

# Bioresource Technology

## Biosurfactants: The Green Generation of Speciality Chemicals and Potential Production Using Solid-State Fermentation (SSF) Technology

--Manuscript Draft--

<b>Manuscript Number:</b>	BITE-D-20-06080R2
<b>Article Type:</b>	Review article
<b>Keywords:</b>	Biosurfactants; glycolipids; solid-state fermentation; industrial applications; speciality chemicals
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<b>Abstract:</b>	<p>Surfactants are multipurpose products found in most sectors of contemporary industry. Their large-scale manufacturing has been mainly carried out using traditional chemical processes. Some of the chemical species involved in their production are considered hazardous and some industrial processes employing them categorised as “having potential negative impact on the environment”. Biological surfactants have therefore been generally accepted worldwide as suitable sustainable greener alternatives. Biosurfactants exhibit the same functionalities of synthetic analogues while having the ability to synergize with other molecules improving performances; this strengthens the possibility of reaching different markets via innovative formulations. Recently, their use was suggested to help combat Covid-19. In this review, an analysis of recent bibliography is presented with descriptions, statistics, classifications, applications, advantages, and challenges; evincing the reasons why biosurfactants can be considered as the chemical specialities of the future. Finally, the uses of the solid-state fermentation as a production technology for biosurfactants is presented.</p>

Dear Editors,

Please find attached a paper entitled “**Biosurfactants: The Green Generation of Speciality Chemicals and Potential Production Using Solid-State Fermentation (SSF) Technology**” for consideration for publications in Bioresource Technology.

Biosurfactant production has become quite an important type of molecules, highly sought after by a wide range of industrial processes and the production technologies are in some instances the main bottle neck.

This review therefore presents the case for Biosurfactants as the specialty chemicals for countless future industrial applications. An analysis of the state of the art with recent bibliography is presented with descriptions, statistics, classifications, and applications; evincing the reasons why biosurfactants can be considered as the chemical specialities of the future. Finally, we discuss the use of the solid-state fermentation as a production technology gap for future biosurfactants that may present a solution to future production and research.

We feel this area of importance and high curiosity for microbiologists enough to be considered for publication by Bioresource Technology.

Kindly note that:

1. An abstract with content and details have been submitted to the Editor-in-Chief (Professor A. Pandey) who approved submission.
2. All the authors mutually agree for submitting their manuscript to BITE
3. The manuscript has not been submitted earlier to BITE
4. The Subject Classification selected is 60: MICROBIAL PRODUCTS

Yours truly,

Ibrahim M Banat

Response to the second revision request

Dear Professor Pandey

Kindly find attached the second revision for this review in which we have carried out the requested revisions as follows:

Remove Fig 1; no such details on number of publications, etc should be given even in text.

Figure 1 was removed, and details of number removed from text

Remove Fig 2 by giving details in text only.

Figure 2 was removed, and details given in text

Refs can be maximum 150; delete older ones or those from books.

References were reduced to 150

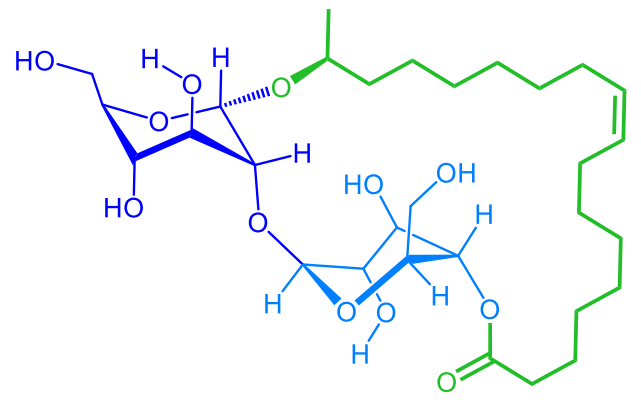
Page length can be maximum 50.

Length was reduced to 50 pages

Production



Biosurfactants



Applications

Pharmaceuticals



Bioremediation

Oil recovery



Cleansing

Cosmetics



Solid-state fermentation (SSF)

vs submerged fermentation



Perspectives



## **Highlights**

- Biosurfactants are becoming the specialty chemicals for many future industrial applications.
- Solid-state fermentation can mitigate some drawbacks of submerged liquid fermentation.
- Industry shows growing interest in biosurfactants for use in different markets.
- Covid-19 may boost future inclusion of biosurfactants in personal care products.

1 **Biosurfactants: The Green Generation of Speciality Chemicals and Potential Production**  
2  
3 **Using Solid-State Fermentation (SSF) Technology**  
4

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26

27 **Abstract**

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29 Surfactants are multipurpose products found in most sectors of contemporary industry. Their  
30 large-scale manufacturing has been mainly carried out using traditional chemical processes.  
31  
32 Some of the chemical species involved in their production are considered hazardous and some  
33 industrial processes employing them categorised as “having potential negative impact on the  
34 environment”. Biological surfactants have therefore been generally accepted worldwide as  
35 suitable sustainable greener alternatives. Biosurfactants exhibit the same functionalities of  
36 synthetic analogues while having the ability to synergize with other molecules improving  
37 performances; this strengthens the possibility of reaching different markets via innovative  
38 formulations. Recently, their use was suggested to help combat Covid-19. In this review, an  
39 analysis of recent bibliography is presented with descriptions, statistics, classifications,  
40 applications, advantages, and challenges; evincing the reasons why biosurfactants can be  
41 considered as the chemical specialities of the future. Finally, the uses of the solid-state  
42 fermentation as a production technology for biosurfactants is presented.  
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1 **Keywords**

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3 Biosurfactants, glycolipids, solid-state fermentation, industrial applications, speciality  
4  
5 chemicals.

6  
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53 **1. Introduction**

54  
55 The increasing concern of the industry about sustainable processes and exploitation of  
56 eco-friendly products are the driving force for the growing interest on biosurfactants (BS).  
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1 Indeed, they are generally considered as less toxic and more biodegradable species than their  
2 synthetic counterparts and can be obtained from sustainable sources (Marchant and Banat,  
3 2012). This interest is supported by the numerous successful applications of biosurfactants in  
4 recent years, not only in the replacement of conventional surfactants, but also because they  
5 exhibit own specific activities which find their use in different industrial sectors (Kumari et al.,  
6 2018; Rincón-Fontán et al., 2020; Sun et al., 2019; Zhao et al., 2018). However, production  
7 costs associated with BS, especially regarding the downstream processes, are still the main  
8 technological limitation for industrial exploitation (Banat et al., 2014b). Numerous studies have  
9 been focused on cost reduction, one example is the alternative approach of solid-state  
10 fermentation (SSF) which offers interesting perspectives as it is strongly tied to the concept of  
11 valorisation of biomass (e.g. the use of agro-industrial byproducts for culture media) and the  
12 reduction of downstream liquid volume treatment. This work aims to provide a comprehensive  
13 overview of the contemporary state on the subject of biosurfactants, advantages, disadvantages,  
14 challenges and production techniques, with particular emphasis on solid-state fermentation,  
15 critical process variables and a comparison with liquid fermentation. We also present some  
16 appealing applications of biosurfactants for different industrial sectors that have been the object  
17 of research and interest in the last five years including pharmaceutical, personal care,  
18 bioremediation and oil applications and some strategies for market access.

## 2. Surfactants: definitions, classifications and challenges

25 Surfactants are amphipathic chemical compounds constituted by two different molecular  
26 motifs: one hydrophilic and one lipophilic. The hydrophilic part is a polar moiety with strong  
27 affinity to polar substances such as water, while the lipophilic (or hydrophobic) shows affinity  
28 to non-polar media such as oils and fats; so, they can simultaneously interact with both polar  
29 and nonpolar substances. This dual characteristic allows surfactants to reduce the interfacial  
30 tension and confers desirable properties such as detergency, emulsifying activity, foaming,



1 mixing and dispersion (Teixeira Souza et al., 2018). Such features are highly demanded in  
2  
3 almost all sectors of the contemporary industry.  
4

5 Surfactants can be categorized according to their origin, electrostatic status, and their  
6  
7 hydrophilic and lipophilic balance (HLB); these categories are presented in Table 1. By origin,  
8  
9 surfactants can be synthetic, semi-synthetic, or biological (biosurfactants). Most of the known  
10  
11 surfactants worldwide fall in the synthetic and semi-synthetic type. Chemical surfactants mostly  
12  
13 comprise ethylene oxide and propylene oxide copolymers and alkoxyated derivatives of  
14  
15 alkylphenol, sorbitan esters, alcohols, and amines. Oleochemicals come from vegetable oils and  
16  
17 fats that are subsequently modified through chemical processes. The lipophilic component is  
18  
19 provided by a natural oil, while the ulterior synthesis conforms the hydrophilic moiety. Thus,  
20  
21 oleochemical surfactants are also called semi-synthetic surfactants or bio-based products.  
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23

24 Among recognized producers worldwide of both synthetic and semi-synthetic surfactants we  
25  
26 find Akzo Nobel (Netherlands), BASF (Germany), Clariant (Switzerland), Croda (UK), Dow  
27  
28 (USA), Evonik (Germany), Huntsman (USA), Oxiteno (Brazil), Procter & Gamble (USA),  
29  
30 Rhodia (France), Sabic (Saudi Arabia), Sasol (South Africa), Shell (Netherlands), Solvay  
31  
32 (Belgium) and Stepan (USA).  
33  
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### 36 **2.1. Drawbacks and challenges of conventional surfactants**

37 Surfactants are the key components in the formulation of a variety of products for many  
38  
39 different applications. However, their manufacturing has negatively impacted the environment  
40  
41 for many years. Most of the raw materials derived from crude oil show high profiles of  
42  
43 ecotoxicity and low profiles of biodegradability, e.g. alkylphenols and aromatic compounds (Li  
44  
45 et al., 2013). This is the reason why such chemical species are regulated by many international  
46  
47 organizations such as the Environmental Protection Agency (EPA), the Occupational Safety and  
48  
49 Health Administration (OSHA), the National Institute for Occupational Safety and Health  
50  
51 (NIOSH), the Department of Transportation (DOT) and The Food and Drug Administration  
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53 (FDA), all in the USA; and the European Chemicals Agency (ECHA) in Europe. Some  
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55 regulatory registries or laws include the ECL Restricted Substances List in South Korea, the  
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1 Industrial Safety and Health Law (ISHL) in Japan, the Work Place Hazardous Material  
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3 Information System (WHMIS) in Canada, the Toxic Substances Control (TSCA) in USA, and  
4  
5 many more, including international inventories of chemical substances. The negative potential  
6  
7 of surfactants has been temporarily attended by the introduction of bio-based products, e.g.  
8  
9 lauryl alcohol derivatives, which attenuate the synthetic nature and hazardousness of traditional  
10  
11 chemical technologies. Nonetheless, the sacrifice of natural resources and subsequent stages of  
12  
13 conventional chemical transformations are always required for manufacturing bio-based  
14  
15 products. This category of “greener” semi-synthetic products has experienced significant growth  
16  
17 in recent years in Europe and North America as a result of expanding applications in personal  
18  
19 care, home care, and agrochemicals. In addition to safety risks associated with the use of raw  
20  
21 materials, intermediaries, and finished products, other challenges to industry may lie in the  
22  
23 emission of billions of kilograms of carbon dioxide (CO<sub>2</sub>) into the atmosphere, contributing to  
24  
25 the undesirable climate change (Griffin et al., 2018). Consequently, the chemical industry is  
26  
27 now compelled to implement strategies for manufacturing more sustainable products through  
28  
29 more eco-friendly processes (Zimmerman et al., 2020) without sacrificing product efficiencies.  
30  
31 This should make biotechnological production of biosurfactants a more desirable technological  
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33 alternative that will resolve such drawbacks in the long term.  
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### 41 **3. Biosurfactants: attributes and interest**

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43 Biosurfactants (BS), also known as microbial (biological) surfactants, are secondary  
44  
45 metabolites that are produced at the end of the exponential growth phase of microorganisms  
46  
47 including bacteria, yeasts, and moulds. Biologically speaking, these molecules are involved in  
48  
49 cell development, biofilm formation, and regulation of osmotic pressure. They are also  
50  
51 implicated in cell survival under disadvantageous circumstances such as the need to use non-  
52  
53 bioavailable hydrophobic substances (like hydrocarbons) as their sole carbon source. In this last  
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55 case, excreted BS are involved in the diffusion of this inaccessible substrate through the cell  
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57 membrane (Santos et al., 2016). BS can either be excreted or remain inside the cell. Because BS  
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1 are produced through complex enzymatic reactions, such biomolecules often have complex  
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3 chemical structures that can be modulated through careful selection of the culture medium or via  
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5 genetic engineering (Brück et al., 2019).  
6

### 7 **3.1. Scientific and technological interest**

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9  
10 The large advantages of versatile BS over synthetic products have drawn the attention of  
11  
12 industrial research. Biosurfactants can be produced from renewable sources, generally have low  
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14 toxicity, are highly biodegradable, often display better environmental compatibility, and usually  
15  
16 remain stable at wide ranges of pH and temperatures (Das and Kumar, 2018; Gaur et al., 2019).  
17  
18 Increased environmental awareness among consumers combined with new environmental  
19  
20 legislations has provided further impetus for serious consideration of BS as possible alternatives  
21  
22 to existing products (Marchant and Banat, 2012). This has been evidenced by the significant rise  
23  
24 of publications not only of scientific articles, but also of patents on the topic. The following is a  
25  
26 summary of the search for bibliographic information from the database SciFinder of Chemical  
27  
28 Abstract Service (CAS). The keyword “biosurfactants” was used, and repeated references were  
29  
30 considered as one. From 1963 to mid-September 2020 the count of publications was around  
31  
32 10,130. Although publishable activity on BS dates back > 57 years, the boom came up within  
33  
34 the last twenty years. Only in the last five years, around 40% of the total known bibliography on  
35  
36 BS has been reported; it is estimated that at least two documents on BS are published every day.  
37  
38 This increase could be the result of the global growth of scientific literature in recent years,  
39  
40 including reports on biotechnological products and processes. However, it is important to note  
41  
42 that the trend shown by patentable activity (technological interest) in BS has not been  
43  
44 proportional. The interest in patenting seems to be gaining ground over time. In early 2000s,  
45  
46 around 10% of the body of publications were patents while, in the last five years, that  
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48 proportion has doubled. This shows a growing and accelerated interest for the industrial  
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50 exploitation.  
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57 Among the institutions with significant publishable activity one finds: East China  
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59 University of Science and Technology, Chinese Academy of Sciences, Hunan University  
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1 (China), Locus IP Company LLC (USA), Zhejiang University (China), China National  
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3 Petroleum Corporation, Jiangnan University (China), and the Ghent University (Belgium).  
4

5 This spreading concern in BS has economic impact as the global surfactant market  
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7 accounted for USD 43.6 billion in 2017, and is projected to reach USD 66.4 billion by 2025,  
8  
9 registering a compound annual growth rate (CAGR) of 5.4% from 2018 to 2025 (Shasttri and  
10  
11 Sumant, 2018). Biosurfactants however, account for a small percentage of the global surfactant  
12  
13 market; and the precise value is unknown due to the incorrect indiscriminate use of the term  
14  
15 “biosurfactant” by some producers of environmental-friendly synthetic surfactants and the  
16  
17 practices of some market research companies which include oleochemicals under this category.  
18  
19 Some of the few known producers in the world of authentic biosurfactants are Evonik  
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21 (Germany), Ecover (Belgium), Jeneil Biotech (USA), Saraya (Japan), AGAE (USA), Soliance  
22  
23 (France; now Givaudan, Swiss), GlycoSurf (USA), TensioGreen (USA), NatSurfact (now  
24  
25 Stepan, USA), Rhamnolipid (USA), MG Intobio (South Korea), Victex (China), and Kingorigin  
26  
27 (China) (Glam Research, 2020).  
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31

### 32 **3.2. Classification of biosurfactants**

33

34 Biosurfactants are amphiphilic molecules formed by two contrasting moieties: one  
35  
36 hydrophilic and one lipophilic. The hydrophilic part of BS is constituted of carbohydrates,  
37  
38 amino acids, proteins, phosphates, carboxylic acids, or alcohol motifs. The lipophilic fragments  
39  
40 are chains of carbon atoms as in fatty acids. Both molecular components are assembled via  
41  
42 linking biochemical functionalities such as ethers (C–O–C), amides (N–C=O), and esters (O–  
43  
44 C=O). According to the kind of each moiety, BS are frequently classified as glycolipids,  
45  
46 lipopolysaccharides, lipopeptides, phospholipids, and fatty acids; each group has specific  
47  
48 physicochemical features and physiological roles (Henkel and Hausmann, 2019). Among all  
49  
50 types of BS, glycolipids have the potential to be produced on a large scale due to their  
51  
52 convenient yield compared to other BS such as lipoproteins. It is expected that BS produced at  
53  
54 higher yields will lead to lower costs of production (Dhanarajan and Sen, 2014). Glycolipids are  
55  
56 the consequence of the condensation of fatty acids (lipids) and carbohydrates. Their names are  
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1 taken from the identity of the carbohydrate. Ergo, glycolipids containing sophorose are called  
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3 sophorolipids; those containing rhamnose are named rhamnolipids; those with trehalose,  
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5 trehalose-lipids, and so on. From all glycolipid types, sophorolipids and rhamnolipids have been  
6  
7 among the most studied species (Funston et al., 2016; Irorere et al., 2017). Sophorolipids can be  
8  
9 found as a variety of structures; the most known architectures are open and cyclic arrangements  
10  
11 (Delbeke et al., 2016). Open sophorolipids are those displaying the chemical functionality of  
12  
13 carboxylic acid (COOH) at the end of the lipophilic chain. Cyclic forms are those having an  
14  
15 ester functionality as a result of the condensation between the fatty acid and one of the hydroxyl  
16  
17 motifs of the sophorose. Cyclic esters are called lactones. In this way, there are acidic and  
18  
19 lactonized forms of sophorolipids. Other molecular variations are (i) the presence or absence of  
20  
21 acetyl groups on the carbohydrate moiety, (ii) the length of the alkyl chain, (iii) the number of  
22  
23 unsaturation in the fatty chain, (iv) the position of the hydroxyl group in the fatty chain, (v) the  
24  
25 position of the hydroxyl group of the carbohydrate that is etherified with the fatty alcohol, and  
26  
27 (vi) the position of the hydroxyl group of the carbohydrate that is esterified with the fatty acid in  
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29 lactone forms, among others.  
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#### 37 **4. Potential applications**

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39 Because BS reduce surface tension exactly in the same way as chemical and oleochemical  
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41 surfactants do, they can find the same application niches. The following section describes some  
42  
43 recent examples of applications of BS in different industrial sectors.  
44

##### 45 **4.1 Pharmaceutical applications of biosurfactants**

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47 Due to their anti-adhesion and enzyme inhibition effects, BS can be useful active  
48  
49 ingredients in fungicides, bactericides, insecticides, antivirals, among others. For this reason,  
50  
51 these have been used in many studies that aim to introduce BS into therapeutic applications  
52  
53 (Fracchia et al., 2015). As mentioned above, BS are naturally produced by microorganisms  
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55 during biofilm formation, smoothing the architecture of microcolonies and maintaining the  
56  
57 channels needed for distributing vital fluids. These attenuating properties of isolated BS can be  
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1 useful to ensure the anti-adherence of human pathogens and, therefore, to inhibit their capacity  
2  
3 to form biofilms at potential sites of infection (Anjum et al., 2016; Sun et al., 2018).  
4

5 In view of their amphiphilic nature, many of the antibiotic effects of BS involve damage  
6  
7 to lipid bilayers constituting cell membranes. Some BS allow the formation of pores in  
8  
9 membranes which leads to disequilibrium in ion exchange and consequently to cell death. It also  
10  
11 has been shown that another bactericidal mechanism exhibited by some BS involves the  
12  
13 generation of intracellular reactive oxygen species (ROS) (Gaur et al., 2020). Moreover, to  
14  
15 avoid the recurrence of resistant microbial strains, the combination of more than one type of  
16  
17 active molecule may be desirable, rather than one (single) molecule (Wani and Ahmad, 2020).  
18  
19 The antimicrobial properties of BS have been investigated in synergistic combination with other  
20  
21 species, e.g. caprylic acid (Díaz De Rienzo et al., 2016) and silver and iron oxide nanoparticles  
22  
23 (Khalid et al., 2019), among others.  
24  
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27 Some BS also exhibit antiviral properties. Particularly, surfactin can prevent infection of  
28  
29 epithelial cells caused by enveloped viruses –including herpes viruses– inhibiting membrane  
30  
31 fusion between virus and host cells. This inhibition can be carried out against porcine epidemic  
32  
33 diarrhoea virus at low BS concentrations without cytotoxicity (Yuan et al., 2018). Surfactin  
34  
35 produced by the probiotic *Bacillus subtilis* also showed efficiency in the inactivation of  
36  
37 transmissible gastroenteritis coronavirus (Wang et al., 2017).  
38  
39  
40

41 Very recently, the use of BS has been suggested to combat SARS-CoV-2, responsible for  
42  
43 the Covid-19 pandemic, a situation which has resulted in strong worldwide ongoing negative  
44  
45 impacts on health, societies, and economies. It is noted that BS could help to mitigate  
46  
47 transmission, incubation, and disease development (Smith et al., 2020). Since coronaviruses,  
48  
49 like any other viruses, are completely dependent on their lipid membrane that keeps and stores  
50  
51 their genetic information and viral machinery (proteins and enzymes), biosurfactants have the  
52  
53 ability to deactivate SARS-CoV2 through a simple membranal disruption such as most  
54  
55 detergents do. Another possible mechanism of action could be the destabilization of viral  
56  
57 envelope and membrane proteins, such as the spikes of glycoprotein that allows the virus to  
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1 anchor itself to human cell receptors, e.g. angiotensin-converting enzyme 2 (ACE2) receptor  
2  
3 (Mittal et al., 2020). Therefore, the use of BS as active agents in handwashing and cleaning  
4  
5 products can prevent the spread of the virus. Moreover, these can also be adequate excipients or  
6  
7 adjuvants for medicines to treat symptoms and be good elements in the production of antiviral  
8  
9 masks.  
10

11  
12 Besides the antimicrobial and antiviral properties, BS can find other health applications  
13  
14 such as immunomodulation, both activation and suppression of immune system and anticancer  
15  
16 effects, e.g. inhibition of the cell cycle, apoptosis, inhibition of the metastatic capacity of  
17  
18 tumour cells, etc. (Guerfali et al., 2019; Sajid et al., 2020).  
19  
20

#### 21 **4.2 Personal care applications of biosurfactants**

22

23 The emulsifying properties of BS are attractive for the formulation of cosmetics and  
24  
25 personal care products, because these can fulfil all the critical functions of synthetic surfactants  
26  
27 (Vecino et al., 2017) and modulate the rheological properties of such formulations (Xu and  
28  
29 Amin, 2019). For example, a BS obtained from *Lactobacillus paracasei* managed to stabilize an  
30  
31 emulsion consisting of essential oils and natural antioxidants just as the commercial surfactant  
32  
33 sodium dodecyl sulfate does (Ferreira et al., 2017). Another example is the study of replacement  
34  
35 commercial chemical surfactants such as sodium laureth sulfate by BS in shampoos (Fernández-  
36  
37 Peña et al., 2020). Other works have introduced BS in mixtures with chemical surfactants for  
38  
39 cleansing agents (Brigitte et al., 2017a; Hartung et al., 2013). Here, the ratio  
40  
41 biosurfactant/surfactant plays a critical role in the properties of the final formulation.  
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45 A biosurfactant extract from corn steep liquor –an agricultural byproduct– showed  
46  
47 promising results as a cosmetic formulation agent. This extract exhibited interesting surface-  
48  
49 active properties. It appeared to be a suitable co-stabilizer for nanoemulsions and nanocrystals  
50  
51 increasing dermal penetration. Besides its advantages as a formulation agent, the extract also  
52  
53 exhibited antioxidant and skin protective properties (Knoth et al., 2019). Moreover, BS  
54  
55 appeared to be effective stabilizing agents of vitamin C in cosmetic formulations (Rincón-  
56  
57 Fontán et al., 2020). Finally, synergistic effects in association with zinc oxide against  
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1 *Cutibacterium acnes* have been found, allowing the reduction of zinc concentrations in  
2  
3 formulations to treat acne (Rodríguez-López et al., 2020).  
4

5 As previously mentioned, BS may be used in cosmetics, not only for their formulation  
6  
7 properties but also for their valuable bioactivities. In this regards, yeast biosurfactants showed  
8  
9 promising results to treat dermatophytosis caused by *Trichophyton mentagrophytes* (Sen et al.,  
10  
11 2020). A mouthwash containing a mixture of biosurfactants from *Pseudomonas aeruginosa* and  
12  
13 *Candida bombicola* (now *Starmerella bombicola*) was effective against cariogenic  
14  
15 microorganisms and was significantly less toxic than commercially available mouthwashes  
16  
17 (Farias et al., 2019). Commercially speaking, Henkel AG & Co. KGaA (Germany) owns  
18  
19 various patents involving BS for cosmetic formulations, e.g. soap, scrub and emulsion agents  
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21 (Brigitte et al., 2017b; Schelges and Tretyakova, 2017a, 2017b, 2017c).  
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#### 25 **4.3 Biosurfactants in household cleaning**

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27 Surface activities are inherent in cleaning agents and the trend of substitution of  
28  
29 surfactants by BS has been of great interest for household cleaning products. Many studies  
30  
31 highlight the potential use of BS in cleaning formulations (Fei et al., 2020). This substitution  
32  
33 may however be partial, to reduce the initial quantity of synthetic compounds. This way, a  
34  
35 lipopeptide biosurfactant used as a laundry detergent showed promising results and additively  
36  
37 worked with a commercial detergent (Bouassida et al., 2018). From a perspective of stain  
38  
39 removal action, microorganisms that simultaneously can produce good yields of both enzymes  
40  
41 and biosurfactants by using the same culture medium are particularly valuable as their crude  
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43 extracts exhibit synergistic effects (Hmidet et al., 2019). Thus, Bhang et al. (2016) produced  
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45 keratinolytic protease, amylase, and a biosurfactant from *B. subtilis* using a single optimized  
46  
47 medium. It is known that water temperature affects the effectiveness of detergents; therefore,  
48  
49 lowering water temperatures may result in an economic and environmental challenge for  
50  
51 cleaning treatments. Synthetic surfactants can crystallize at lower temperatures and therefore  
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53 lose their surface activities at conditions where BS remain active. Consequently, processes  
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55 involving BS can be carried out at lower temperatures. Following this idea, a special focus is  
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1 given to BS-producing microorganisms isolated from cold environments (Perfumo et al., 2018).  
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3 Unilever (Rotterdam-London, Netherland-United Kingdom) has various patents related to  
4  
5 household products, some of them are: laundry comprising BS and lipases from *Psychromonas*  
6  
7 *ingrahamii* –a psychrophilic bacterium– active at low temperatures (De Rose et al., 2017a,  
8  
9 2017b); cleaning fluids comprising mixtures of surfactants and BS (Jones and Stevenson, 2016),  
10  
11 and BS mixtures to protect coloured or dyed substrates from dye transfer during exposure to  
12  
13 aqueous cleansing solutions (Torodov Petkov and Stevenson, 2016).  
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#### 16 **4.4 Biosurfactants in oil recovery**

17  
18 Petroleum also known as ‘black gold’ is the main source of energy for the contemporary  
19  
20 world. Crude oil is found below the earth's surface or trapped inside rocks as a result of the  
21  
22 transformation of accumulated organic matter into sediments from the geological past located in  
23  
24 different parts of the earth where it can be extracted by well drilling. The natural pressure of an  
25  
26 oil reservoir makes it flow from the bottom to the surface. However, as the extraction  
27  
28 progresses, this pressure decreases, and the application of liquids or gases is required to re-  
29  
30 pressurize and obtain the remaining oil. During these operations, formulated chemical agents  
31  
32 that can perform lubrication, wetting, demulsification, corrosion inhibition, wax inhibition, flow  
33  
34 improvement, among others, are normally required. These products are called oilfield chemicals  
35  
36 and are typically made up of polymeric materials of petrochemical origin whose raw materials,  
37  
38 intermediates, or final components are regulated due to their ecotoxicity profiles. In recent  
39  
40 years, the possibility of replacing chemical compounds with BS has been investigated (Ke et al.,  
41  
42 2019; Sivasankar and Suresh Kumar, 2017; Zhao et al., 2018).  
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48 Recent examples are the inventions of Locus Oil IP Company, who has patented  
49  
50 compositions and methods for oil recovery (Farmer et al., 2019, 2018b), upgrading heavy crude  
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52 oil (Farmer et al., 2018a), and removing paraffins (Farmer et al., 2020). Another example is  
53  
54 Baker Hughes Inc. (Houston, USA) who owns various biosurfactants-related patents. One  
55  
56 involves the application of a mixture of BS to prevent the corrosion inside the wells during  
57  
58 treatments (Gunawan et al., 2015) and other involves the addition of BS to hydrocarbon-based  
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1 fluids to reduce their viscosity (Campbell and Weers, 2016). Varjani and Upasani (2016)  
2  
3 showed that a thermo- and halo-tolerant rhamnolipid produced by *P. aeruginosa* could improve  
4  
5 the oil recovery over the residual oil saturation of 8.82% in a core flooding system. Surfactin,  
6  
7 when applied to the oil recovery process exhibits interesting pH dependency, e.g. emulsification  
8  
9 in alkaline conditions and demulsification in acidic conditions. When in presence with surfactin,  
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11 oil can thus undergo two different behaviours through a simple pH adjustment (Long et al.,  
12  
13 2017).  
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#### 16 **4.5 Biosurfactants for bioremediation and pollutants removal**

17  
18 Remediation of aquatic and terrestrial polluted environments is a challenging topic as  
19  
20 physical collection methods often allow a limited recovery, and chemical methods can generate  
21  
22 new damages to the environment (Dave, 2011). In this context, bioremediation offers attractive  
23  
24 perspectives. Bioremediation may be carried out following two mechanisms: (i) one direct  
25  
26 which involves the presence of the microorganism that degrades *in situ* the contaminant through  
27  
28 its metabolism and (ii) one indirect that involves the use of microbial compounds (such as  
29  
30 biosurfactants) to modify the physicochemical properties and help the recovery of the  
31  
32 contaminant (Francis and Nancharaiiah, 2015). When speaking of bioremediation, one often  
33  
34 thinks of contamination of environments with hydrocarbons, which, incidentally, are these  
35  
36 environments where many biosurfactant-producing microorganisms have been isolated (Datta et  
37  
38 al., 2018; Pi et al., 2017; Sarkar et al., 2017; Sun et al., 2019). Biosurfactants, secreted by  
39  
40 microorganisms and released into the hydrophobic medium, increase hydrocarbon  
41  
42 bioavailability to the (same) microorganisms. In other words, the degradation of hydrocarbons  
43  
44 in the presence of microorganisms is enhanced by the production of BS (Xue et al., 2019). For  
45  
46 that matter, strategies of biostimulation and bioaugmentation of native microorganisms showed  
47  
48 promising results in the degradation of sludge generated in an oil refinery (Roy et al., 2018).  
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54 Amongst the various chemical molecules, polycyclic aromatic hydrocarbons (PAH),  
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56 produced from incomplete combustion, are particularly recalcitrant (Patel and Patel, 2020). A  
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58 strain of *P. aeruginosa* was efficient degrading crude oil and PAHs such as pyrene and  
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1 fluoranthene. Biosurfactants isolated from this same strain showed interesting results in  
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3 hydrocarbons remobilization from oil-contaminated soils (Chebbi et al., 2017). Another strain  
4  
5 of *Acinetobacter baumannii* isolated from a petroleum-contaminated soil was able to degrade  
6  
7 pyrene. The growth of this strain on PAH as the sole carbon source was accompanied by BS  
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9 production (Gupta et al., 2020). Selected strains can this way being combined in a bacterial  
10  
11 consortium to improve the degradation of multiple PAH present in crude oil (Kumari et al.,  
12  
13 2018). Direct volatile hydrocarbon removal can also be carried out using BS. Saponins in  
14  
15 biotrickling filters significantly participated in hexane removal (Tu et al., 2015). It also has been  
16  
17 reported that a biofilm containing a strain of *Pseudomonas* functioned as a (bio)filter for volatile  
18  
19 organic compounds both degrading hexane and producing biosurfactants. Microorganisms that  
20  
21 produce BS from hydrocarbons are valuable in biofilter systems because these can remove the  
22  
23 pollution, avoiding biomass accumulation (He et al., 2020). Among the indirect bioremediation  
24  
25 mechanisms involving BS, phytoremediation is a cost-effective technique to treat soils  
26  
27 contaminated with crude oil. Liao et al. (2016) have shown that the addition of BS to the  
28  
29 contaminated soil improved the oil degradation by the soil microorganisms in the presence of  
30  
31 corn (*Zea mays* L.) through increased hydrocarbons accessibility and that rhamnolipids  
32  
33 favoured the PAH uptake in the plant roots. A study revealed that the supplementation of BS  
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35 from *Pseudozyma sp.* to a medium containing crude oil as sole carbon source, improved the oil  
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37 degradation by *Pseudomonas putida* up to 46% (Sajna et al., 2015).  
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43 Composting is also an efficient way of handling biologically active and potentially  
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45 hazardous species to obtain stabilized inert materials with potential fertilizer properties. The  
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47 addition of BS during the chicken manure composting process was reported to improve the  
48  
49 overall final qualities of such compost in terms of sanitization (higher peak of temperature  
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51 during the thermophilic phase), fertilization (higher seed germination index), formation of  
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53 humic acids (lower E4/E6 ratio) and organic matter degradation (higher cellulase activity). A  
54  
55 metagenomic study revealed that these improved qualities were the consequence of increased  
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57 diversity in microbial communities and subsequent diversity in metabolism, especially that  
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1 related to carbohydrate metabolism (Yin et al., 2019). Still during chicken manure composting,  
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3 it has also been showed that the addition of rhamnolipids significantly reduced the relative  
4  
5 abundance of the antibiotic resistance genes among the microbial communities through a  
6  
7 mechanism of decrease of the bioavailability of the heavy metals present in the environment  
8  
9 (Zhang et al., 2016).

10  
11 Following the same idea, the addition of rhamnolipids during the fermentation of waste  
12  
13 activated sludge improved the hydrolysis and acidification processes, notably by favouring the  
14  
15 growth of functional microorganisms compared to the action of synthetic surfactants (Zhou et  
16  
17 al., 2015). This process, when supplemented with free nitrous acid and tea saponin (a  
18  
19 biosurfactant) improved the sludge solubilization, reducing the fermentation time and improving  
20  
21 the short-chain fatty acids production (Xu et al., 2016). When considering anaerobic treatment  
22  
23 of sludge, it has been shown that a pre-treatment consisting in the biomass disintegration using a  
24  
25 biosurfactant-producing strain of *Planococcus jake* of a previously deflocculated sludge  
26  
27 improved the biodigestibility (Kavitha et al., 2015). Pre-treatment of waste activated sludge  
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29 with BS also enhanced the release of phosphorus, thus facilitating its recovery from the sludge  
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31 (He et al., 2016).  
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## 39 **5 Solid-state fermentation (SSF) for biosurfactant production**

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41 There are currently two ways to produce BS: the submerged fermentation (SmF), also  
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43 called liquid fermentation, and the solid-state fermentation (SSF). SSF is a microbial process  
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45 occurring mostly on the surface of solid materials that have the property to absorb or contain  
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47 water, with or without soluble nutrient (He et al., 2019; Pandey, 2003). SmF is the best-known  
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49 methodology in the scientific literature and patents while SSF occupies a still very small but  
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51 emerging space. Both techniques can use the same producing microorganisms, but results can  
52  
53 be significantly different due to the large differences in conditions between the two types of  
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55 cultivation regimen. Moreover, for a given bioprocess, SSF is often known to reduce the global  
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57 cost in comparison to liquid fermentation (Sadh et al., 2018). The low water volume in SSF has  
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1 a large impact on the economy of the process mainly due to smaller bioreactor size, reduced  
2 downstream processing, and lower sterilization costs. Besides, many SSF processes focus on the  
3 utilization of cheap agro-industrial byproducts as a culture medium (Venil et al., 2017).  
4  
5 Although, it is not limited to SSF as studies using SmF for BS production also involve agro-  
6 industrial byproducts valorisation (Kourmentza et al., 2018; Moya Ramírez et al., 2015;  
7 Radzuan et al., 2017; Sharma et al., 2020).

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10 To observe tendencies, an exhaustive study has been carried out on the scientific  
11 literature to highlight the microorganism type, biosurfactant type, production scale, and  
12 bioreactor type related to the BS production using SSF (Figure 1). One-third of research articles  
13 involves using strains of fungi and two-third for bacteria (Figure 1A). This is interesting  
14 because SSF processes traditionally involve more fungi than bacteria for their ability to grow at  
15 lower water activity values. Indeed, the low moisture content in the SSF medium means that  
16 fermentation can theoretically only be carried out by a limited number of microorganisms,  
17 mainly yeasts and fungi. This is justified by the fact that the evolution of higher fungi take place  
18 on solid growth substrates. Another interesting observation is that around 70% of the  
19 microorganisms that have been used were unicellular, whereas SSF processes are generally  
20 claimed to be optimized for filamentous growth which can penetrate through the interparticle  
21 spaces into the depth of the solid medium. The invasion of the solid matrix is optimized by both  
22 the hydrolytic enzymes secretion and the application of a mechanical force at the apex of the  
23 hypha, increasing the surface contact (King et al., 2017). The nutrients translocation within the  
24 filamentous web is also better adapted to cope with nutrient-poor areas to seek resources in  
25 media where nutrients are heterogeneously distributed. The two classes of BS produced through  
26 SSF are glycolipids and lipopeptides, with 26% of undetermined BS (Figure 1B). The  
27 production scale involves up to 80% laboratory-scale ranging from 0 to 250 g of medium, it is  
28 thus not surprising that 58% of the articles used flasks for microbial cultures (Figure 1C and D);  
29 only 2% of the scientific studies of BS production involve a scale ranging from 5 to 10 kg.  
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## 5.1 Important factors for biosurfactant production in SSF

The most common factors influencing BS production may be physical (like temperature) or chemical (like pH and the nutrient sources). SSF involves microbial cultures in the near absence of free running water. Therefore, its moisture content is also of critical importance.

### 5.1.1 Effect of pH on biosurfactant production

The pH values of the medium strongly affect many enzymatic, secondary metabolites production and transport of various components across the cell membrane. It is well-known that fungi can adapt to more acidic conditions than most bacteria. Concerning BS production in SSF, optimal pH conditions follow this trend. The best pH values for *A. fumigatus* and *P. djamor* are 4.5 and 5.5, respectively (Velioğlu and Ürek, 2016). Biosurfactant productions involving yeasts like *Starmerella bombicola* require pH values around 6 (Cerde et al., 2019; Jiménez-Peñalver et al., 2016). Regarding bacteria like *P. aeruginosa* and *B. subtilis*, optimal pH values range from neutral to slightly basic (7–8) (Zhu et al., 2013).

### 5.1.2 Effect of temperature on biosurfactant production

Temperature is a critical parameter in SSF. Indeed, as the bioprocess progresses, metabolic heat is generated by the microorganism and because the solid media generally exhibit low thermal conductivities, the heat may accumulate in the medium leading to detrimental temperature increase and thermal gradients (Pandey, 2003). As optimum activity of each type of microorganisms takes place in a relatively well-defined range of temperatures, at which these operate most efficiently. BS production is depending on temperature. Filamentous fungi and yeasts require optimum values between 25°C and 30°C for optimal BS production (Jiménez-Peñalver et al., 2016; Lourenço et al., 2018). Rhamnolipid production requires optimal temperatures ranging from 30°C to 37°C following the strain of *P. aeruginosa* (Wu et al., 2017). Temperature greatly affects lipopeptide production, as in the case of *Bacillus cereus* (Nalini et al., 2016).

### 5.1.3 Effect of moisture content on biosurfactant production

Like temperature, the initial moisture content of the medium may vary as the bioprocess progresses and the microorganism consumes the water and/or it is evaporated by the metabolic heat leading to water gradients in the bioreactor. The water content of the medium is therefore a critical factor in SSF systems as it affects the microbial growth rate and extent on the substrates and determines the product yield; low water content could retard cell growth and metabolite production. The water content of the medium is a critical factor in SSF systems that affects the microbial growth rate, extent on the substrates and determines the product yield. Low water content could retard cell growth and metabolite production. Appropriate water content would provide an ideal microenvironment for supporting growth and enhancing metabolite production (Zhu et al., 2013). BS producing filamentous fungi like *A. fumigatus* and *T. versicolor* and yeasts like *S. bombicola* exhibit optimal moisture values between 44.7% and 50% (Jiménez-Peñalver et al., 2016; Lourenço et al., 2018). These moisture values are particularly important because lower values tend to limit the growth of potential contaminants in SSF. Bacteria often require higher values of moisture (generally fungi and yeast have water activity requirements of around 0.5–0.6 and bacteria around 0.8–0.9) that may generate alternative issues of solid medium compaction leading to reduced gaseous transfers and favouring contamination (He et al., 2019); although successful examples of SSF carried out with bacteria do exist (Costa et al., 2018).

### 5.1.4 Effect of the medium composition on biosurfactant production

Several carbon substrates have been used in many investigations. Indeed, the type and quantity of BS are influenced by the carbon source. Both the composition and concentration of the carbon source seem to be essential factors of BS congeners, yields and physicochemical properties. Yet, hydrophobic carbon sources were reported to be better than hydrophilic ones in promoting BS production (Ismail et al., 2015). The importance of hydrophobicity of the carbon sources for BS production is underlined by the fact that many producing microbes are commonly isolated from soils or water contaminated with hydrophobic wastes. BS production

1 however generally requires a medium containing the simultaneous presence of a hydrophilic and  
2  
3 a hydrophobic carbon sources in the culture medium (Teixeira Souza et al., 2018). As a  
4  
5 consequence, many solid media employed in SSF use oil containing byproducts from the oil  
6  
7 extraction industry, or include vegetal oils –like sunflower seed oil– or crude oils –like diesel–  
8  
9 in their final compositions (Jiménez-Peñalver et al., 2016).

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11  
12 Another important consideration to develop a production bioprocess is media consistency,  
13  
14 quality, and availability in sufficient quantities. For that matter, it is interesting to supply solid  
15  
16 byproducts directly from their producing industries to ensure availability of large quantities with  
17  
18 standardized composition. Table 2 shows a list of solid media used for BS production by SSF in  
19  
20 the scientific literature and highlights the diversity of potential of biomass valorisation.  
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23 In SSF, the physical properties of the media are also essential to provide an environment  
24  
25 suitable for microbial growth and metabolite productions, BS production being generally an  
26  
27 aerobic process. Factors such as the shape and size of solid particle, medium porosity, mass and  
28  
29 energy transfers, hydrophilic and hydrophobic nature of solid particles are usually  
30  
31 interdependent and essential to consider in SSF. A good medium texture is therefore essential  
32  
33 and some media include solid support like wheat straw or sugarcane bagasse (El-Housseiny et  
34  
35 al., 2019; Zhu et al., 2013). These solid compounds only act as supports; they are not used  
36  
37 directly in the nutrition of the microorganism but create a suitable physical environment  
38  
39 reducing the porosity changes during the process. Fungal development may clog the empty  
40  
41 spaces in the medium, the support material therefore, maintains the physical properties  
42  
43 favourable for mass and heat transfers to take place, allowing for example, the gaseous phase  
44  
45 inside the interparticle void to be regenerated in oxygen while it is consumed by the fungus  
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48 (Carboué et al., 2018).  
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### 51 **5.1.5 Effect of nutrient supplementation on biosurfactant production**

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54 Natural byproducts have complex compositions and usually provide multiple nutrients to  
55  
56 microorganisms that grow on them. However, these compounds are usually present in  
57  
58 suboptimal quantities and, in this case, nutrient supplementation is needed to provide all  
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1 necessary compounds for optimum growth and production (Soccol et al., 2017). Some examples  
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3 of supplements are copper and iron. Indeed, iron is recognized to be an important enzyme  
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5 activator, specifically for the isocitrate lyase, an enzyme involved in cell growth on hydrophobic  
6  
7 substrates. Each microorganism has its optimal cultivation conditions and requires specific  
8  
9 compounds depending on the expected products. Thus, many studies imply experimental  
10  
11 designs to search for the factors with significant positive effect and their optimal levels to  
12  
13 increase BS production (Jiménez-Peñalver et al., 2018).  
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## 16 **5.2 Scale up of SSF process for the production of biosurfactants**

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18 The scaling up of SSF process is often considered a technological bottleneck because the  
19  
20 mass and energy transfers issues, leading to the accumulation of heat and the apparition of water  
21  
22 and gas gradients inside the bioreactor bed, are amplified as the process scale increases (Lopez-  
23  
24 Ramirez et al., 2018). In the case of aerobic bacterial metabolism which involves an important  
25  
26 part of BS producing microorganisms, the metabolic heat generated could even be more  
27  
28 amplified (bacteria generally grow faster than fungi).  
29  
30

31 Two mechanisms are usually presented as critical in managing heat removal at higher  
32  
33 SSF production scales: cooling with water evaporation and agitation. Water evaporation is an  
34  
35 endothermic process, for that reason, aeration with water-saturated air can be used to remove the  
36  
37 metabolic heat produced during microbial growth and also to avoid the medium drying  
38  
39 (Saucedo-Castañeda et al., 1994). Agitation counters the energy and mass gradients formation  
40  
41 through bed mixing. The combination of both mechanisms is often required at industrial scales,  
42  
43 as agitation facilitates the homogenization of the system and thus an equal distribution of the  
44  
45 moisture and oxygen through the medium. In general, bioreactors are classified depending on  
46  
47 the mechanisms of aeration and agitation. The type of microorganism strongly influences the  
48  
49 choice of bioreactor. Filamentous microorganisms, for example, may be particularly sensitive to  
50  
51 the shear forces (generated during agitation) that lead to the detachment of microorganisms  
52  
53 from the solid medium, damage to mycelia, and ultimately to the reduction of BS production.  
54  
55 So, it is often necessary to find a compromise between mass and energy transfers qualities and  
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1 mycelial damages due to agitation. Some studies have reported good productions of spores and  
2 enzymes with filamentous fungi belonging to the genera *Aspergillus* and *Trichoderma* in  
3 agitated SSF systems with lower rotational speeds – ranging from 1 to 6 rpm – and/or  
4 intermittent mixings (Carboué et al., 2019; Finkler et al., 2017; Lopez-Ramirez et al., 2018).  
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10 Even though, reports of BS production using SSF at higher scales are very scarce (Figure  
11 1C). Ano et al., (2009) carried out SSF in an agitated and aerated bioreactor on 2 kg of dry  
12 weight okara to produce iturin A using a strain of *B. subtilis*. Biosurfactant production was  
13 significantly reduced when mixing was carried out during the process, probably due to the  
14 reduction of biofilm formation. Under static conditions, they observed an important temperature  
15 gradient across the bed during the process but were able to control it with aeration of humidified  
16 air. The production of BS was very low when compared to those obtained at lower scales using  
17 the same substrate and strain. In another investigation at a laboratory scale (100 g of medium),  
18 Jiménez-Peñalver et al. (2016) showed that intermittent mixing increased the bioavailability of  
19 the substrates to the yeast and led to an increased production of sophorolipids. This result  
20 indicates that the process could be scaled up, not only to improve the yield but also to reduce  
21 channelling and overheating problems. To date, the largest production scale reported in the  
22 literature was achieved using a *Bacillus amyloliquefaciens* strain producing surfactins, in a  
23 forcefully aerated and agitated (rotational speed of 50 rpm) bioreactor on 9.25 kg of solid  
24 medium (Zhu et al., 2013). Interestingly, they did not observe any difference in the production  
25 yields between the static laboratory scaled culture and the agitated pilot-scaled culture,  
26 highlighting the absence of detrimental agitation effects on their process despite a higher  
27 rotational speed than the ones used for filamentous microorganisms.  
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50 Interestingly, the fact that BS producing microorganisms are a major part unicellular  
51 (bacteria and yeasts) may also change the established paradigm stating that the SSF is more  
52 adapted for filamentous microorganisms, allowing successful scale up through the  
53 implementation of stronger agitation regimes, thanks to a higher tolerance of unicellular  
54 microorganisms to the shear forces reducing heat and mass transfer limitations. We can  
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1 therefore think that BS production not only benefits from the scientific advances made in SSF,  
2  
3 but also actively participates to develop the discipline.  
4

### 5 **5.3 Comparison of SSF vs SmF for the production of biosurfactants**

6  
7 Many investigations have highlighted that BS are produced in higher quantities using SSF  
8  
9 in comparison to SmF, using same culture conditions e.g. temperature, nutrients concentration,  
10  
11 agitation, etc. The product yield of hydrophobins (in mg/g of biomass) produced by *P. ostraeus*  
12  
13 for example, was two-fold higher when produced in SSF than in SmF. Indeed, these proteins  
14  
15 were secreted during adhesion of mycelia to solid support as these molecules are responsible for  
16  
17 surface hydrophobicity of mycelia during binding to the hydrophobic substrate (Kulkarni et al.,  
18  
19 2020). Rhamnolipids production in SSF was also reported at threefold higher than in SmF by  
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21 El-Housseiny et al., (2019). Mizumoto et al. (2006) also observed similar results, as they  
22  
23 obtained an iturin A production tenfold higher using SSF than that in SmF. They explained this  
24  
25 difference with various hypotheses: as a secondary metabolite, iturin A is produced after the  
26  
27 exponential growth phase, when nutrients become scarce in the medium. In SmF, the nutrients  
28  
29 and oxygen are homogeneously distributed abundantly in the liquid medium, leading their  
30  
31 bacterial strain to produce biomass rather than iturin A. In SSF systems, however, nutritional  
32  
33 stress may be observed, as nutrients uptake becomes a limiting step due to heterogeneities in the  
34  
35 liquid phase, thus promoting the secondary metabolism. Another hypothesis was based on the  
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37 biological structure differences observed between the two types of process: in SSF, there is the  
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39 formation of a biofilm that may be more suitable for iturin A production than the planktonic  
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41 form of liquid culture.  
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48 The higher production obtained for BS in SSF compared to SmF however, is not a  
49  
50 general rule: Sitohy et al. (2010) have shown that SmF gave higher quantities of BS using  
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52 bacteria and yeast cultures than when SSF was used. This effect relies mostly on the strain and  
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54 the culture conditions used, hence, ideally, a comparison should be made between SSF and SmF  
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56 for every process, not only including quantitative and qualitative considerations, but also  
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58 economical ones to choose the best option. Besides, it is also interesting to mention that these  
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1 studies reporting better production of BS using SSF rather than SmF were mainly carried out at  
2 laboratory scale.  
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5 At present, the technological aspects, especially regarding process control, are more  
6 developed for the SmF than for SSF, and although there are counter-examples, SmF is the most  
7 prevalent cultivation method used in the fermentation industry (Prado et al., 2016). As an  
8 example, Novozymes, which is the world-leading enzyme producer, currently applies SmF for  
9 cellulase production (Hansen et al., 2015). Industrial BS productions make no exception and are  
10 still widely carried out using SmF (Brumano et al., 2016). The main reason for the lack of  
11 industrial-scaled SSF bioreactor is the absence of efficient mathematical models backing the  
12 bioreactor designs and automated control system that could successfully represent and  
13 overcome the heterogeneity of the bioreactor bed with respect to the heat and mass transfers  
14 (Arora et al., 2018). Technical issues specific to the BS production in SmF however are  
15 particularly encountered at the industrial scale. One of them is the production of foam because  
16 of the important air-liquid interface. In SmF, liquid phase constitutes the culture medium and in  
17 the case of BS production, an excess of foam is often generated during the fermentation,  
18 especially at intense agitation and aeration which is generally the case of microorganisms that  
19 produce BS (predominantly under aerobic conditions), favouring the dispersion at the interface  
20 between phases and the risk of contaminations. Microorganisms are carried into the foam layer  
21 by froth flotation; thus, foaming may decrease the effective biocatalyst concentration in the bulk  
22 liquid, affecting the global bioprocess performance and often creating interferences with the  
23 measurement and sampling material. As a consequence, many industrial-scaled BS productions  
24 processes require the use of antifoam agents that increases the overall cost of the process. SSF  
25 enhance O<sub>2</sub> transfer without foam production mitigating the risk of contamination. The use of  
26 SSF, involving reduced amounts of free water, therefore could be an alternate method for BS  
27 production (Krieger et al., 2010).  
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56 Regarding the downstream process, the extraction in SSF is usually considered more  
57 difficult than in SmF because of the complex nature of the agro-industrial byproducts used as  
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1 solid medium that display a bigger diversity of interaction (medium-product and medium-  
2 solvent) which as a result can lead to the presence of residual impurities in the crude extract.  
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4 Nonetheless, in practice, the required degree of purity for a product depends on the intended  
5 application (Singhania et al., 2009). Thereby, some sectors like health or cosmetics, e.g.  
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7 involving direct contact with living beings including humans, are particularly sensitive to the  
8 composition and require very high grades of purity. For this type of finer applications, improved  
9 separation techniques such as preparative HPLC will be required; even these types of  
10 applications will require using more refined raw materials. On the other hand, the direct use of  
11 fermented medium or crude extracts without further purification is appropriate for many other  
12 applications ranging from oil industry to pollutant removal or general environmental  
13 applications. In the case where microorganisms would still be present and active in the product  
14 (e.g. dry fermented medium containing conidia), it may be important to carry out the bioprocess  
15 with GRAS microorganisms.  
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30 It has been widely mentioned that production costs associated with BS production,  
31 especially the downstream processes, can limit their general application (Jimoh and Lin, 2019;  
32 Najmi et al., 2018). Because of that, an important emphasis is put on the search for mechanisms  
33 to decrease the total cost of the process. In the last five years, innovative technologies of  
34 integrated process of fermentation and simultaneous extraction have emerged, involving for  
35 example integrated gravity-based separation processes that greatly improve productivity,  
36 process cycles and production costs (Dolman et al., 2019). Although developed for SmF,  
37 integrated these separation processes can be envisaged in the future for SSF, particularly in the  
38 case of continuous or semi-continuous processes involving a plug-flow bioreactor with  
39 separated fermentation and extraction compartments which is similar to the process developed  
40 by Gibbons et al. (1988) for a semicontinuous production and extraction of ethanol.  
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## 6 Future potentials

The future of biosurfactant uses is huge because they cover and sometimes exceed the scope of synthetic surfactants. Their implementation in productive and daily life will involve most ecological aspects such as (i) reducing exposure of dangerous chemical substances, not only for humans but also for plants and animals, (ii) reducing carbon footprint, and (iii) boosting the circular economy. Small amounts of these biological surfactants have been shown to produce the same effects in terms of surface activities as commercial chemical surfactants. Therefore, finished surfactant bio-based products (detergents, shampoos, soaps, cleaning agents, etc.) could simply contain biosurfactants in very small quantities either as main components or adjuvant components that enhances overall activity. Using modest but significant loads of biosurfactants into formulations automatically would reduce the scale of their production and consequently the scale of their purification processes (if necessary). This approach can bring biosurfactants to markets in a shorter time than expected. Not too many commercial examples are emerging nowadays in different parts of the world.

The generation of innovative technologies for production, control, separation, purification, characterization, and performance evaluations shows a promising future for biotechnological exploitation of biosurfactants. Many studies therefore work on overcoming the technological bottlenecks and, amongst them, SSF can be an interesting candidate. Although the publishable activity on BS production via SSF is more recent and does not follow the same exponential tendency as SmF, it is likely to think that this culture technique will turn into something established in the near future in the biotechnological industry. This will not only take place in the academic world, but also as a relevant strategy for chemical industry to produce biobased products with interesting yields, lower costs and through a possible valorisation of byproducts and industrial wastes. The advances in SSF are accompanied by the evolution of chemical engineering, modelling and biotechnology, which contribute to understanding and decreasing specific technological limitations (e.g. heat removal) and facilitating its integration in processes at pilot and industrial scales (Jin et al., 2020, 2019; Pessoa et al., 2019; Zeng et al., 2019).

1 Processes involving microorganisms with good tolerance to higher agitation may also hold an  
2  
3 interesting potential approach for scale-up.  
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5 Finally, the increase of good and efficient relationships between academia and industry  
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7 worldwide, mediated by the public opinion on a better recognition of the sustainability aspect in  
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9 the production practices will also contribute to this impulse. The current battle against the  
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11 Covid-19 may become a good example as a driving force behind such evolution.  
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## 16 **7 Conclusions**

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18 Biosurfactants is a fascinating topic for innovation, research and sustainable development.  
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20 Interest has become notable in recent years due to the discovery of new applications in relevant  
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22 industrial sectors and the growing urgency of greener industrial processes. However, the use of  
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24 biosurfactants on a large scale is still limited by competitive production costs. Consequently, the  
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26 imminent replacement of synthetic materials by biosurfactants is not realistic, but a partial and  
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28 progressive introduction of biosurfactants is more likely to occur in small quantities via  
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30 innovative formulations that will play essential roles in the transition from the petrochemical  
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32 industry towards the circular economy.  
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## 39 **8 Acknowledgments**

40  
41 We thank the National Council of Science and Technology of Mexico (CONACyT) and  
42  
43 Polioles S.A. de C.V. for supporting the PEI innovation projects: 221262, 231833 and 250609.  
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45 The Metropolitan Autonomous University is also thanked for the post-doctoral fellowship of  
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**Table 1.** Classification of surfactants by origin, ionic status, and hydrophilic-lipophilic balance (HLB).

<b>Category</b>		<b>Example</b>
Origin	synthetic	nonylphenol ethoxylates
	oleochemical	lauryl alcohol ethoxylates
	biosurfactants	glycolipids
Ionic status	cationic (+)	ammonium salts
	ionic anionic (-)	lauryl sulfates
	zwitterionic (+/-)	betaines
	non-ionic	oxirane and 2-methyloxirane copolymers
HLB	01 – 03	antifoaming agents
	03 – 08	w/o emulsifiers
	07 – 10	wetting agents
	08 – 16	o/w emulsifiers
	13 – 16	detergents
	16 – 19	solubilizing agents

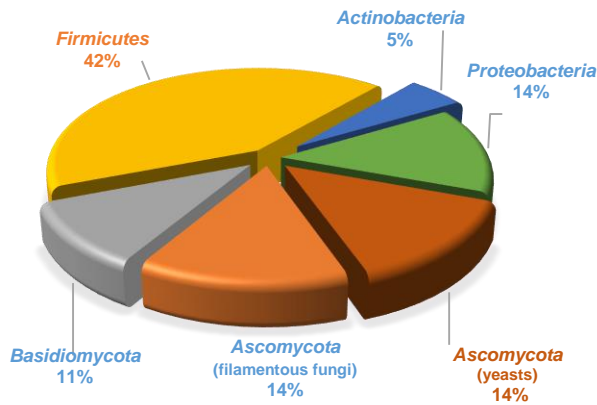
**Table 2.** Producer microorganisms, biosurfactants, solid media and type of reactor for known SSF.

Microorganism	Type of BS	Complemented solid medium	Bioreactor	Reference
<i>Aspergillus fumigatus</i>	Undetermined	Defatted rice bran and husk and diesel oil	Flask	(Martins et al., 2009)
		Rice bran	Flask	(Castiglioni et al., 2013)
<i>Aspergillus niger</i>	Undetermined	Wheat bran, corncob and sugarcane molasse	Flask	(Kreling et al., 2020)
		Banana stalk powder		(Asgher et al., 2020)
<i>Bacillus amyloliquefaciens</i> XZ-173	Surfactin	Soybean flour and rice straw	Flask	(Zhu et al., 2012)
			Forcefully aerated and agitated bioreactor (9 kg)	(Zhu et al., 2013)
<i>Bacillus cereus</i> SNAU01	Lipopeptide	Peanut oil cake	Flask	(Nalini et al., 2016)
<i>Bacillus subtilis</i> DM-03	lipopeptides	Potato peels	Flasks	(Das and Mukherjee, 2007)
<i>Bacillus subtilis</i> NB22	Iturin A	Okara	8 L Bottle (3 kg)	(Ohno et al., 1996)
<i>Bacillus subtilis</i> RB14-CS	Iturin A	Okara	Flask	(Mizumoto and Shoda, 2006)
<i>Bacillus subtilis</i> SPB1	Lipopeptides	Tuna fish flour and potato waste flour	Flask	(Mnif et al., 2013)
<i>Bacillus subtilis</i> iso 1	Iturin A	Defatted soybean meal, wheat bran and rice husk	Column	(Piedrahíta-Aguirre et al., 2014)
<i>Bacillus subtilis</i> TrigorCor 1448	Iturin A, fengycin	Wheat middlings	Packed-bed bioreactor	(Pryor et al., 2007)
<i>Candida guilliermondii</i>	Undetermined	Okara	Flask	(Sitohy et al. 2010)

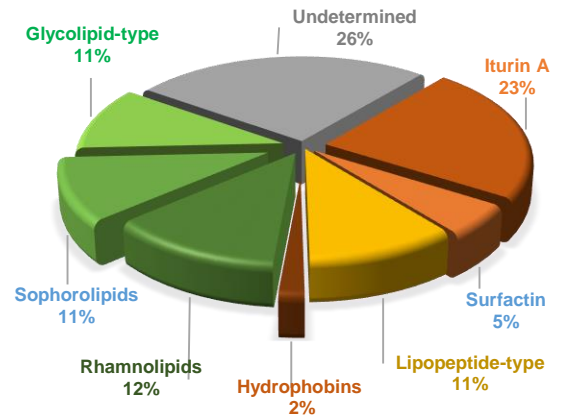
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<i>Candida tropicalis</i>	Undetermined	Instant noodle waste	Flask	(Ribeaux et al., 2020)
<i>Nocardiopsis</i> sp. MSA13A	Undetermined	Treated molasses from distillery	Flask	(Kiran et al., 2014)
<i>Nocardiopsis lucentensis</i> MSA04	Glycolipids	Wheat bran	Flask	(Kiran et al., 2010)
<i>Pleurotus ostreatus</i>	Undetermined	Sunflower seed shell and sunflower seed oil	Flask	(Velioğlu and Ürek, 2016)
<i>Pleurotus ostreatus</i>	Hydrophobin like proteins	Sesame and coconut oil cake	Flask	(Kulkarni et al., 2020)
<i>Pseudomonas aeruginosa</i> IRMD-2010	Rhamnolipids	Oil-corn germ meal and corn bran	Packed-bed bioreactor	(Ranjbar and Hejazi, 2019)
<i>Pseudomonas aeruginosa</i> UFPEDA 614	Rhamnolipids	Sugarcane bagasse and corn bran	Flask	(Camilios-Neto et al., 2011)
<i>Pseudomonas aeruginosa</i> RG18	Undetermined	Rapeseed meal, wheat bran and glycerol	Flask	(Wu et al., 2017)
<i>Pseudomonas aeruginosa</i> SS14	Rhamnolipid	Rice distillers dried grain	Flask	(Borah et al., 2019)
<i>Serratia rubidaea</i> SNAU02	Rhamnolipid	Mahua oil cake	Flask	(Nalini and Parthasarathi, 2014)
<i>Starmerella bombicola</i>	Sophorolipids	Polyurethane foam impregnated with molasses and stearic acid	Packed-bed bioreactor	(Jiménez-Peñalver et al., 2018)
		Hygienised digestate supplemented with external sugar and fat sources		(Cerda et al., 2019)
<i>Starmella bombicola</i> ATCC 22214	Sophorolipids	Winterization oil cake, sugar beet molasses	Packed-bed bioreactor	(Jiménez- Peñalver et al., 2020)
		Winterization oil cake, sugar beet molasses and wheat straw		(Jiménez-Peñalver et al., 2016)
<i>Trametes versicolor</i> CECT 20817	Undetermined	Winterization oil cake, sugar beet molasses and wheat straw	Flasks	(Rodríguez et al., 2020)
		Two-phase olive mill waste, wheat bran and olive stones		(Lourenço et al., 2018)

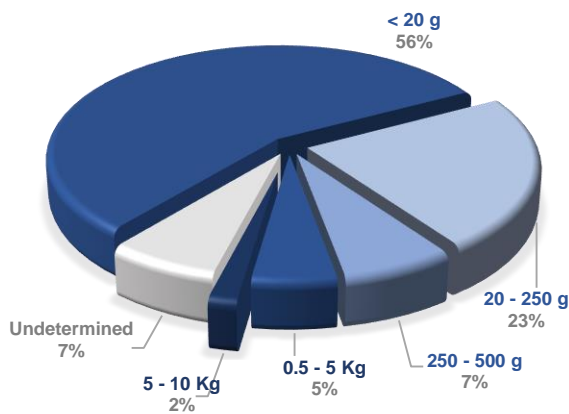
(A) Phyla of microorganisms used in SSF to produce biosurfactants



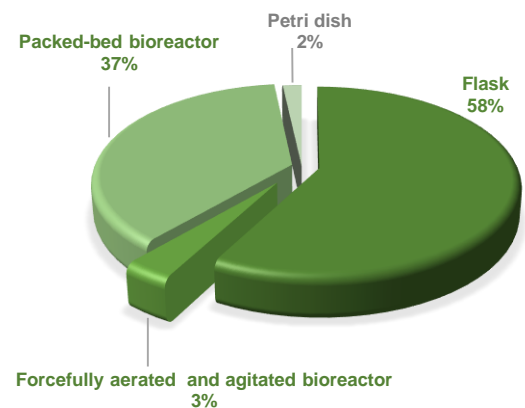
(B) Biosurfactants produced in SSF



(C) Scale of production of biosurfactants in SSF



(D) Type of bioreactor used for production



**Figure 1.** Analysis of the scientific literature involving biosurfactants production using SSF: A, phylum of the producing microorganism; B, class of obtained biosurfactants; C, scale of production (in g of solid medium); D, type of bioreactor employed to perform the SSF.

Declaration of interest

All authors declare no financial of personal with any organization that could influence this work.