Regulatory status quo and prospects for biosurfactants in pharmaceutical applications

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The concept of going 'green' and 'cold' has led to utilizing renewable resources for the synthesis of microbial biosurfactants that are both patient and eco-friendly. In this review, we shed light on the potential and regulatory aspects of biosurfactants in pharmaceutical applications and how they can significantly contribute to novel concepts for the Coronavirus 2019 (COVID-19) vaccine and future treatment. We emphasize that more specific guidelines should be formulated to regulate the approval of biosurfactants for human use. It is also crucial to implement a risk-based approach from the early research and development (R&D) phase in addition to establishing more robust standardized techniques and assays to evaluate the characteristics of biosurfactants.

Keywords: Microbial biosurfactants; Nanoformulations; COVID-19; EMA; FDA

Introduction

Surface-active agents, also known as tensides, amphiphiles, or surfactants, are substances that tend to preferentially accumulate at the boundary (i.e., interface) between two phases and reduce surface tension [1]. Surfactants have a significant role in both the pharma and nonpharma fields. These agents impart a variety of applications, including, but not limited to, enhancing the solubility, increasing the permeability, improving the dissolution rate, and enhancing the stability of the colloid [2–4].

However, the toxicity of surfactants resulted in a worldwide alert followed by various regulations. There are still concerns about their biodegradability, safety, and eco-friendliness. Hence, increased efforts are devoted to developing 'green' surfactants that are safe not only for customers (patients) and the environment, but also from an economic and productivity perspective, being 'benign-by-design'. The rational combination of both green chemistry (GC) and Quality-by-Design (QbD) principles provides a dual advantage for the robust design of patient-friendly and environmentally benign surfactants.

Surfactants in pharmaceutical applications

Given the remarkable physical properties of surfactants, resulting from their distinctive molecular features, various surfactant-based delivery systems for systemic and/or localized delivery have been developed. These include microspheres, micro/nano-based drug carriers [polymeric and lipid-based micro/nanocarriers, micro/nanoemulsions, self-emulsifying drug delivery systems (SEDDS), liposomes, solid lipid nanoparticles (SLNs), niosomes and micro/nanogels], novel powders, hydrogels, and polymeric micelles [1,5–9]. The ever-increasing number of poorly soluble and/or permeable drugs requires the development of effective and safe surfactants to enhance the bioavailability of these drugs. In addition to enhancing the solubility or stability of the drug in liquid, semisolid, and solid preparations, surfac-

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tants also have a vital role in controlling the particle size of nano-based drug delivery systems [10]. Furthermore, surfactants can have direct effects on biological membranes, thus affecting membrane integrity and altering drug transport across the membrane [6,11,12]

Given their wetting effect, surfactants have been used in tablet and capsule formulations to enhance the wetting and disaggregation of drug particles, which, in turn, maximizes the surface area of particles available for dissolution [13,14]. In suppository formulations, surfactants are widely used not only to improve the wetting and water-absorption properties of the suppository, but also to keep insoluble substances suspended in a fatty base suppository [15]. In addition, these agents can improve the permeability and rectal absorption of loaded drugs. In pulmonary drug delivery systems, surfactants are used as stabilizing agents for suspensions, solubilizing agents, absorption enhancers, and lubricants as metering mechanisms of metered-dose inhalers [16]. Moreover, surfactants are used in topical formulations (creams, lotions, and micro/nanoemulsions) as emulsifying agents and also as penetration enhancers [in monomeric concentrations or concentrations above critical micelle concentration (CMC)] in skin patches and gels for topical application [17].

In addition to their utilization as excipients to enhance the physical and chemical characteristics of the pharmaceutical formulations, surfactants can be applied to improve the efficacy or bioperformance of the pharmaceutical product, such as promoting the actions of antimicrobial drugs or laxatives [18].

Urgent need for green surfactants

The overall impact of applying surfactants in pharmaceutical formulations is complex and perhaps beyond their intended purpose. The toxicological effects of surfactants were assessed in 1967 by Elworthy and Treon [19]. Non-ionic surfactants are generally considered to be less toxic, with more solubilizing power under biological conditions [20–22]. Many synthetic surfactants have been used in pharmaceutical formulations without being approved by regulatory bodies for human use, showing toxic effects. Several studies reported several toxic effects of surfactants that disrupt vital functions in the human body, examples of which are provided in Table 1 [20,23–30].

TABLE 1

Examples of toxic effects of surfactants used in drug formulations.

Surfactant example	Toxic effect
CTAB, Tweens, Triton x100, Myrj S40, Pluronic F68	Alters enzymatic activity in liver and intestine
STDC, CTAB, SDS	Disrupts membrane integrity
CTAB, DOC, DSS, Brij 35, Tween 80	Alters drug absorption rate, intestinal transit, and gastric emptying
SDS, STS	Surfactant–protein interactions and subsequent solubilization of insoluble membrane-bound protein
SLS, CTAB	Affects skin integrity, causing protein denaturation and loss of water-binding capacity
CTAB, ADBAC	Affects eye mucosa (inflammation, edema, photophobia, purulent exudate, and irritation)
Triton X100, Span 20, Brij 56, CTAC	Hemolysis activity

[a]Abbreviations: ADBAC, alkyl dimethyl benzyl ammonium chloride; CTAB, cetyl trimethyl ammonium bromide; CTAC, cetyl trimethyl ammonium chloride; DOC, sodium deoxycholate; DSS, dioctylsulfusuccinate; SDS, sodium dodecyl sulfate; SLS, sodium lauryl sulfate; STDC, sodium taurodeoxycholate; STS, sodium tetradecyl sulfate.
[b]Based on [12,20,23–30].

The toxicity level of synthetic surfactants is structure and concentration dependent [12,26,29,31]. Different classes of surfactant cause various toxic effects and to different extents, from altering membrane permeability because of disrupting membrane integrity, skin damage, severe mucous membranes and eye irritation, to inactivation of enzymes, such as esterase and phosphatase, and pseudomembrane and ulcer formation. Higher concentrations of surfactants can damage the epithelial cells of the mouth, pharynx, and upper airway.

Cationic surfactants have the highest toxicity level, followed by anionic, zwitterionic (or amphoteric), and non-ionic surfactants, Disruption of cell membrane integrity by cationic surfactants has been widely reported, and this limits their pharmaceutical application. A recent paper assessing the cytotoxicity of cationic SLNs against five human cell lines showed that the type of cationic lipid is a risky formulation parameter that impacts the cytotoxic profile of the SLNs, with CTAB-SLNs being highly cytotoxic even at low concentrations [22]. To avoid the toxicity of synthetic surfactants, enhancing the biodegradability and chemical recyclability of new surfactants using renewable resources by an environmental-friendly process is urgently needed [32]. In addition, the focus on enhancing the stability and efficacy of surfactants is one way to minimize their concentrations in drug products.

The concept of going 'green' and 'cold' has led to the utilization of renewable resources for 'green' surfactant synthesis that are both patient and eco-friendly. GC emphasizes the need to eliminate or minimize toxic effects on health as well as the environment during the synthesis and analysis of new compounds [33]. To this end, significant progress in biotechnology, stricter regulatory requirements and industrial expectations regarding the toxicity and cost of newly synthesized surfactants, as well as the enhanced ecological consciousness among customers (patients), have delivered additional stimulus for the development of green surfactants to be used for pharmaceutical applications and also in other industries (i.e., food, cosmetics, and petroleum industries) [34,35].

The past few decades demonstrated an enormous effort devoted to the development of green biosurfactants naturally produced by microorganisms (yeasts, bacteria, and some filamentous fungi) when grown on water-miscible or oily substrates [34]. Various renewable raw materials, particularly triglycerides, carbohydrate sources, and organic acids (produced by fermentation), serve as starting materials in green surfactant synthesis, of which triglycerides/sterols contribute to the hydrophobic part. By contrast, sugars/amino acids contribute to the hydrophilic part of these surfactants [33].

These biobased amphiphilic molecules are able to reduce surface and interfacial tensions using the same mechanisms as chemical surfactants. Moreover, they have several advantages making them superior to chemically synthesized surfactants, including higher biodegradability, lower toxicity (lower CMC), better surface and interfacial activity, higher selectivity and,

hence, greater safety and biocompatibility, not to mention their eco-friendliness and stability [34,36]. They also ascend in soil, which should expand their use for other industrial fields beyond pharmaceutical applications. In addition, by using biological systems, different structural types of surfactant can be produced (Table 2), such as glycolipids, lipopeptides, lipoproteins, lipopeptides, lipopolysaccharide–protein complexes and polysaccharide–protein–fatty acid complexes, which cannot be produced simply by chemical processes [36]. However, the complex nature of their head groups (amino acids in lipopeptides and saccharides in glycolipids) can lead to challenging characterizations of their structure because they are adaptable to different structures triggered via pH, temperature, or other environmental changes [32]. Furthermore, the high manufacturing costs of raw materials and bioprocessing, in addition to low productivities, will eventually limit the industrial-scale production of biosurfactants [37,38]. Fig. 1 presents a strengths, weaknesses, opportunities, and threats (SWOT) analysis of the application of biosurfactants in the pharmaceutical industry.

TABLE 2

Major classes of microbial biosurfactants and examples of surfactant-producing microorganisms.[a]

Surfactant class	Examples	Microorganism
Glycolipids	Rhamnolipids	*Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas chlororaphis*, *Bacillus subtilis*, *Renibacterium salmoninarum*
	Cellobiolipids	*Ustilago maydis*
	Sophorolipids	*Candida bombicola*, *Candida apicola*
	Trehalipids	*Rhodococcus* spp., *Tsukamurella* spp., *Arthrobacter* spp.
	Mannosylerythritol lipid A	*Candida antartica*, *Kurtzmanomyces* spp., *Pseudozyma fusifornata*
Lipopeptides	Viscosin	*Pseudomonas fluorescens*, *Leuconostoc mesenteroides*
	Serrawettin	*Serratia marcescens*
	Gramicidins	*Bacillus brevis*, *Brevibacterium brevis*
	Polymyxins	*Bacillus polymyxa*, *Brevibacterium polymyxa*
	Iturin	*B. subtilis*
	Peptide-lipid	*Achromobacter* spp.
	Subtilisin	*B. subtilis*
	Surfactin	*B. subtilis*
	Lichenysin	*Bacillus licheniformis*
Fatty acids, phospholipids, and neutral lipids	Fatty acids Neutral lipids Phospholipids	*Nocardia erythropolis*, *Thiobacillus thiooxidans*, *Candida lepus*, *Acinetobacter* spp., *Pseudomonas* spp., *Micrococcus* spp., *Mycococcus* spp., *Candida* spp., *Penicillium* spp., *K. pneumoniae*
Polymeric biosurfactants	Emulsan	*Acinetobacter calcoaceticus*
	Alasan	*Acinetobacter radioresistens*
	Biodispersan	*A. calcoaceticus A2*
	Liposan	*Candida lipolytica*
	Mannan lipid protein	*Candida tropicalis*
	Carbohydrate-lipid-protein	*Debaryomyces polymorphus*
	Protein PA	*P. aeruginosa*
Particulate biosurfactants	Vesicles	*Acinetobacter* spp.

[a] Based on [38–41].

Compared with their chemical counterparts, which are classified based on the head group, biosurfactants are commonly categorized according to their molecular weight and by their microbial origin and chemical composition (Table 2). They are divided according to their molecular weight into low (glycolipids, lipopeptides, flavolipids, and phospholipids) and high (polysaccharides, proteins, lipoproteins, lipopolysaccharide–protein complexes, and polysaccharide–protein–fatty acid complexes) molecular weight [38–40]. Biosurfactants are mainly produced by microbial cultures, where bacteria of the genera *Pseudomonas* and *Bacillus* are good biosurfactant producers, whereas *Candida bombicola* and *Candida lipolytica* are the most widely used yeasts for biosurfactants production [39]. A crucial advantage of using yeasts, such as *C. lipolytica, Saccharomyces cerevisiae*, and *Kluyveromyces lactis*, resides in their 'generally regarded as safe' (GRAS) status [34,41].

Regulatory aspects of potential novel surfactants

The use of surfactants has become crucial in several pharmaceutical applications, especially nanoformulations. Given that safety has always been the most crucial requirement when dealing with pharmaceutical products, regulatory bodies in the USA and Europe have published guidelines regarding the safety evaluation of new excipients, such as surfactants.

The US Food and Drug Administration (FDA) guidelines emphasize that the use of surfactants at a predefined concentration in drug development requires appropriate justification. The FDA also highlights the importance of performing risk–benefit assessments of proposed new excipients to be used in drug products. It has published a guidance document for industry on the conduct of nonclinical studies for the safety evaluation of new pharmaceutical excipients [42]. The guidance recommends performing all pivotal toxicology studies for the potential new excipient in accordance with state-of-the-art protocols and Good Laboratory Practice regulations.

The safety pharmacology studies must be conducted according to ICH guidelines S7A. For potential excipients intended for short-term use, at least one of the following safety evaluations is recommended: (i) acute toxicology studies performed in both a rodent species and a mammalian nonrodent species by the route of administration intended for clinical use (*CDER Guidance for Industry Single Dose Acute Toxicity Testing for Pharmaceuticals*); (ii) the absorption, distribution, metabolism, and excretion of the excipient should be studied following administration by the clinically relevant routes to the same species that are used in the nonclinical safety studies (ICH guidelines S3A and S3B); (iii) the standard battery of genetic toxicology studies to be conducted according to ICH guidance S2B; (iv) 1-month repeat-dose toxicology studies should be performed in both a rodent species and a mammalian nonrodent species by the route of administra-

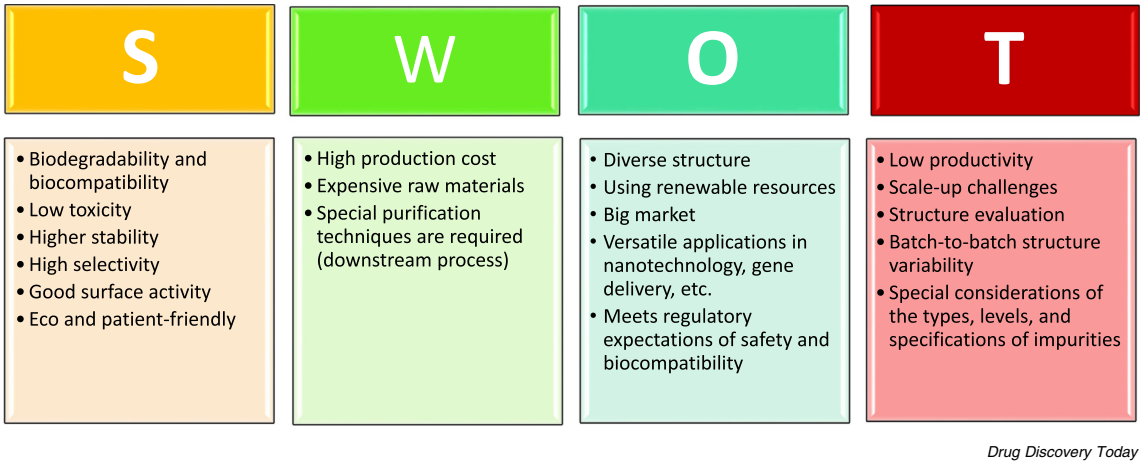

FIGURE 1
Strengths, weaknesses, opportunities, and threats (SWOT) analysis of biosurfactants for pharmaceutical application.

tion intended for clinical use. It is important that the studies include complete clinical pathology, histopathology, and toxicokinetic analysis; and finally (v) reproductive toxicology of the excipient should be evaluated according to ICH guidelines S5A and S5B.

In addition to conducting all the aforementioned safety studies (except the 1-month repeat-dose toxicology studies), 3-month repeat-dose toxicology studies are recommended to be performed to evaluate the potential excipient intended for intermediate use in both a rodent species and a mammalian nonrodent species by the appropriate route of administration. Additional studies might also be required (e.g., involving parenteral administration).

If the potential excipient is intended for long-term use, the following safety evaluations are recommended: (i) all the aforementioned studies regarding excipient intended for short-term and intermediate use; (ii) 6-month repeat-dose toxicology study be performed in a rodent species by the appropriate route; (iii) chronic toxicology studies must be performed in a mammalian nonrodent species by the appropriate route; and (iv) their carcinogenic potential should be evaluated according to ICH guideline S1A.

For a potential excipient intended for use in injectable, topical (dermal, intranasal, intraoral, ophthalmic, rectal, or vaginal), or pulmonary drug products, the safety evaluations should include the following: (i) all the previously mentioned studies, as appropriate, using the appropriate route of administration; (ii) sensitization study (e.g., guinea pig maximization study or murine local lymph node assay); (iii) for excipients intended for injectable use, the following considerations might be appropriate: (a) *in vitro* hemolysis study to be conducted at the intended concentration for i.v. administration (bolus and/or infusion); (b) plasma concentrations of creatinine kinase to be determined at the intended excipient concentration for intramuscular or subcutaneous administration can provide information on potential muscle damage; and (c) protein binding in relation to local site tolerability; (iv) for excipients intended for topical use, toxicology studies by both the intended clinical route and the oral or parenteral route might be required when clinical pharmacokinetic studies show that patients would experience systemic exposure to the excipient or its metabolite; and (v) for topical dermal products and ophthalmic products, ocular irritation studies might also be required.

In Europe, European Medicines Agency (EMA) guidelines on excipients including surfactants (emulsifiers, solubilizers, permeation enhancers, stabilizers, or preservatives) for marketing authorization of medicinal products for human use [43] state that the pharmaceutical development section should include an explanation of the choice of the excipient and its concentration, and how the properties of the excipient can affect the drug product performance or manufacturability. In addition, compatibility studies of the excipient with the drug and, where relevant, other excipients should be also established. Likewise, data concerning residual solvents should be submitted in accordance with the *Note for Guidance on Impurities: Residual Solvents* (CPMP/ICH/283/95).

For excipients described in the *European Pharmacopoeia*, or in the pharmacopoeia of an EU Member State, justification of specifications will normally not be required. Regarding novel excipients, full details of the manufacture, characterization, and controls with cross references to supporting safety data should be provided according to the drug substance format, and the following should be taken into consideration: (i) any bibliographical data on the chemistry and on the toxicology and the field in which the product is already used; (ii) the international specifications (FAO/WHO/JECFA), and other publications, such as the Food Chemical Codex; (iii) for medicinal products for cutaneous use, data on the ingredient used in cosmetic products; (iv) data concerning the toxicology of the novel excipient according to the dosage form and the route of administration of the medicinal product (if applicable) in Module 4, the safety section of the dossier; and (v) documentation on the chemistry of novel excipients (origin, manufacturing process, structure, physicochemical properties, identification and purity tests, stability data, impurities, residual solvents, etc.), taking as its basis the *CPMP Guideline on the Chemistry of New Active Substances* (CPMP/QWP/130/96).

Given that biosurfactants are produced from microbial culture, there are also special impurity tests, in addition to the general guidelines for the toxicity assessment of new excipients, that

should be considered. ICH Q3D (R1) [44] and ICH Q6B guidelines [45] on elemental impurities should be followed to control the elemental impurities in biotechnologically derived products. These ICH guidelines describe the characterization and analytical specifications of biotechnological-based products with a focus on controlling potential residual impurities. In addition, the *European Pharmacopoeia Monograph* [46] and the *United States Pharmacopeia General Chapter* [47] provide specific guidance for controlling host cell-derived impurities, host cell proteins (HCPs), and other bioprocess-related impurities by using appropriate risk-management strategies.

A combination of well-established upstream processes along with the selection of appropriate purification techniques (precipitation, filtration, centrifugation, extraction, etc.) in the downstream process should be optimized according to a risk-based approach to maximize the efficacy and minimize the level of residual undesired impurities produced from the fermentation process, while developing a scale-up cost-effective production method.

COVID-19 standpoint

Polymeric and lipid-based nanocarriers (NCs), including SLNs, liposomes, and SEDDS, have significant potential in biologics delivery, including vaccines. Given their physicochemical properties, NCs can protect biological drugs, such as RNA and DNA, from degradation, enhancing the permeability across biological barriers while providing controlled and targeted release [48]. Lipid-based NCs have proven potential to be translated into the clinic in terms of COVID-19 vaccine development. Over the past year, many potential gene-based vaccines loaded into lipid NCs have been developed against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Such vaccines have a leading role in the global response to the COVID-19 pandemic [49].

SLNs are colloidal carriers (50 nm–1 μm) that contain solid lipids and are distributed in an aqueous surfactant solution [50]. Liposomes are spherical bilayered phospholipid structures (20 nm–10 μm) surrounding an inner aqueous core, whereas niosomes are liposomes made with nonionic surfactants. SEDDS are isotropic thermodynamically stable mixtures of oil, solid, or liquid surfactants, solvents, and co-solvents/surfactants that can emulsify spontaneously to produce oil-in-water (o/w) nanoemulsions of ~100 nm or less in size upon being introduced into an aqueous phase, such as biological fluids, under gentle agitation [6]. In addition to these lipid NCs, polymeric NCs containing polymers such as PLGA [a co-polymer of PLA and poly glycolic acid (PGA)], approved by the FDA for therapeutic use in humans, have also shown promising results as carriers for several biologics, including vaccines [50].

Some studies highlighted the dual antiviral efficacy and targeted drug delivery of nanoformulations, such as charged liposomes and polysaccharide-capped gold nanoparticles [51,52], which can offer the potential for treatments of COVID-19. Given their abovementioned unique properties, biosurfactants can shape the future of nanopharmaceutical development and help to shorten the regulatory pathway of novel vaccine-based nanoformulations [53]. Biosurfactants offer new possibilities for designing safe, effective, and cost-effective nanosystems that meet the stakeholder expectations (patients, regulatory bodies, and industry). In addition to utilizing them as excipients, there is a pressing need to assess the anti-COVID-19 efficacy of microbial biosurfactants *in vitro* and *in vivo* [54].

Concluding remarks and general opinion

Biosurfactant production by microorganisms is a crucial strategy for applying GC concepts and advances in the environmental sustainability of the 21st century. Their biodegradability and green production from renewable-resource substrates give biosurfactants the potential to eventually replace their chemically synthesized counterparts.

According to regulatory guidelines in pharmaceutical development, the design of a new biosurfactant to be used in drug products should be based not only on its efficacy, but also on its safety, stability, pharmacokinetics profile, and physicochemical and biological compatibility with the other components of the delivery system (drug and excipients) to guarantee acceptance to be administered by the predefined clinically relevant routes.

The development of quality green biosurfactants should be fostered by emerging cost-effective technologies, selecting renewable materials, designing novel scale-up bioprocessing and developing characterization techniques. Given that large-scale industrial production can significantly hamper the application of biosurfactants, various strategies have been applied to improve production economics of biosurfactants [40,47,55], including using inexpensive raw substrates, optimization of media components and growth conditions, in addition to applying design of experiments for statistical optimization of media components, upscaling process, downstream process.

Regardless of the success in biosurfactant production at the lab level and their potential in pharmaceutical formulations, the scale-up production of biosurfactants is limited because the composition of the final product is affected by many factors [55,56]. In addition, as with other bio-based products, the batch-to-batch structure variability of biosurfactants can affect its quality attributes, including the safety and efficacy of the final product. This should be carefully considered while designing novel biosurfactants and controlled by combining QbD and risk management with advanced structure analytical tools [Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and chromatographic methods] and in-process control. To this end, implementing the QbD approach from the early bioprocessing stage provides a robust strategy to be followed to control risk factors when designing novel surfactants for human use. QbD is a science- and risk-oriented approach that provides a piece of comprehensive knowledge, yielding high-quality products without extensive regulatory burden. By directing the focus toward building the quality in each step of bioprocessing of biosurfactants, QbD can guarantee saving time and effort while meeting stakeholder expectations (regulatory, patients, and industry) of the new surfactants. QbD-based submission is recommended by regulatory authorities [57] and will enable a smooth clinical translation of new biosurfactants. The elements of a pharmaceutical QbD approach are described in the relevant guidelines of the International Council of Harmonization (ICH), namely ICH Q8 (R2) (Pharmaceutical Development), ICH Q9 (Quality Risk Management), and ICH Q10 (Pharmaceutical Quality System) [58–60].

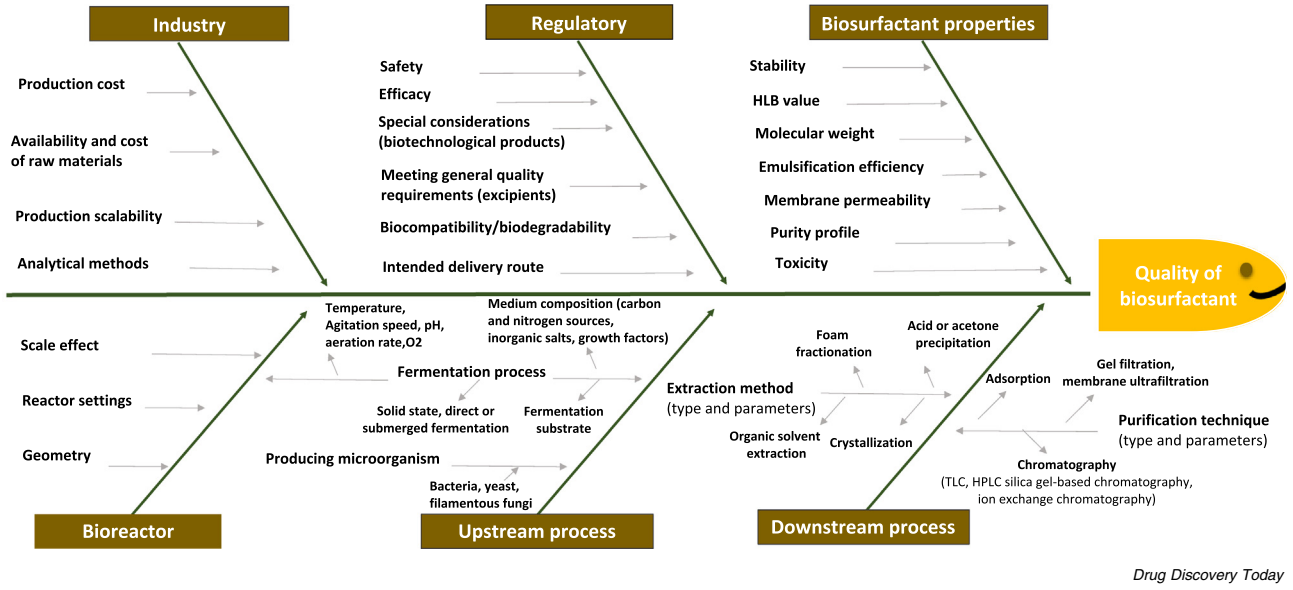

FIGURE 2
Ishikawa fishbone diagram for evaluating the risk factors affecting biosurfactant development.

As a part of QbD, initial risk assessment helps identify and prioritize the risk of all crucial process parameters (CPPs) that can impact the quality of the final product [61]. The Ishikawa diagram in Fig. 2 represents the crucial process parameters that can affect biosurfactant production.

More specific guidelines and regulations should be formulated for utilizing biosurfactants in the pharmaceutical industry. Moreover, additional robust standardized techniques and assays must be established to evaluate the critical quality attributes of biosurfactants. Besides considering the risk-based approach, biosurfactant development needs a multidisciplinary approach with thorough consideration of all the relevant knowledge from physiology, genetics, biochemistry, microbiology, and clinical and toxicology science.

Declaration of Competing Interest

None declared by authors.

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