1

General Views on Natural Surfactants

1.1 Introduction

This book has been written with the view of presenting a state-of-the-art bridge between the academic research, the commercial achievements, and the future opportunities of naturally occurring surfactants. The title of the book is natural surfactants, but in the text we sometimes also use the terms naturally occurring surfactants or biosurfactants for the amphiphiles produced by plants, animals, or microorganisms through a naturally occurring biosynthesis. The term "bioamphiphile" has also been proposed in the literature [1].

Biosurfactants are not to be confused with bio-based surfactants that are man-made surfactants produced by chemical reactions from natural building blocks, such as sucrose and sorbitan esters of fatty acids, alkylpolyglucosides, and fatty alcohol ethoxylates built from naturally derived fatty alcohols and bio-based ethylene oxide, or other synthetic surfactants containing different proportions of moieties obtained from nature.

Bio-based surfactants are excluded from this book, because they represent a totally different reality than the biosurfactants. Bio-based surfactants are carbon copies of petrochemical surfactants, made from building blocks of natural origin, but involving chemical reactions, processing conditions, and catalysts that are anything but natural. Because of the raw material origin, they can claim sustainability compared to their petrochemical brothers, but they fall short of being entirely natural. We have dedicated a short section to this category just to describe more completely their features and to position them correctly vis-à-vis the biosurfactants and the petrochemical surfactants.

The term "green surfactants" has become popular and is frequently used by surfactant-producing companies and by formulators, such as the detergent and personal care industries. The term is vague, however, and can mean anything from biosurfactants to man-made surfactants with a reasonable share of the building blocks coming from natural sources. We have avoided the term "green surfactants" in this book.

References to patents are reduced to a minimum as these are not scientific documents, nor do they provide useful information on commercial aspects. The few patent references quoted are because of their conceptual value rather than for scientific or commercial interest.

Regulatory information is immensely complex as it is country and application dependent and in various stages of legal development (and therefore in a constant goalpost moving mode). Only if something is approved or forbidden for a very clear reason, a firmer

Natural Surfactants: Science, Technology, and Industrial Aspects, First Edition. Guido Bognolo and Krister Holmberg.

statement on regulatory acceptance/nonacceptance can be made. For this reason, only a few references are reported in the book.

The information given in the different chapters on regulatory matters and commercial issues represents the situation known to the authors at the moment of writing.

1.2 Surfactants from Nature

Nature is a forge of surface-active agents and produces scores of substances with amphiphilic structure with considerable surface and/or interfacial activity, some of which have been exploited for a long time. For sure, the people who inhabited the Blombos caves in South Africa around 100 000 years ago did not know that they were dealing with colloid chemistry when they prepared the color mixtures, of which the remains have been recently discovered. But they did, and they must have used some natural ingredients in the process. Closer to our times, polysaccharides from acacia trees (e.g. *Acacia nilotica*, *Acacia senegal*, and *Vachellia (Acacia) seyal)*, commonly referred to as Gum Arabic were used in the preparation of inks by the ancient Egyptians. By reference to these products and the effects that can be obtained from them, modern researchers have used Gum Arabic to separate agglomerates of carbon nanotubes into individual fibers [2].

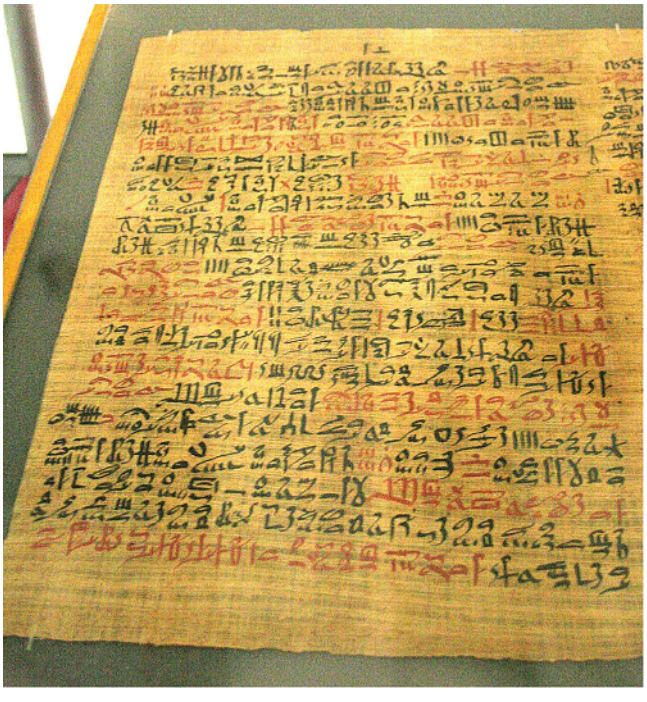

Figure 1.1 *The Ebers Papyrus*, ca. 1550 BCE. (*Source:* Einsamer Schütze / Wikimedia Commons / CC BY-SA 3.0.)

The Ebers Papyrus (ca. 1550 before the Christian era [BCE]), see Figure 1.1, along with the *Kahun Gynaecological Papyrus* (ca. 1800 BCE), the *Edwin Smith Papyrus* (ca. 1600 BCE), the *Brugsch Papyrus* (ca. 1300 BCE), and the *London Medical Papyrus* (ca. 1300 BCE) are among the oldest preserved documents of ancient Egyptian medicine. They contain recipes for the treatment of various diseases that involve the use of both natural and man-made surface-active agents (essentially soap) for their preparation [3].

Surface-active agents are produced by plants and macro- and microorganisms in an amazing variety of molecules and in relatively small quantities for functions that are often not fully understood yet. Several hundred species have been isolated from the two principal vegetal sources, *Quillaja saponaria* and *Yucca schidigera*, and from the other numerous plants producing saponins (e.g. bouncing bet, (*Saponaria officinalis*), a few from animals (e.g. starfish (Asteroidea), and sea cucumber (Holothuroidea)). Phospholipids and bile acids are found in the bodies of animals and humans, where they facilitate pulmonary functions and ensure food absorption, respectively. A large variety of sugar lipids and lipoproteins are synthesized by bacteria, fungi, and yeasts.

With the exception of saponins, which have been used for centuries in many countries around the world to wash clothes, and the phospholipid lecithin, isolated for the first time in 1845 and purposedly used as emulsifier in food since then (but certainly already used long before not as an isolated product but via the main product containing it, i.e. egg yolk), until recent years there was no interest in natural surfactants.

The use of saponins for washing clothes had fallen into oblivion with the advent of broader availability, first of soaps and later of synthetic surfactants. However, saponins are being proposed again by regional formulators because of their performance, natural connotation, and ecological profile. They have found a wide variety of profitable niche markets, ranging from aquaculture and adjuvants for vaccines to foaming agents in nonalcoholic drinks. These are specialty applications where synthetic surfactants are inadequate. An example of a niche segment where the peculiar effects of saponins are exploited is aquaculture. The hemolytic properties of saponins were recognized long ago and saponin extracts were spread on rivers and ponds to daze or kill fish, making their gathering easy. This practice is still common with primitive tribes and, in more sophisticated ways, in industrial aquaculture in developed countries. In fact, saponins are toxic to some aquatic species but not to others, e.g. shrimps and shellfish in general. Not only are they nontoxic but they also have nutritional value. They have become an important ingredient in fisheries, killing predators on one side and contributing to the growth of the valuable breed on the other. The historical source of saponins, *Quillaia saponaria*, was inadequate to respond to the growing demand and new plant sources, such as *Yucca schidigera*, and new cultivation methods have been introduced.

Lecithin is also entering new markets as its phospholipids are essential ingredients to produce the high added value organized structures, liposomes, and ethosomes, capable of encapsulating and controlling the penetration or the release of drugs or cosmetic actives for pharmaceutical and personal care formulations.

While the presence of surface-active species in plants and animals had been recognized long ago, it was only in 1946 that an amphiphilic molecule produced by a microorganism was mentioned [4] and identified a few years later by Jarvis and Johnson as the metabolites of *Pseudomonas* species [5].

In 1961, Gorin et al. described the oil formed during fermentation by a strain of *Torulopsis magnoliae* that mainly consisted of partly acetylated 2-O-β-D-glucopyranosyl-D-glucopyranose units attached β-glycosidically to 17-L-hydroxyoctadecanoic and 17-L-hydroxy-9-octadecenoic acids, the first sophorolipids isolated [6]. In 1968 came the discovery of surfactin, the biologically active secondary metabolite first identified in cultures of *Bacillus subtilis* strains [7].

Surfactants from microorganisms, notably the lipopeptide surfactin, are used in medical and pharmaceutical research. Sophorolipids and rhamnolipids are increasingly included in cosmetic preparations, and have become an ingredient for mass consumers, the former in household cleaners and the latter in hand dishwashing formulations.

Many other surface-active species produced by microorganisms were identified in the years after the initial discoveries, and with them came a better selection of the producing strains and the progressive improvement of the production process, such that there are now on offer microbial surfactants in quantities and at prices that eventually enable their use in commercial formulations.

On the back of these achievements, the chemical industry is beginning to take a real interest in biosurfactants. Many major surfactant producers like BASF, Dow, Sasol, Solvay, and Evonik, as well as formulators like Unilever and Ecover, have biosurfactants in their selling range, in their research programs, or in their mass consumer products. All this means that the volumes of biosurfactants produced are steadily increasing. Early manufacturers such as Holiferm and Evonik increase their capacities, and new ones emerge.

1.3 Natural Surfactants Versus Bio-Based Surfactants

After years of warning messages from the scientific community, the public opinion worldwide has gained consciousness and has started to react to the reality that our planet is warming up, that the climate is changing, and that in the absence of concrete and immediate actions major disruptions ahead are to be expected. And there is a growing consensus on the cause of this phenomenon: the ever-increasing emission of carbon dioxide from the use of fossil fuels, i.e. petroleum, gas, and coal. In many countries, large segments of the population have developed an almost instinctive revulsion against anything with a "petro" connotation and an equally instinctive, sometimes even irrational, attraction for what looks natural, sustainable, renewable, in one word, "green." Something that has eventually coalesced around that magic and captivating word "bio."

The surfactants industry did not have much "bio" in its basket when this movement began to gain momentum and started to impact on consumer choices, reversing the trend of the previous generations. In fact, since the early years of the twentieth century in the industrialized countries, there had been a progressive penetration of synthetic detergents in all the activities of an evolving society, from household cleaning and personal care to the agriculture and the industry in general. All human activities consumed more and more petrochemical products: alkylbenzene, alkylphenol, ethylene oxide, synthetic fatty alcohols, paraffins, and olefins. Only a generation ago, the surfactants industry relied on nonrenewable raw materials for most of the production.

Looking *a posteriori* it is evident that, in the absence of facts, the first response from the surfactant producers was haphazard to say the least. It took the form of highlighting to the consumers the natural/sustainable content of their products. An example of such an approach is the document, shown in Table 1.1, published by Cognis (at that time not yet part of BASF) in 2008 for some of their personal care products, showing the classification of their products based on the content of natural moieties.

Table 1.1 shows that few molecules could have the label "100% natural" and those that did were biological actives rather than surface active agents. A more thought-after response came when the surfactant producers realized that they could create the new label "bio-based" to attach to their surfactants by switching the raw materials from petrochemical to natural sources.

Fatty alcohol ethoxylates became bio-based if made from "a natural fatty alcohol" and "bio-ethylene oxide," regardless of the fact that the "natural" raw materials, fatty alcohol, and ethylene oxide were produced via nonnatural chemical processes involving consumption of nonrenewable resources. To produce a fatty alcohol a triglyceride oil is normally converted to a fatty acid methyl ester in a process called methanolysis, consuming methanol and energy, and the methyl ester is subsequently hydrogenated, using hydrogen that does not exists in nature and has to be petrochemically produced, consuming energy and generating offal, without considering the use of land, fertilizer, pesticides for growing the plants, or the feeding for growing the animals, for the production of the triglycerides, not to mention the energy consumption involved in the harvesting and working-up of the crop.

Bio-ethylene oxide is made by dehydration of ethanol produced from sugar canes or sugar beet (again use of land, etc.) followed by oxidation with a silver catalyst at high temperature and pressure (again energy, offal, and chemical consumption). Few people are probably aware of the fact that 50% of the silver extracted globally every year is immobilized in catalysts used to produce ethylene oxide.

The European Commission for Standardization has defined a bio-based surfactant as follows [8]:

- *Wholly bio-based:* >95% bio-based carbon; surfactants where all raw materials can be considered as bio-based
- *Majority bio-based:* 95–50% bio-based carbon; surfactants where the majority of the raw material is bio-based
- *Minority bio-based:* 50–5% bio-based carbon; surfactants where a minor part of the raw material is bio-based
- *Non–bio-based:* <5% bio-based carbon; surfactants where no raw material is bio-based

The percentage of bio-based carbon is usually calculated based on data from the surfactant supplier. It can also be determined experimentally by isotope analysis. Carbon in nature consists of 99% ^{12}C, 1% ^{13}C, and a tiny amount (1 per 10^{12} carbon atoms) of ^{14}C. The ^{14}C isotope is radioactive with a half-life of 5730 years. This means that it has more or less completely disappeared in fossil petroleum. Thus, an isotope analysis of the carbon in a linear alcohol made by oligomerization of ethylene will show a negligible amount of ^{14}C while the same alcohol obtained from a triglyceride by methanolysis followed by hydrogenation of the methyl ester will give an abundance of ^{14}C of 1 per 10^{12} carbon atoms. The methodology

Table 1.1 Cognis' classification model based on the content of natural ingredients in their personal care products (2008). (*Source:* Cognis release, reprinted from author's archives.)

Category	Description	Product examples	Chemical structure/INCI
Cognis naturals	From 100% natural, renewable feedstocks; physically purified by water, alcohol, or energy treatment.	Coviox® T50	Tocopherol
		Aloveria®	*Aloe barbadensis*
		Generol® R	*Brassica campestris* (rapeseed) Sterols
Cognis natural modified	From 100% natural, renewable feedstocks; chemically processed using catalysts or other reaction aids.	Cetiol® OE	Dicaprylyl ether
		Plantacare® 818 UP	Coco-glucoside
		Glucopon® 600 CSUP	Aqueous dispersion of alkylglucosides based on natural fatty alcohol C10–C16, free of preservatives
Cognis ecohybrids	From a mixture of natural, renewable, and synthetic feedstocks, combined in a chemical process. Higher level of natural C-atoms.	Lamesoft® Care	PEG-4 distearyl ether (and) sodium laureth sulfate (and) distearyl ether (and) dicaprylyl ether
		Cosmedia® DC	Dimethylcarbonate copolymer
		Texapon® N70	Sodium laureth sulfate
Cognis hybrids	From a mixture of natural, renewable, and synthetic feedstocks, combined in a chemical process. Lower level of natural C-atoms.	Plantapon® LC7	Sodium lauryl glucose carboxylate (and) lauryl glucoside (CTFA)
		Dehypon® LS 54	Fatty alcohol C12-C14 with approx. 5 moles EO and approx. 4 moles PO
		Cosmedia® CTH (E)	Polyquaternium-37 (and) propylene glycol (and) Dicaprylate/dicaprate (and) PPG-1 trideceth-6 (CTFA)
Cognis synthetics	From synthetic feedstocks, chemically processed.	Ultragel® 300	Polyquaternium-37 (CTFA)
		Polyquart® Ampho 149	Aqueous solution of an acrylic copolymer
		Dehyquart® E-CA	Hydroxycetyl Hydroxyethyl dimonium chloride (CTFA)

is the same as archaeologists use to determine the age of organic artifacts, where it is often referred to as radiocarbon dating or carbon-14 dating.

In the literature, and in particular in the promotion material from raw materials producing companies, the term "bio-attributed" is sometimes seen. The label "bio-attributed" for a given product indicates that the use of renewable feedstock has been ascribed to that product using the "mass balance approach." The mass balance approach provides a means to calculate and declare that an equivalent amount of biogenic carbon was used compared to nonbiogenic carbon, but gives no indication of the total quantity of biogenic substitution. Thus, "bio-attributed" is not necessarily synonymous with "bio-based."

Abstracting from the chemicals involved in the raw material preparation and in the surfactant synthesis the following surfactant classes are examples of amphiphiles that could claim the label "wholly bio-based" if produced with the appropriate raw materials, such as natural fatty alcohols and bio-ethylene oxide (although the presence of certain groups, such as sulfate, sulfonate, ammonium, and phosphate, may make the "wholly bio-based" attribution questionable):

- Fatty alcohol ethoxylates
- Fatty alcohol sulfates, sodium salts
- Fatty alcohol ether sulfates, sodium salts
- Alkylpolyglucosides
- Sucrose esters
- Natural fatty amine ethoxylates
- Natural fatty amide ethoxylates
- Sorbitan esters
- Polysorbates
- Fatty acid esters of glycerol and of other naturally occurring polyols
- Castor oil ethoxylates
- Fatty amino acid surfactants
- Phosphate ester ethoxylates
- Natural fatty amine oxides
- Fatty acid sulphosuccinates and their ethoxylated derivatives

In general, switching surfactant raw materials, such as a C12–14 alcohol, from a petrochemical to an oleochemical origin is not an issue, as for most applications, there is no noticeable difference in performance. However, given the choice cosmetic formulators will use bio-based products. In special formulations, for example, those used in crop protection or for pharmaceutical purposes, a change in ingredients may require re-registration.

To the best of knowledge, as of autumn 2023 in the United States, only two companies, Croda and Clariant, produce bio-ethylene oxide, and they do so exclusively for in-house consumption. This drive to nature was probably prompted, at least in part, by the prohibitive cost of transporting ethylene oxide from the US Gulf, where it is produced, to the ethoxylation sites of these companies in the Northeast of the country, combined with the risk of disruptions in the transport chain (e.g. stricter regulations or limited availability of rail cars certified for EO transport). India is the only other country in the world where bio-ethylene oxide is produced on an industrial scale. India Glycol has been producing it since 1989 (India Glycol Ltd. Company Summary – India Infoline).

In July 2022, INEOS (London, UK) launched bio-attributed ethylene oxide which is claimed to have 100% substitution of fossil feedstock and give 100% greenhouse gas saving

compared to ethylene oxide produced in the conventional way, as certified by both RSB (Roundtable on Sustainable Biomaterials) and ISCC+ (International Sustainability & Carbon Certification Plus) (INEOS press release, July 27, 2022).

During recent years, there has been an interest in making bio-based surfactants from a wide range of natural raw materials, some with quite a complex structure, which have been synthesized. The driving force for such investigations, apart from scientific curiosity, has been to find new and green building blocks for surface-active molecules, in particular amino acids and peptides. This has resulted in scientific publications of high standard with respect to preparative organic chemistry. However, these amphiphiles are also man-made in the laboratory by organic – or sometimes bioorganic – synthesis and are therefore examples of bio-based surfactants, not natural surfactants. Thus, they are outside the scope of this book.

1.4 Advantages and Disadvantages of Naturally Occurring Surfactants

Much literature has been produced portraying natural and biosurfactants as the Holy Grail that will enable to correct all the wrongdoings of the synthetic surfactants, perhaps on the back of the negative connotation of anything that has to do with chemistry and in particular petrochemistry, or maybe by ignorance of the facts. Whatever the reason, the reality is that it has contributed to a distorted, false image of the benefits of biosurfactants.

A few observations are appropriate and may hopefully contribute to a more realistic picture.

First, biosurfactants will never be able to completely replace the synthetic surfactants. The best evidence is that synthetic surfactants were developed and encountered a rapid and widespread acceptance because of the shortcomings of the sustainable surfactants previously used, in particular, soaps.

Another reason is that while the synthetic surfactants comprise all categories, i.e. anionic, nonionic, cationic, and zwitterionic, the majority of the naturally occurring surfactants are essentially nonionic with a weak anionic character conferred to some of them by a carboxylate moiety. And in surfactancy terms, a carboxylate group does not have the power of a sulfate or sulfonate group.

It should also be recognized that a key feature of nature, with consequences on its products, is variability. The chemistry and structure of its produce depend, for example, on the soil composition, the meteorological conditions, and the identity of the producing organisms. Sometimes, small differences in congener molecular structure can result in significant differences in functionality. For example, sophorolipid congeners can possess the same length and chemical structure of the fatty acid chain but can exist either in the lactonic or the acidic form. The lactonic form has strong antimicrobial properties that are missing in the acidic form.

A feature of some of the synthetic surfactants is the suitability of one single product for a large range of different applications. Alkylbenzene sulfonates or alkylphenol ethoxylates can cover, if not all, at least a great number of applications and application conditions from the point of view of the physical chemical performance. The fact that in practice other surfactants or combinations of surfactants are often used instead is a matter of toxicology,

environmental impact, cost effectiveness, consumer habits, regulatory, etc. Before their use was restricted because of environmental and human safety concerns, one could find alkylphenol ethoxylates in household detergents and cosmetic products, as well as in crop protection, textiles, paints, paper, and metalworking formulations. Their safer replacements can also be used in a broad, though considerably reduced, range of applications. None of the naturally occurring surfactants discovered so far has shown such versatility.

The main advantage of naturally occurring surfactants is the sustainability, in the sense that they are the result of the natural transformation of renewable substrates. There is the widespread perception that since they are a produce of nature, they must be good, overlooking that some of the most potent poisons, such as curare and strychnine, are naturally occurring, respectively from *Chondrodendron tomentosum* and *Strychnos nux-vomica,* not to mention the taxine alkaloids, that can be found in garden's hayes, such as those of *Taxus baccata* (English yew), *Taxus cuspidata*, *Taxus canadensis*, *Taxus floridana*, and *Taxus brevifolia* (Pacific yew or Western yew).

It is generally believed that natural surfactants must be readily biodegradable and safe to humans and the environment, but this lacks scientific demonstrations, as discussed later.

Biosurfactants are natural products produced by microorganisms colonizing a variety of environmental niches; some can maintain their effectiveness even under much adverse conditions. Certain biosurfactants have been shown to maintain their physical chemical activity at high temperatures and within a pH range of 3–12 and salt concentrations up to 10%. Most synthetic anionic surfactants are deactivated by high concentrations of divalent cations such as calcium and magnesium, while ethoxylated nonionic surfactants are sensitive to monovalent cations.

For sure, natural surfactants do not need for their production hazardous and harmful chemicals such as SO_3, HCl, HBr, HF, oleum, H_2SO_4, H_3PO_3, ethylene oxide, epichlorohydrin, just to mention a few. They also do not require organic solvents for their synthesis. But it should not be overlooked that high biosurfactant yields sometimes require pathogenic species (e.g. *Pseudomonas aeruginosa* for producing rhamnolipids or *Bacillus subtilis* for surfactin) or genetic modification (again for the production of rhamnolipids). This is just to say that many of the generally accepted beneficial features of biosurfactants may originate from debatable premises.

There are much less toxicological data reported for the natural surfactants than for the synthetic surfactants, for which there is also a wealth of information from in-use conditions. The knowledge available suggests in general, a favorable toxicological profile for the natural surfactants, save for the strong hemolytic effect and the sternutatory effect of the powder saponins, but the field is still in its infancy. Also, the regulatory for most biosurfactants ranges from ill-defined to nonexistent.

Table 1.2 summarizes the most relevant strengths and weaknesses of naturally occurring surfactants compared with synthetic surfactants.

1.5 Production Process of Natural Surfactants

Natural surfactants of plant and animal origin can be isolated with traditional technologies involving solvent (generally water) extraction, filtration to separate impurities, and possibly concentration and drying. Biosurfactants from microorganisms involve a more

Table 1.2 Strengths and weaknesses of natural surfactants.

Criterion	Strength	Weakness
Environmental impact	x	—
Social acceptability	x	—
Sustainability	x	—
Availability	—	x
Cost	—	x
Heterogenicity of raw materials	x	—
Product consistency	—	x
Competitivity for food production	—	x
Process yield	—	x
Biodegradability	x	—
Performance	—	x
Global surfactancy	—	x
Versatility	—	x
Tolerance to external environment	x	—
Physical chemical characterization	—	x
Formulation expertise	—	x
Toxicity	Ill defined	Ill defined
Regulatory	Ill defined	Ill defined

complex process with the intervention of several variables, as discussed below. Most of these biosurfactants are exolipids, although in some cases they can be cell-bound. The excretion occurs in certain phases of the fermentation cycle: the late exponential growth or the stationary phase.

The surfactant-producing microorganisms can be divided into three groups with respect to hydrocarbon utilization and the synthesis of extracellular lipids:

- Microorganisms that produce biosurfactants exclusively from hydrocarbons, e.g. some strains of *Arthrobacter* sp., *Corynebacterium* sp., and *Nocardia* sp.
- Microorganisms that produce biosurfactants from both hydrocarbon and water-soluble substrates. This is by far the largest category, the best-known example being the rhamnolipids-producing *Pseudomonas aeruginosa*.
- Microorganisms that produce biosurfactants exclusively from water-soluble substrates. Surfactin-producing *Bacillus subtilis* is one example.

Figure 1.2 shows the microorganisms that produce the most promising biosurfactants: *Candida albicans* for the sophorolipids, *Pseudomonas aeruginosa* for the rhamnolipids, and *Bacillus subtilis* for the lipopeptide surfactin.

The microorganisms feeding on hydrocarbons attracted much attention in the early days of the biosurfactants development, in the expectation that they could assist in oil recovery and/or in soil bioremediation, which has happened only to a moderate extent. Today, the

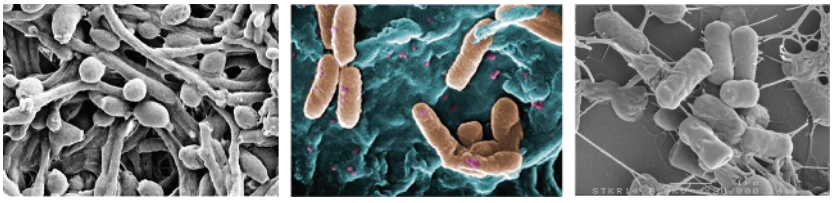

Figure 1.2 The three microorganisms producing the most promising biosurfactants visualized using scanning electron microscopy. (The left image is from Vader1941 / Wikimedia Commons / CC BY-SA 4.0; the middle image is from Janice Carr / Pixnio / Public domain; and the right image is from Reproduced with permission from Zweers et al. (2008) / Springer Nature / CC BY 2.0.)

commercially most successful biosurfactant, a rhamnolipid, is produced by genetically modified *Pseudomonas putida*, grown on corn glucose.

There is a distinction between the biological processes leading to the formation of biosurfactants in nature and their laboratory or industrial production. This section will focus on the latter, while the biological pathways are discussed separately in the chapters on the different classes of biosurfactants. The natural biosynthesis generally yields biosurfactants with specific properties in concentrations of a few grams/liter or less, which makes their costs unacceptable except for a few extremely high added value applications. However, the production yield, the productivity, the chemical structure, the composition, and the physical chemical properties of the biosurfactants can be modified to a certain extent by genetic, biological, or chemical manipulation.

1.5.1 Selection of the Microorganism

The selection of the microorganism is perhaps the most critical step for the production of a biosurfactant, as it will be decisive for the yield, with only limited (if any) information to guide the choice [10]. So, the first step in the process is the choice of microorganism, using criteria of interest to the researcher. Strains of interest need to be harvested from a suitable source and then isolated using various culture techniques. Strains are usually selected that efficiently feed on the raw materials chosen, preferentially as the sole energy source. These can be refined raw materials, such as glucose, but they can also be crude products if the objective is to reduce the cost of the feed, while retaining an acceptable yield. The strains that give the highest cell density at a certain optical density are retained for further optimization. Several tests can be performed at this point, e.g. Gram-staining analyses, motility, shape (e.g. rod-shaped, coccoid, bacilli), catalase (or other enzyme) sensitivity, and genetic identification using 16S rDNA gene sequence analysis. A phylogenetic tree is useful for detecting evolutionary relationships.

1.5.2 Medium Composition

The main parameters to consider are:

Carbon source: The carbon source is the primary variable to consider in the production of biosurfactants, as it directly influences the growth of the microorganism, as well as the structure and yield of the target biosurfactant molecule. A variety of carbon sources

have been studied, ranging from crude oil to refined hydrocarbons of different molecular weight, glycerol, glucose, and their mixtures [11, 12], as well as carbon sources derived from waste products from industrial processes [13]. While in the case of more or less pure raw material, the prime objective is to maximize yield and productivity, the drive for using waste raw material is to reduce production costs, both directly and because of the added value of reducing or eliminating disposal problems and costs. The use of waste raw material would add to the sustainability of biosurfactants through their contribution to a circular economy.

The company Evonik reports that glucose from corn, nongenetically modified, is the carbon source for the industrial production of their rhamnolipid (Evonik management, personal communication).

Nitrogen source: The nitrogen source can be of inorganic origin, such as ammonium nitrate and ammonium sulfate, or organic, such as urea, amino acids, and yeast extract. The choice of nitrogen source depends on the composition of the medium and the producing microorganism [14]. An example of where the nitrogen source affects biosurfactant production has been identified in *Bacillus* species. Increased surface activity or emulsifying efficiency of *Bacillus* biosurfactants is linked to the use of organic or inorganic nitrogen sources, respectively, during growth [15]. When both organic (yeast extract) and inorganic (ammonium nitrate) sources are added simultaneously to the medium, there is synergism between the two sources in the production of biosurfactants [16]. Nurfarahin et al. have reviewed various nutrients and elements considered in the formulation of a production medium describing, among others, the effect of organic and inorganic nitrogen sources in the production of biosurfactants [17].

C/N ratio: The ratio between the carbon source and the nitrogen source is an important parameter for the production yield. The C/N ratio determines the endpoint of the exponential microorganism growth and the onset of the metabolic process. However, the optimal C/N ratio varies widely depending on the strain, as well as on the carbon and nitrogen source, and needs to be established experimentally for each individual biosynthesis.

Fermentation medium: A typical medium for the production of biosurfactants will consist of a source of carbon and energy (ideally glucose, but hydrocarbons, oils, and degraded products from other industrial processes may also be employed) and a source of nitrogen. It further contains potassium dihydrogen phosphate and/or dipotassium hydrogen phosphate to control the pH and serve as a source of phosphorous. The medium may also contain microelements like Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, Mo^{2+}, and Mn^{2+}. Although not critical, the choice and level of the microelements must be optimized in each specific case.

Biosurfactants are produced mostly in the stationary stage of the fermentation process when the feed is no longer used for producing cells but is metabolized into surface-active molecules. Ideally, the process should be performed under conditions such that cells are produced as fast as possible and in volumes as high as possible. From this phase of exponential growth, the system should move to a stationary stage and remain in that producing stage for as long as possible.

The possibility of recovering the cells and using them for the exclusive production of metabolites has been investigated and holds promise.

Subsanguan et al. studied the possibility of maintaining the biosurfactant-producing activity of a strain of a GRAS ("generally recognized as safe" by the US Food and Drug Administration) lactic acid bacterium, *Weissella cibaria* PN3. The bacterium was immobilized on a commercial porous carrier. For biosurfactant production, 2% soybean oil was used as the carbon source. After 72 hours, the immobilized cells were reused by replacing the production medium. The extracellular and cell-bound biosurfactants were extracted from the resulting cell-free broth and cell pellets, respectively. To maintain biosurfactant production activity, the immobilized cells were reactivated every three production cycles by incubating the washed immobilizing carriers in LB medium (LB stands for Luria-Bertani broth) for 48 hours. The maximum yields of purified extracellular and cell-bound biosurfactants were 1.46 g/L and 1.99 g/L, respectively, and were achieved in the fourth production cycle. The repeated biosurfactant production of nine cycles was completed within one month, while only 2 g of immobilized cells/L were applied. The extracellular and cell-bound biosurfactants had comparable surface tension values (31–33 mN/m); however, their CMC values were different, 1.6 and 3.2 g/L, respectively [18]. Although the total quantity of biosurfactants produced was not large (24.67 g/L for the sum of extracellular and cell-bound biosurfactants), the process is relatively cheap, given that two types of biosurfactants were produced simultaneously, and no new bacterial inoculum was required. Furthermore, the yields were higher than observed in other studies [19, 20].

However, there are some disadvantages of cell immobilization for long-term usage, such as cell inactivation during the process, mass transfer limitations, and accumulation of toxic metabolites or inhibitor products inside the carrier [21].

1.5.3 Feeding Strategies

The feed can be supplied in one (batch feeding) or sequential installments (fed-batch feeding) according to one of the following strategies.

1.5.3.1 Pulse Feed

The nutrient solution is added in separate installments at fixed time intervals. The advantage is the simplicity of the feed addition; the disadvantage is that the optimal feed volume and the feed interval times have to be optimized by trial and error and may vary with time. In addition, the yield and productivity are lower compared with other strategies.

1.5.3.2 Constant Feed Rate

A constant feed rate strategy involves adding a nutrient solution at a fixed rate throughout the process. The advantage of this strategy is that it is easy to implement and monitor, and it can prevent substrate accumulation and inhibition. The disadvantage is that it does not account for the changes in the biomass concentration, the specific growth rate, and the product formation rate during the process. Therefore, it may not be optimal for maximizing the productivity or the product quality.

1.5.3.3 Exponential Feed Rate

An exponential feed rate strategy is based on the principle of maintaining a constant specific growth rate of the microorganisms or cells. It involves adding a nutrient solution at a rate

that is proportional to the biomass concentration. The advantage of this strategy is that it can achieve a high biomass yield and a high product concentration, as well as reduce the risk of overflow metabolism and by-product formation. The disadvantage is that it requires accurate measurement and control of the biomass concentration, which can be challenging at a large scale. Moreover, it may not be suitable for processes that require a low or variable specific growth rate.

1.5.3.4 Feedback Control Feed Rate

A feedback control feed rate strategy is a more advanced and flexible feeding method for fed-batch bioprocesses. It involves adding a nutrient solution at a rate that is adjusted according to the feedback from one or more process variables, such as substrate concentration, dissolved oxygen, pH, or product concentration. The advantage of this strategy is that it can adapt to the dynamic behavior of the bioprocess and optimize the feeding rate according to the desired objectives. The disadvantage is that it requires a complex and robust control system, as well as reliable and accurate sensors and actuators.

As an example, Bouassida et al. compared batch and fed-batch strategies for biosurfactant production by *Bacillus subtilis* SPB_1. Experiments of fed-batch fermentations were carried out through three different glucose feeding strategies, namely the pulsed, the constant feed rate, and the exponential feeding. The comparison between different fermentation processes revealed that the fed-batch process is a more efficient cultivation strategy than the batch process in terms of cell biomass and biosurfactant production and productivity. Among the three different feeding strategies, the exponential feeding process achieved the highest biosurfactant concentration, an almost twofold increase compared to batch fermentation [22].

Industrially, biosurfactant production is mostly carried out under submerged fermentation (SmF), which implies cultivating microorganisms in a liquid nutrient medium. This is because of history: SmF was used in the early days and therefore more knowledge has been accumulated. In the case of SmF, there is also a possibility of stricter and simpler control over various parameters, whereas there is a knowledge gap for the solid-state fermentation process (SSF). SSF is performed on a solid substrate with a low moisture content, with the advantage that relatively low energy is consumed. The required water content in SSF can be provided by the feeding medium and is absorbed by the substrate in a solid matrix. This offers advantages for the transfer of oxygen to support the growth of microorganisms. Several agricultural wastes, such as rice straw, sugarcane bagasse, wheat straw, rice hulls, and corn cobs, are used as substrate for SSF. One possible approach to produce biosurfactant is to utilize SSF with substrates derived from other industrial processes. For example, in two days of fermentation using a simple downstream process with 5.58 g of soy flour and 3.67 g of rice straw, 50.01 mg/g of dry lipopeptide was produced by *Bacillus amyloliquefaciens* XZ-173 in SSF [23].

In addition, through SSF and using molasses as substrate, a strain of *Bacillus subtilis* producing 74 mg/g of biosurfactant has been reported [24]. Eras-Muñoz et al. have investigated the sustainable production of sophorolipids through an SSF process, which seems to be a promising alternative for re-valorizing industrial or food waste that cannot be exploited by SmF. Industrial wastes like sunflower oil cakes, waste-water from the cleaning of industrial equipment used in the production of cosmetics and household cleaners, and

organic fractions of municipal solid wastes were used as feeding substrates. A production of 0.033 g/L/h was achieved under optimum conditions, with a yield of 0.047 g/g for the diacetylated lactonic C18:1 and of 0.141 g/g for the sophorolipid crude extract [25]. Bouassida et al. conducted a comparative study of SmF and SSF for the production of lipopeptides using Aleppo pine waste and confectionery effluents as cheap substrates. The SmF gave a yield of 0.0172 g/g versus 0.0276 g/g for the SSF, thus showing the superiority of SSF under the test conditions [26].

The above cases demonstrate the feasibility of using SSF to produce lipopeptides and glycolipids, saving operational costs related to SmF. However, more work is needed before SSF is ready for large-scale processing, notably in terms of reaction times (longer than with SmF) and heat transfer. Bioreactor design and sustainability are some critical limiting factors for SSF, which need to be tailored based on the existing advancements in SSF techniques.

1.5.4 Biosurfactants Transport

One of the key contributors to reaching high productivity titers is the transport of biosurfactants outside the microbial cell environment where they are synthesized. Apart from the obvious economic advantage of an increased efficiency of the biosynthesis, a high productivity also lowers downstream processing costs and reduces the risks of product toxicity.

The biosurfactants transfer from the interior of the cells to the exterior in yeasts (mannosylerythritol lipids, cellobiose lipids, sophorolipids) and bacteria (trehalose lipids and rhamnolipids) fermentation is mediated by transport proteins and has been addressed in several works. Yet, in contrast to the yeast glycolipids, the genetic basis of transporters of bacterial glycolipids is largely unknown.

Apart from moving biosurfactant molecules to the extracellular environment, transport proteins also intervene in many intracellular steps along the biosynthetic pathway. In fact, the eukaryotic producers of glycolipids present the compartmentalization of building blocks within the cell, hence the importance of intramolecular transport of feedstuff and of synthesized moieties.

The direct and indirect involvement of transport proteins in the production of biosurfactants has been recently reviewed by Claus et al. [27].

1.5.5 Recovery and Purification

Gravity is generally sufficient to separate the liquid phase containing the biosurfactants from the biomass.

The supernatant liquid can be used as such for applications that do not demand high purity, but it must be noted that the impurities are likely to affect the physical and chemical properties of the isolate. If high purity is demanded, ultrafiltration or solvent extraction has to be used, which negatively impacts the production costs. The solvent to be used depends upon the biosurfactant and may be hydrocarbons, such as *n*-heptane or *n*-octane, alcohols such as 1-octanol, ethyl acetate, acetone, and chloroform/methanol blends.

Ultrafiltration was used in a one-step process to purify and concentrate the lipopeptide surfactin and rhamnolipids from the culture supernatant fluids. Surfactants form micelles at concentrations above the critical micelle concentration and the micelles can be

retained by relatively high molecular weight cut-off membranes. Lower molecular weight impurities, such as salts, free amino acids, and small peptides, are then easily removed [28].

The performance of a laboratory-scale fixed-bed adsorption column for surfactin isolation from the culture medium over commercial active carbon was assessed by Montastruc et al. Using methanol elution in up-flow mode in the column at 25 °C gave a surfactin recovery of 94% [29].

1.6 Raw Materials in Natural Surfactants Production

For the following discussion, the term "raw materials" must be viewed in a broad sense and encompass not only the classical chemicals necessary for plants, animals, or microorganisms to grow but also all other materials consumed in their life cycle and the physical space needed to accommodate their growth. Saponins and soy lecithins require acreage for growing the plants, yolk lecithin needs poultry to lay eggs, the most performing fermentation processes for microbial surfactants rely on feedstock like glucose or corn syrup, again the produce of agriculture.

Time ago, there was much concern and debate about the use of agricultural resources, and acreage in particular, to produce natural fatty alcohols, one of the major feedstocks for synthetic nonionic and anionic surfactants. So, in all likelihood with the advent of natural surfactants the issue will only be shifted, but it will remain: is it ethical to use soil and agricultural resources, for example corn crops (see Figure 1.3) to produce surfactants and their raw materials rather than to feed people?

Figure 1.3 Corn, one important raw material source for biosurfactants. (*Source:* Silverije / Wikimedia Commons / CC BY-SA 3.0.)

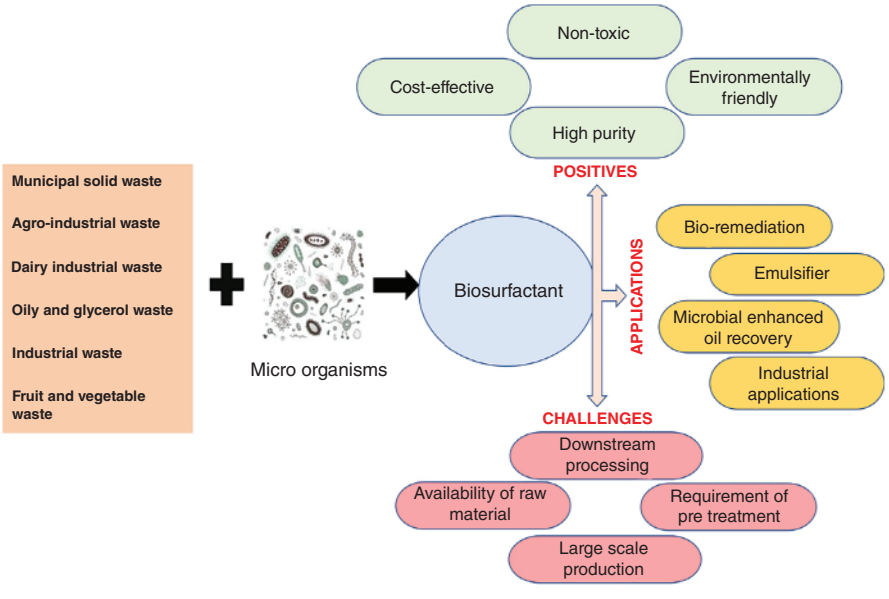

Figure 1.4 Biosurfactants from microorganisms using different wastes as raw material. (From Mohanty et al. [30]. Mohanty, S.S., Koul, Y., Varjani, S., et al. (2021) / A critical review on various feedstocks as sustainable substrates for biosurfactants production / Springer Nature / Public domain.)

For the microbial production of biosurfactants, the conundrum has been approached, along with the context of reducing production costs, by exploring the use of wastes from domestic or industrial uses or from agricultural products processing.

Figure 1.4 is an illustration of the concept of using wastes of different types for biosurfactant production, but it is fair to anticipate that so far, the efforts have not been very successful. It has been demonstrated that biosurfactants can be produced from wastes, but for the time being, not economically. The yields are insufficient and the production costs for commercial volumes always exceed the savings from the reduced feedstock costs.

Different types of wastes have been explored for different types of natural surfactants. This is described and discussed in some detail in the chapters devoted to the individual biosurfactants.

1.7 Life Cycle Analysis of Natural Surfactants

It is an almost omnipresent claim associated to naturally occurring surfactants that they are environmentally friendly, based on the imaginative consideration that they are a product from nature, built on renewable resources, easily biodegradable, and that they return to nature as nonnoxious catabolites. Yet, for years, the supporting factual evidence has been lacking. It is only in the last few years that scientific principles have begun to be implemented in the assessment of the environmental impact of naturally occurring surfactants.

Life cycle analysis (LCA) methodologies provide a scientifically valid technique for evaluating the inputs, outputs, and potential environmental consequences of a product

system throughout its entire life cycle [31]. LCA enables a comprehensive understanding of the environmental impact of products, quantifying the contribution of the various production stages and providing data for establishing principles of sustainable production while enabling the identification of the most environmentally friendly products among a range of alternatives [32]. However, it must be noted that the boundaries of LCA are elastic. One may use different start- and endpoints, such as cradle-to-grave, cradle-to-cradle, gate-to-gate, or cradle-to-gate. The gate-to-gate approach is conveniently used for comparative studies of different production processes.

For example, a gate-to-gate LCA by Aru et al. for the production of biosurfactants from oily waste by a diculture of *Pseudomonas* sp. and *Azotobacter vinelandii* showed that electricity supply was the major source of pollution during the production process [33]. Impact values for global warming potential and eutrophication, and acidification potential were also determined.

In studies by Hu et al., a dynamic life cycle analysis (dLCA) was used to iteratively evaluate the environmental impact of sophorolipid production with the aim of identifying the most suitable feedstock among food waste, textile waste, and bakery waste. Food waste was identified as the preferred feedstock. The authors concluded that fed-batch fermentation of food waste integrated with in situ separation techniques resulted in the lowest environmental impact, mainly due to the relatively low cumulative energy demand and global warming potential [34, 35]. In addition, energy consumption was identified as accounting for over 80% of the total impact in sophorolipids production.

Kopsahelis et al. conducted a gate-to-gate LCA of sophorolipids and rhamnolipids. The authors found that biogenic emissions (CO_2) and thermal energy and electricity needs during the synthesis of the biosurfactants were the major contributors to the environmental effects. The environmental impact of sophorolipids synthesis was 22.7% higher than that of rhamnolipids. The reason for this is that production of sophorolipids is more time consuming than that of rhamnolipids and, as a result, it requires more thermal energy in order to maintain the culture medium at a steady temperature [36].

Schonhoff et al. studied biosurfactants produced from two waste products from the sugar industry, molasses and sugar beet pulp. They found that production of mannosylerythritol lipids had a lower environmental impact than that of rhamnolipids due to differences in microbial properties and process designs. The choice of substrate did not play an essential role [37].

The literature on LCA is still modest. Most of the recent works cover the production of single biosurfactants, comparing the effect of different feedstocks, or of different products (generally two) from the same feedstock. Comparative studies of naturally occurring surfactants versus synthetic surfactants are missing, and therefore, the widespread claim of the superiority of naturally occurring surfactants over synthetic surfactants as far as environmental impact still rests on immaterial considerations.

1.8 Production Costs of Naturally Occurring Surfactants

Only general principles are given here; figures for specific natural surfactants are reported, when available, in the respective chapters.

The production costs of naturally occurring surfactants vary considerably depending upon their origin, whether animal/vegetal or from microorganisms because in the former the process is extractive, while in the latter it is fermentative, generally followed by an isolation step.

The cost components include:

- plant capital (land, equipment, construction)
- extraction (for animal/plant-derived surfactants) or fermentation (for microbial surfactants)
- isolation and purification (homogenization, precipitation, filtration, centrifugation, solvent recovery, drying, etc.)

and the parameters involved are feedstock, yield, energy, chemicals, labor, and sundries.

Various cost models have been developed, which however are largely based on extrapolations, as the actual figures for industrial production either do not exist, or in the few instances when they do, they are proprietary intellectual property. Schonhoff et al. have produced a comprehensive study of the industrial production of rhamnolipids and mannosylerythritol lipids by extrapolating laboratory and pilot plant data. This is further discussed in the chapter on mannosylerythritol lipids [38].

In the production of biosurfactants, electricity is by far the major contributor to the variable costs, approaching in some instances 80% of the total [39]. However, simulation of different scenarios with aeration units providing higher oxygen transfer rates and adjusting power input to match oxygen uptake can significantly decrease the electricity consumption [40].

For many years, the costs of biosurfactants have been prohibitively high, making them totally noncompetitive against synthetic surfactants, even in a cost/effectiveness perspective. However in the case of rhamnolipids, advances in the production technology have shifted the balance. Although still remaining on the edge of cost/effectiveness, the progress has opened their use in mass consumer brands. Here there are still opportunities unexploited for further reducing production costs. This may involve identification of new producing strains as well as genetic engineering. Advances in formulation technology will also be of importance. Together, this development is likely to result in broadly recognizable cost-effectiveness progress, hence a deeper industrial penetration.

1.9 Natural Surfactants are Chiral

There is currently a considerable interest in chiral surfactants, i.e. amphiphiles with one or more chiral centers. For self-assembling species such as natural surfactants, there is chirality in the individual molecules but also in the self-assemblies. Highly organized chiral supramolecular nanostructures, such as fibers, ribbons, tubes, and helices, have been described [41–43]. Applications that have been proposed for such chiral surfactant aggregates include enantiomeric catalysis and chiral separation [44, 45].

Synthesis of chiral surfactants is cumbersome and difficult to perform in large scale. Since natural surfactants are chiral molecules, they are attractive alternatives to the synthetic chiral amphiphiles. Extraction and purification of biosurfactants is usually less demanding

than organic or bioorganic synthesis of chiral surfactants. This is discussed and exemplified in a recent review on chiral surfactants [46].

1.10 A Glimpse into the Future

The commercial opportunities for naturally occurring surfactants are evolving under several influences, such as public opinion perceptions, environmental concern, and regulatory constraints.

While naturally occurring surfactants will never replace in full the synthetic surfactants, they could achieve a much broader market penetration if two conditions are realized:

- The availability of even larger volumes at even reduced prices. Cost-effectiveness and availability are achieved in certain circumstances, but for the time being, this is the exception, not the rule.
- Substantial progress in formulation technology, resulting from both a better knowledge and understanding of the physical and chemical properties of natural surfactants and an extended testing under real-use conditions. More specifically, the possible synergies between natural and synthetic surfactants have been only marginally studied until now and published information on the topic is virtually nonexisting.

For naturally occurring surfactants to become real winners they will have to make substantial entries in the major application, the household detergents, over and above what already achieved in hand dishwashing products (see Figure 1.5). Although there is no published information, there are reasons to believe that certain natural surfactants and notably

Figure 1.5 Biosurfactants are already used in hand dishwashing liquids and in household cleaners. (Courtesy of Joakim Holmberg.)

Figure 1.6 The cosmetic industry has recorded the first commercial entry for biosurfactants. (*Source:* Wikimedia Commons / CC BY-SA 3.0.)

the saponins, may have the potential, probably in combination with synthetic surfactants, to solve today's two major challenges for domestic laundry detergents:

- stains removal
- energy conservation (shorter washing cycles at lower temperature)

These are discussed in the section on potential applications in the chapter on saponins.

It is obvious that a massive entry into the household detergents will require volumes far in excess of what can be supplied today or can be envisaged in a foreseeable future, and this is a serious matter that needs attentive consideration, as discussed in section 1.6.

Outside the household laundry detergents, there are other markets open to natural surfactants. The cosmetic industry has already responded favorably (see Figure 1.6). It has become an established consumer of saponins, sophorolipids, and rhamnolipids and is eying with interest to the mannosylerithritol lipids. But it is fair to recognize that what has happened so far has been just scratching the surface. There are many more opportunities ahead, driven both by objective benefits and the emotional appeal that the natural/sustainable/renewable connotation elicits.

In the authors' opinion, agriculture/crop protection has received less attention than it deserves. While there is no evidence that biosurfactants on their own can show pesticidal and fungicidal activity superior to that of existing synthetic actives, they could perform additively or synergistically with plant protection actives, while enabling formulations in convenient delivery forms, for example for aerial spray(see Figure 1.7).

Soil remediation is a realistic and environmentally beneficial application target if it can be demonstrated beyond doubt that biosurfactants not only leach soil contaminants but

Figure 1.7 An aerial crop duster applying a fine mist. (Image credit: Stephan Krause / Wikimedia Commons / Public domain.)

Figure 1.8 Remediated soil can be returned to agriculture. (*Source:* SURASIT / Adobe Stock Photos.)

also enhance their ultimate degradation into nonnoxious catabolites, thus enabling the re-start of agricultural exploitation (see Figure 1.8). Unless this is happening, any surfactant, no matter if bio, bio-based, or petrochemical, will only move the issue from one place to another, from soil to aquifers. A confirmation that this enhanced biodegradation is possible comes, among others, from a recent study by Patowary et al. [47].

Figure 1.9 Microbial enhanced oil recovery, where biosurfactants are generated in situ, may have a future. (Image credit: Ficelloguy / Wikimedia Commons / CC BY-SA 3.0.)

Microbially enhanced oil recovery, often written M-EOR (or MEOR), is the technique of injecting a surfactant-producing microorganism into the reservoir to generate in-situ production of biosurfactants. The crude oil is normally the sole carbon source necessary, but a nitrogen source and other nutrients need to be injected together with the microorganism. The microorganism is supposed to generate amphiphiles that will reduce the interfacial tension between the crude and the injection water to very low levels, of the order of 10^{-3} mN/m or lower, which is key for successful mobilization of the oil (see Figure 1.9). The microorganism should also decompose the oil into shorter hydrocarbons, which will facilitate the recovery due to lower viscosity of the oil. In successful cases, the decomposition is so powerful that a substantial amount of gas is also generated. M-EOR field trials of this type are currently being performed in large scale in China and the results so far are promising. Injecting ex-situ generated biosurfactants into the reservoir does not hold promise, however. That would fall short from cost and maybe also from technical reasons.

It is an undeniable fact that natural surfactants are today (2025) playing a real, sustained role in colloid chemistry and its industrial realizations, a step change from the situation of just a few years ago. The renewed interest of the industry, the public opinion, and the regulatory drive for sustainability, the improved knowledge gained in understanding their physical and chemical properties and in-use performance, the progress in formulation technology, and the positive impact of process improvement for both cost reduction and availability are positive steps forward for a promising future.

As a final remark, natural surfactants may be the shot in the arm that the surfactant industry is in need of. The volumes of surfactants on the market are impressive and growing but from a technological point of view, the surfactant industry is in many ways a sunset industry. Most of the important types of surfactants that we use today appeared on the market during the half-century 1920–1970. The important processes involved in surfactant production, such as alkylation of aromatics, development of processes to make synthetic alcohols, sulfonation, sulfation, alkoxylation, etc., were developed and commercialized during this

period. After 1970, only a few new products have reached the market on a large scale, the paraffin sulfonates and the ester quats being the most important exceptions. The advent of these two surfactant classes also dates long back. They were both produced already in the 1930s, although on a relatively small scale.

There have also been substantial innovations during the last half-century related to silicone surfactants, acetylenic surfactants, and fluorinated surfactants, but these are small volume, specialty products. In addition, silicone surfactants and fluorinated surfactants will probably experience problems in the future because they face problems in meeting the increasingly strict environmental regulations.

In short, there is time for something new in the surfactant arena. Natural surfactants may be the answer in a longer-term perspective.

References

1 Bacille, N. (2023). Are microbial *biosurfactants* actually only *surfactants*? *Current Opinion in Colloid and Interface Science* 68: 101747.

2 Bognolo, G. (2023). *Surface Active Agents – Historical Perspectives and Future Development*. Boca Raton, FL: CRC Press.

3 Guerini, V. (1909). *A History of Dentistry: From the Most Ancient Times Until the End of the Eighteenth Century*. Philadelphia, PA: Lea & Febiger.

4 Bergström, S., Theorell, H., and Davide, H. (1946). Pyolipic acid, a metabolic product of *Pseudomonas pyocyanea*, active against *Mycobacterium tuberculosis*. *Archives of Biochemistry* 10: 165–166.

5 Jarvis, F.G. and Johnson, M.J. (1949). A glycol-lipide produced by *Pseudomonas aeruginosa*. *Journal of the American Chemical Society* 71: 4124–4126.

6 Gorin, P., Spencer, J., and Tulloch, A. (1961). Hydroxy fatty acid glycosides of sophorose from Torulopsis magnolia. *Canadian Journal of Chemistry* 39: 846–855.

7 Arima, K., Kakinuma, A., and Tamura, G. (1968). Surfactin, a crystalline peptidelipid surfactant produced by *Bacillus subtilis*: isolation, characterization and its inhibition of fibrin clot formation. *Biochemical and Biophysical Research Communications* 31: 488–494.

8 Tropsch, J. (2017). A journey to standardization of bio-based surfactants in Europe. *Inform* 28: 20–22.

9 Zweers, J.C., Barak, I., Becker, D. et al. (2008). Toward the development of *Bacillus subtilis* as a cell factory for membrane proteins and protein complexes. *Microbial Cell Factories* 7: 10.

10 Jaime, J.B., Romero, L.G., and Leon, J.C. (2023). Selection of biosurfactant-producing microorganisms. In: *From Biorefineries Production to Versatile Applications* (ed. P.R.F. Marcelino, S.S. da Silva, and A.O. Lopez) Chapter 2. Chichester, UK: Wiley.

11 Lee, D.W., Lee, H., Kwon, B.-O. et al. (2018). Biosurfactant-assisted bioremediation of crude oil by indigenous bacteria isolated from Taean beach sediment. *Environmental Pollution* 241: 254–264.

12 Patowary, K., Patowary, R., Kalita, M.C., and Deka, S. (2017). Characterization of biosurfactant produced during degradation of hydrocarbons using crude oil as sole source of carbon. *Frontiers in Microbiology* 8: 1–14.

13 Liepins, J., Balina, K., Soloha, R. et al. (2021). Glycolipid biosurfactant production from waste cooking oils by yeast: review of substrates, producers and products. *Fermentation* 7: 136.

14 Jimoh, A.A. and Lin, J. (2019). Enhancement of *Paenibacillus* sp. D9 lipopeptide biosurfactant production through the optimization of medium composition and its application for biodegradation of hydrophobic pollutants. *Applied Biochemistry and Biotechnology* 187: 724–743.

15 Liang, X., Shi, R., Radosevich, M. et al. (2017). Anaerobic lipopeptide biosurfactant production by an engineered bacterial strain for *in situ* microbial enhanced oil recovery. *RSC Advances* 7: 20667–20676.

16 Abbasi, H., Hamedi, M.M., Lotfabad, T.B. et al. (2012). Biosurfactant producing bacterium, *Pseudomonas aeruginosa* MA01 isolated from spoiled apples: physicochemical and structural characteristics of isolated biosurfactant. *Journal of Bioscience and Bioengineering* 113: 211–219.

17 Nurfarahin, A.H., Mohamed, M.S., and Phang, L.Y. (2018). Culture medium development for microbial-derived surfactants production – an overview. *Molecules* 23: 1049.

18 Subsanguan, T., Khondee, N., Nawavimarn, P. et al. (2020). Reuse of immobilized *Weissella cibaria* PN3 for long-term production of both extracellular and cell-bound glycolipid biosurfactants. *Frontiers in Bioengineering and Biotechnology* 8: 751.

19 Joy, S., Rahman, P.K.S.M., Khare, S.K. et al. (2019). Statistical and sequential (fill-and-draw) approach to enhance rhamnolipid production using industrial lignocellulosic hydrolysate C_6 stream from *Achromobacter* sp. (PS1). *Bioresource Technology* 288: 121494.

20 Bustos, G., Arcos, U., Vecino, X. et al. (2018). Recycled *Lactobacillus pentosus* biomass can regenerate biosurfactants after various fermentative and extractive cycles. *Biochemical Engineering Journal* 132: 191–195.

21 Partovinia, A. and Rasekh, B. (2018). Review of the immobilized microbial cell systems for bioremediation of petroleum hydrocarbons polluted environments. *Critical Reviews in Environmental Science and Technology* 48: 1–38.

22 Bouassida, M., Mnif, I., and Ghribi, D. (2023). Enhanced biosurfactant production by Bacillus subtilis SPB_1 using developed fed-batch fermentation: effects of glucose levels and feeding systems. *Bioprocess and Biosystems Engineering* 46: 555–563.

23 Zhu, Z., Zhang, F., Wei, Z. et al. (2013). The usage of rice straw as a major substrate for the production of surfactin by *Bacillus amyloliquefaciens* XZ-173 in solid-state fermentation. *Journal of Environmental Management* 27: 96–102.

24 Al-Dhabi, N.A., Esmail, G.A., and Arasu, M.V. (2020). Enhanced production of biosurfactant from *Bacillus subtilis* strain Al-Dhabi-130 under solid-state fermentation using date molasses from Saudi Arabia for bioremediation of crude-oil-contaminated soil. *International Journal of Environmental Research and Public Health* 17: 8446.

25 Eras-Muñoz, E., Gea, T., and Font, X. (2023). Carbon and nitrogen optimization in solid-state fermentation for sustainable sophorolipid production using industrial waste. *Frontiers in Bioengineering and Biotechnology* 11: 1252733.

26 Bouassida, M., Mnif, I., Hammami, I. et al. (2023). *Bacillus subtilis* SPB_1 lipopeptide biosurfactant: antibacterial efficiency against the phytopathogenic bacteria *Agrobacterium tumefaciens* and compared production in submerged and solid state fermentation systems. *Food Science and Biotechnology* 32: 1–15.

27 Claus, S., Sánchez, L.J., and Van Bogaert, I.N.A. (2021). The role of transport proteins in the production of microbial glycolipid biosurfactants. *Applied Microbiology and Biotechnology* 105: 1779–1793.

28 Mulligan, C.N. and Gibbs, B.F. (1990). Recovery of biosurfactants by ultrafiltration. *Journal of Chemical Technology and Biotechnology* 47: 23–29.

29 Montastruc, L., Liu, T., Nikov, I. et al. (2011). Integrated process for production of surfactin (III). Modeling of adsorption column. *Chinese Journal of Chemical Engineering* 19: 357–364.

30 Mohanty, S.S., Koul, Y., Varjani, S. et al. (2021). A critical review on various feedstocks as sustainable substrates for biosurfactants production. *Microbial Cell Factories* 20: 120.

31 Sala, S., Amadei, A.M., Beylot, A., and Ardente, F. (2021). The evolution of life cycle assessment in European policies over three decades. *The International Journal of Life Cycle Assessment* 26: 2295–2314.

32 Valdivia, S., Backes, J.G., Traverso, M. et al. (2021). Principles for the application of life cycle sustainability assessment. *The International Journal of Life Cycle Assessment* 26: 1900–1905.

33 Aru, O.O. and NE, O.I. (2018). Life cycle assessment of the environmental impact of biosurfactant production from oil waste by a diculture of *Azotobacter vinelandii* and *Pseudomona*s sp. *Journal of Bioremediation & Biodegradation* 9: 435.

34 Hu, X., Subramanian, K., Wang, H. et al. (2021). Guiding environmental sustainability of emerging bioconversion technology for waste-derived sophorolipid production by adopting a dynamic life cycle assessment (dLCA) approach. *Environmental Pollution* 269: 116101.

35 Hu, X., Subramanian, K., Wang, H. et al. (2021). Bioconversion of food waste to produce industrial-scale sophorolipid syrup and crystals: dynamic life cycle assessment (dLCA) of emerging biotechnologies. *Bioresource Technology* 337: 125474.

36 Kopsahelis, A., Kourmentza, C., Zafiri, C., and Kornaros, M. (2018). Gate-to-gate life cycle assessment of biosurfactants and bioplasticizers production via biotechnological exploitation of fats and waste oils. *Journal of Chemical Technology and Biotechnology* 93: 2833–2841.

37 Schonhoff, A., Ihling, N., Schreiber, A., and Zapp, P. (2022). Environmental impacts of biosurfactant production based on substrates from the sugar industry. *ACS Sustainable Chemistry & Engineering* 10: 9345–9358.

38 Schonhoff, A., Stöckigt, G., Wulf, C. et al. (2023). Biosurfactants production with substrates from sugar industry. Environmental, cost, market and social aspects. *RSC Sustainability* 1: 1798–1813.

39 Moutinho, L.F., Moura, F.R., Silvestre, R.C., and Romão-Dumaresq, A.S. (2021). Microbial biosurfactants: a broad analysis of properties, applications, biosynthesis, and techno-economical assessment of rhamnolipid production. *Biotechnology Progress* 37: e3093.

40 Oraby, A., Rupp, S., and Zibek, S. (2022). Techno-economic analysis as a driver for optimisation of cellobiose lipid fermentation and purification. *Frontiers in Bioengineering and Biotechnology* 10: 913351.

41 Pi-Boleda, B., Bouzas, M., Gaztelumendi, N. et al. (2020). Chiral pH-sensitive cyclobutane β-amino acid-based cationic amphiphiles: possible candidates for use in gene therapy. *Journal of Molecular Liquids* 297: 11.

42 Berthier, D., Buffeteau, T., Léger, J.-M. et al. (2002). From chiral counterions to twisted membranes. *Journal of the American Chemical Society* 124: 13486–13494.

43 Koc, M.H., Ciftci, G.C., Baday, S. et al. (2017). Hierarchical self-assembly of histidine-functionalized peptide amphiphiles into supramolecular chiral nanostructures. *Langmuir* 33: 7947–7956.

44 Liang, X., Gui, Y., Li, K. et al. (2020). A novel chiral surfactant-type metallomicellar catalyst for asymmetric Michael addition in water. *Chemical Communications* 56: 11118–11121.

45 Liu, L., Sun, D., Li, F. et al. (2019). Enantioselective liquid-liquid extraction of valine enantiomers in the aqueous two-phase system formed by the cholinium amino acid ionic liquid copper complexes and salt. *Journal of Molecular Liquids* 294: 111599.

46 Xiong, Z., Zhou, L., Wang, J. et al. (2024). Chiral surfactants: design, aggregation behaviors and applications. *Current Opinion in Colloid & Interface Science* 73: 101842.

47 Patowary, R., Jain, P., Malakar, C., and Devi, A. (2023). Biodegradation of carbofuran by *Pseudomonas aeruginosa* SO_7: biosurfactant production, plant growth promotion, and metal tolerance. *Environmental Science and Pollution Research* 30: 115185–115198.