashi, K. Igarashi and H. Hagihara, *Appl. Microbiol. Biotechnol.*, 2016, **100**, 9519–9528.
30 K. Ciesielska, S. L. K. W. Roelants, I. N. A. Van Bogaert, S. De Waele, I. Vandenberghe, S. Groeneboer, W. Soetaert and B. Devreese, *Appl. Microbiol. Biotechnol.*, 2016, **100**, 9529–9541.
31 I. N. A. Van Bogaert, D. Buyst, J. C. Martins, S. L. K. W. Roelants and W. K. Soetaert, *Biotechnol. Bioeng.*, 2016, **113**, 2644–2651.
32 K. M. J. Saerens, S. L. Roelants, I. N. Van Bogaert and W. Soetaert, *FEMS Yeast Res.*, 2011, **11**, 123–132.
33 M. Kasture, S. Singh, P. Patel, P. A. Joy, A. A. Prabhune, C. V. Ramana and B. L. V Prasad, *Langmuir*, 2007, **23**, 11409–11412.
34 D. V. R. Kumar, M. Kasture, A. A. Prabhune, C. V. Ramana, B. L. V. Prasad and A. A. Kulkarni, *Green Chem.*, 2010, **12**, 609.
35 S. Singh, V. D'Britto, a. a. Prabhune, C. V. Ramana, A. Dhawan and B. L. V. Prasad, *New J. Chem.*, 2010, **34**, 294.
36 N. Baccile, R. Noiville, L. Stievano and I. Van Bogaert, *Phys. Chem. Chem. Phys.*, 2013, **15**, 1606–1620.
37 M. Sodagari, H. Wang, B. M. Z. Newby and L. K. Ju, *Colloids Surfaces B Biointerfaces*, 2013, **103**, 121–128.
38 C. Valotteau, C. Calers, S. Casale, J. Berton, C. V. Stevens, F. Babonneau, C. M. Pradier, V. Humblot and N. Baccile, *ACS Appl. Mater. Interfaces*, 2015, **7**, 18086–18095.
39 C. Valotteau, I. M. Banat, C. A. Mitchell, H. Lydon, R. Marchant, F. Babonneau, C. M. Pradier, N. Baccile and V. Humblot, *Colloids Surfaces B Biointerfaces*, 2017, **157**, 325–334.
40 G. Moiset, C. A. López, R. Bartelds, L. Syga, E. Rijpkema, A. Cukkemane, M. Baldus, B. Poolman and S. J. Marrink, *J. Am. Chem. Soc.*, 2014, **136**, 16167–16175.
41 H. D. Andersen, C. Wang, L. Arleth, G. H. Peters and P. Westh, *Proc. Natl. Acad. Sci.*, 2011, **108**, 1874–1878.
42 S. Singh, P. Patel, S. Jaiswal, a. a. Prabhune, C. V. Ramana and B. L. V. Prasad, *New J. Chem.*, 2009, **33**, 646.
43 A. S. Cuvier, F. Babonneau, J. Berton, C. V. Stevens, G. C. Fadda, I. Genois, P. Le Griel, G. Péhau-Arnaudet and N. Baccile, *Chem. - An Asian J.*, 2015, **10**, 2419–2426.
44 N. Baccile, P. Le Griel, S. Prévost, B. Everaert, I. N. A. Van Bogaert, S. Roelants and W. Soetaert, *ChemistryOpen*, 2017, **6**, 526–533.
45 D. Kitamoto, T. Morita, T. Fukuoka, M. Konishi and T. Imura, *Curr. Op. Coll. Interf. Sci.*, 2009, **14**, 315–328.
46 J. Penfold, M. Chen, R. K. Thomas, C. Dong, T. J. P. Smyth, A. Perfumo, R. Marchant, I. M. Banat, P. Stevenson, A. Parry, I. Tucker and I. Grillo, *Langmuir*, 2011, **27**, 8867–8877.
47 N. Baccile, M. Selmane, P. Le Griel, S. Prévost, J. Perez, C. V. Stevens, E. Delbeke, S. Zibek, M. Guenther, W. Soetaert, I. N. A. Van Bogaert and S. Roelants, *Langmuir*, 2016, **32**, 6343–6359.
48 N. Baccile, A.-S. Cuvier, S. Prévost, C. V Stevens, E. Delbeke, J. Berton, W. Soetaert, I. N. A. Van Bogaert and S. Roelants, *Langmuir*, 2016, **32**, 10881–10894.
49 P. Dhasaiyan and B. L. V. Prasad, *Chem. Rec.*, 2017, **17**, 597–610.
50 S. Zhou, C. Xu, J. Wang, W. Gao, R. Akhverdiyeva, V. Shah and R. Gross, *Langmuir*, 2004, **20**, 7926–7932.
51 N. Baccile, F. Babonneau, J. Jestin, G. Pehau-Arnaudet and I. Van Bogaert, *ACS Nano*, 2012, **6**, 4763–4776.
52 S. Manet, A. S. Cuvier, C. Valotteau, G. C. Fadda, J. Perez, E. Karakas, S. Abel and N. Baccile, *J. Phys. Chem. B*, 2015, **119**, 13113–13133.
53 Y. Ishigami, Y. Gama, H. Nagahora, M. Yamaguchi, H. Nakahara and T. Kamata, *Chem. Lett.*, 1987, **16**, 763–766.
54 P. Dhasaiyan, P. Le Griel, S. Roelants, E. Redant, I. N. A. Van Bogaert, S. Prevost, B. L. V Prasad and N. Baccile, *ChemPhysChem*, 2017, **18**, 643–652.
55 E. I. P. Delbeke, O. Lozach, T. Le Gall, M. Berchel, T. Montier, P.-A. Jaffrès, K. M. Van Geem and C. V Stevens, *Org. Biomol. Chem.*, 2016, **14**, 3744–3751.