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Advancements in Biosurfactants Research

 Springer

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ISBN 978-3-031-21681-7 ISBN 978-3-031-21682-4 (eBook)
<https://doi.org/10.1007/978-3-031-21682-4>

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The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Microorganisms create a structurally varied category of surface-active chemicals known as biosurfactants. Biosurfactants have been investigated as a potential substitute for synthetic surfactants in numerous industrial processes, including food, medicine, biotechnology, petroleum, oil recovery, biomedical and therapeutic, and bioremediation, due to the rising worldwide need for sustainable solutions. The book will cover a variety of current biosurfactants research advancements and progresses. The book will also cover the most recent academic advances, major applications, and implementation studies from across the world. It will be a valuable resource for research organizations, research institutes, university libraries, and R&D involved in recent surfactant research and development.

The book is divided into four parts, and each part contains numerous chapters. PART 1 explores the “overview and economic aspect of biosurfactants production.” Topics covered in Chapters “Biosurfactants: Types, Sources, and Production” to “Surface Activity and Emulsification Properties of Saponins as Biosurfactants” are types, sources, characterization, purification, biodegradation, and cytotoxic aspects of biosurfactants. PART 2 discusses the biosurfactant’s industrial applications. Topics covered in Chapters “Biosurfactants as Emulsifying Agents in Food Formulation” to “The Role of Biosurfactants in Biofuel Production” are the application of biosurfactants in nanoparticle synthesis, heavy metal remediation, drug absorption, waste treatment, agriculture management, marine sediment remediation of organic pollutants, biofuel production, emulsification, and anti-corrosive applications. PART 3 discusses the biosurfactant’s biomedical applications. Topics covered in Chapters “Role of Biosurfactants in Biocidal Activity and Wound Healing” to “Role of Biosurfactants in Biofilm Prevention and Disruption” are the application of biosurfactants as biocidal, wound healing, and anti-tumor agents. This section also covers the applications of biosurfactants in oral cavity care, and biofilm prevention and disruption. PART 4 discusses the biosurfactant’s commercialization, challenges, and future outlook. Topics covered in Chapters “Advantages and Disadvantages of Biosurfactants over Other Synthetic Surfactants” to “Biosurfactants: Challenges and

Future Outlooks” are the advantages of biosurfactants over synthetic surfactants, commercialization, challenges, and future outlook of biosurfactants.

This book is intended for a very wide-ranging audience working in the fields of advanced surface science, chemistry, colloids and interfaces science, chemical engineering & technology, etc. This book will be an invaluable reference source for libraries in universities and industrial institutions, government and independent institutes, individual research groups, and scientists. Overall, this book is written for scholars and students in academia and industry, working in the field of colloids and interface science, applied and engineering chemistry.

The editors and contributors are renowned researchers and scientists from academia. On behalf of Springer-Nature, we are very thankful to the contributors of all chapters for their amazing and passionate efforts in the making of this book. Our special thanks are dedicated to Dr. Cansu Kaya (Associate Editor) and Mr. Srinivasan Manavalan (Project Coordinator) and the Editorial Team at Springer-Nature for their devoted support and help during this project. In the end, all gratitude goes to Springer-Nature for publishing the book.

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Contents

Part I Overview and Economic Aspect of Biosurfactants Production	
Biosurfactants: Types, Sources, and Production	3
Ruby Aslam, Mohammad Mobin, Saman Zehra, and Jeenat Aslam	
Innovative and Sustainable Production Processes for Biosurfactants . . .	25
Talita Corrêa Nazareth Zanutto, Luís Antonio Lourenço, and Danielle Maass	
Sustainable Production of Biosurfactants Using Waste Substrates	57
Catherine N. Mulligan	
Characterization and Purification of Biosurfactants	79
Mridul Kumar Medhi, Shweta Ambust, Rajesh Kumar, and Amar Jyoti Das	
Biodegradation and Cytotoxic Effects of Biosurfactants	95
Sethuramalingam Balakrishnan, Marimuthu Ragavan Rameshkumar, Chandrasekaran Krithika, Avoodaiappan Nivedha, Dakshinamurthy Thirumal Kumar, and Narasingam Arunagirinathan	
Comparison of Biodegradability, and Toxicity Effect of Biosurfactants with Synthetic Surfactants	117
Natalia Andrade Teixeira Fernandes, Luara Aparecida Simões, and Disney Ribeiro Dias	
Surface Activity and Emulsification Properties of Saponins as Biosurfactants	137
Sweeta Akbari, Nour Hamid Abdurahman, and Viacheslau Kudrashou	

Part II Biosurfactants: Current Industrial Applications

Biosurfactants as Emulsifying Agents in Food Formulation	157
Oluwaseun Ruth Alara, Nour Hamid Abdurahman, John Adewole Alara, Chinonso Ishmael Ukaegbu, Moses Oludayo Tade, and Hassan Alsaggaf Ali	
Application of Biosurfactants as Anti-Corrosive Agents	171
Saman Zehra, Mohammad Mobin, and Ruby Aslam	
Role of Biosurfactants in Nanoparticles Synthesis and their Stabilization	191
Sahibzadi Saira Saleem, Saqib Saleem, and Muhammad Faizan Nazar	
New Trends in the Textile Industry: Utilization and Application of Biosurfactants	215
Júlio Cesar Vasconcelos dos Santos, Andrea Fernanda de Santana Costa, Thayse Alves de Lima e Silva, Leonie Asfora Sarubbo, and Juliana Moura de Luna	
Biosurfactants as an Eco-Friendly Technology in Heavy Metal Remediation	225
Renata Raianny da Silva, Yali Alves da Silva, Thayse Alves de Lima e Silva, Leonie Asfora Sarubbo, and Juliana Moura de Luna	
Biosurfactants and Their Perspectives for Application in Drug Adsorption	237
Thaís Strieder Machado, Bruna Strieder Machado, Danielli Martins Sanderi, Giovana Marchezi, Larissa Crestani, Luciane Maria Colla, and Jeferson Steffanello Piccin	
Role of Biosurfactants in Promoting Biodegradation in Waste Treatment	257
Brian Gidudu and Evans M. N. Chirwa	
Role of Biosurfactants in Agriculture Management	277
Asif Jamal, Muhammad Ishtiaq Ali, Malik Badshah, and Aetsam Bin Masood	
Biosurfactants and Their Benefits for Seeds	309
Luara Aparecida Simões, Natalia Andrade Teixeira Fernandes, Nelson Augusto dos Santos Junior, and Disney Ribeiro Dias	
Role of Biosurfactants in Marine Sediment Remediation of Organic Pollutants	331
Chiamaka Linda Mgbechidinma, Yeting Xie, Xiaoyan Zhang, Guiling Wang, and Chunfang Zhang	

The Role of Biosurfactants in Biofuel Production	371
O. E. Oyetunji, B. C. Kotun, O. A. Thonda, and E. A. Ademola	
Part III Biosurfactants: Current Biomedical Applications	
Role of Biosurfactants in Biocidal Activity and Wound Healing	399
John Adewole Alara, Oluwaseun Ruth Alara, and Nour Hamid Abdurahman	
Biosurfactants as Potential Antitumor Agents	439
C. I. Ukaegbu, S. R. Shah, R. O. Alara, and O. A. Thonda	
Biosurfactants in Oral Cavity Care	461
Avoодаiappan Nivedha, Chandrasekaran Krithika, Ravanasamudram Sundaram Neelakandan, Sethuramalingam Balakrishnan, Marimuthu Ragavan Rameshkumar, and Narasingam Arunagirinathan	
Role of Biosurfactants in Biofilm Prevention and Disruption	481
Chandana Malakar, Suresh Deka, and Mohan Chandra Kalita	
Part IV Biosurfactants: Commercialization, Challenges and Future Outlook	
Advantages and Disadvantages of Biosurfactants over Other Synthetic Surfactants	505
Jyoti Sharma, D. Sundar, and Preeti Srivastava	
Commercialization of Biosurfactants	525
Oluwaseun Ruth Alara, Nour Hamid Abdurahman, John Adewole Alara, Moses Oludayo Tade, and Hassan Alsaggaf Ali	
Biosurfactants: Challenges and Future Outlooks	551
Arif Nissar Zargar, Manoj Kumar, and Preeti Srivastava	

About the Editors

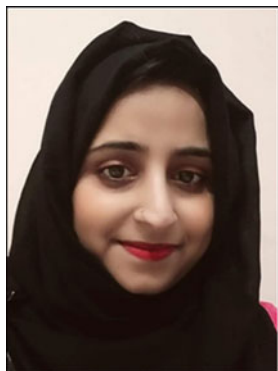


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Part I
Overview and Economic Aspect
of Biosurfactants Production

Biosurfactants: Types, Sources, and Production



Ruby Aslam, Mohammad Mobin, Saman Zehra, and Jeenat Aslam

1 Introduction

A class of amphiphilic chemical molecules known as surfactants, which have both hydrophobic and hydrophilic domains, are essential to nearly every aspect of modern industry. These substances are widely used in various industries, such as agriculture, food, and beverage. Due to their properties, they are also widely used in bioremediation and healthcare. Surfactants are chemical compounds that have a pair of hydrophobic and hydrophilic moieties. The polar moieties are commonly referred to as cationic, anionic, non-ionic, or amphoteric molecules. On the other hand, non-polar moieties are commonly referred to as hydrocarbon chains. Surfactants can also produce microemulsions by combining the two moieties (Nikolova and Gutierrez 2021). The best method to describe a surfactant is to evaluate the force of attraction between liquid molecules, which allows you to determine how well it can modify the surface and interfacial tensions. Effective surfactants reduce surface tension, enabling interactions between molecules with various polarities (Nikolova and Gutierrez 2021).

Most synthetic chemicals are produced from petroleum. This practice is considered harmful to the environment and human health (Rebello et al. 2014). It is therefore contradicts the goals of the Sustainable Development Goals of the United Nations. Various governments have been implementing policies aimed at reducing the use of harmful chemicals. Due to the increasing number of people demanding sustainable materials, many companies are now producing products that are made

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from renewable sources. This is very important as it allows them to reduce their consumption of fossil fuels. One of the biggest challenges that people face when it comes to switching to renewable sources of energy is the availability of biosurfactants. Due to their immense genetic diversity, microorganisms can potentially be used to produce biosurfactants that are more effective than organochemicals.

Biosurfactants are surface-active biomolecules produced by microorganisms that have a variety of uses. Since the first biosurfactant, “surfactin,” was purified and identified in 1968, developments have progressed a lot. Since then, many research groups from all around the world have conducted detailed and successful studies on biosurfactants. Due to their distinctive qualities like specificity, low toxicity, and relative ease of preparation, these surface-active biomolecules have garnered considerable interest and have been used in a variety of industries, including organic chemicals, petroleum, petrochemicals, mining, metallurgy (primarily bioleaching), agrochemicals, fertilizers, foods, beverages, cosmetics, pharmaceuticals, and many others. Biosurfactants are employed as emulsifiers, demulsifiers, wetting agents, foaming agents, spreading agents, functional food components, and detergents.

2 The Superiority of Biosurfactants Over Other Synthetic and Plant-Based Surfactants

Since biosurfactants are largely regarded as a superior alternative to their synthetically produced counterparts, their use and share of the global surfactant market have grown over the past 15 years (Naughton et al. 2019). The chemical structures that make up biosurfactants are extremely diverse, just like those of their synthetic counterparts. They are produced by bacteria that have been raised on either soluble (carbohydrates) or insoluble (oils, residues, and hydrocarbons) substrates (Silva et al. 2014; Bezerra et al. 2018). Biosurfactants would replace synthetic surfactants, reducing lifetime CO₂ emissions by 8% and preventing the release of an estimated 1.5 million tons of CO₂ into the atmosphere (Farias et al. 2021; Rocha e Silva et al. 2019; Banat et al. 2021). Approximately 10% of the world’s surfactant production is currently made up of biosurfactants (approximately ten million tons per year). These organic surfactants are used in a variety of industries, including petroleum, food, pharmaceutical, medical, agricultural, civil (waste and sewage treatment), and pharmaceutical (formulation of moisturizers, lotions, and medicines). The following features make biosurfactants advantageous to synthetic surfactants: They are easier to biodegrade, more environmentally compatible, better at foaming, more specific, and more efficient at extremes in temperature, pH, and salinity. They also have lesser toxicity. Renewable feedstocks can be used to produce them synthetically (Leonie et al. 2022).

Additionally, it has been found that microbial biosurfactants outperform plant-based surfactants in terms of scalability, manufacturing speed, and

multi-functionality. Many plant-based biosurfactants, like saponins, lecithins, and soy proteins, are good emulsifiers but are expensive to produce on an industrial scale and have other questionable properties like solubility and hydrophobicity (Xu et al. 2011).

3 Global Biosurfactant Market

A recent study predicts that the global market for biosurfactants would increase from US\$1.3754 billion in 2020 to US\$1.4427 billion in 2026, with a Compound Annual Growth Rate (CAGR) of 0.8% (The 360 research reports 2021). A different market study estimated that the worldwide biosurfactant market would reach approximately \$5.52 billion by 2022, growing at a CAGR of 5.6% from 2017 to 2022 (Markets and Markets 2017). Asia is becoming a more significant user of biosurfactants due to growing infrastructure and awareness. Sophorolipids (SLs), a class of biosurfactants, were discovered to have the biggest global market share, with the detergent industry dominating the field of product applications. The two leading surfactant producers to enter the biosurfactant market are BASF Cognis (Germany) and Ecover (Belgium). Several companies, such as Sun Products Corporation, AkzoNobel, Croda International PLC, Evonik Industries (Germany), Mitsubishi Chemical Corporation, Saraya, MG Intobio, Urumqi Unite, Jeneil Biosurfactant, are also involved in the production of biosurfactants. However, despite the huge market demand, the production of biosurfactants is still not as competitive as that of its synthetic counterparts. Due to the increasing environmental concerns, the need for sustainable development has become more important. Therefore, the production process must be optimized.

4 Types of Biosurfactants

Biosurfactants can be divided into the following categories based on their polarity, chemical composition, and microbial source:

1. *Classification based on their source of production* (Fig. 1)

(i) *Glycolipids*

They are long-chain aliphatic acids or hydroxy aliphatic acids attached to carbohydrates via an ester group. The majority of biosurfactants are glycolipids. The most well-known glycolipids are sophorolipids, trehalolipids, and rhamnolipids. The sources and qualities of the many glycolipids are discussed here:

(a) *Rhamnolipids*: Rhamnolipids are glycolipids where one or two rhamnose molecules are connected to one or two hydroxydecanoic acid

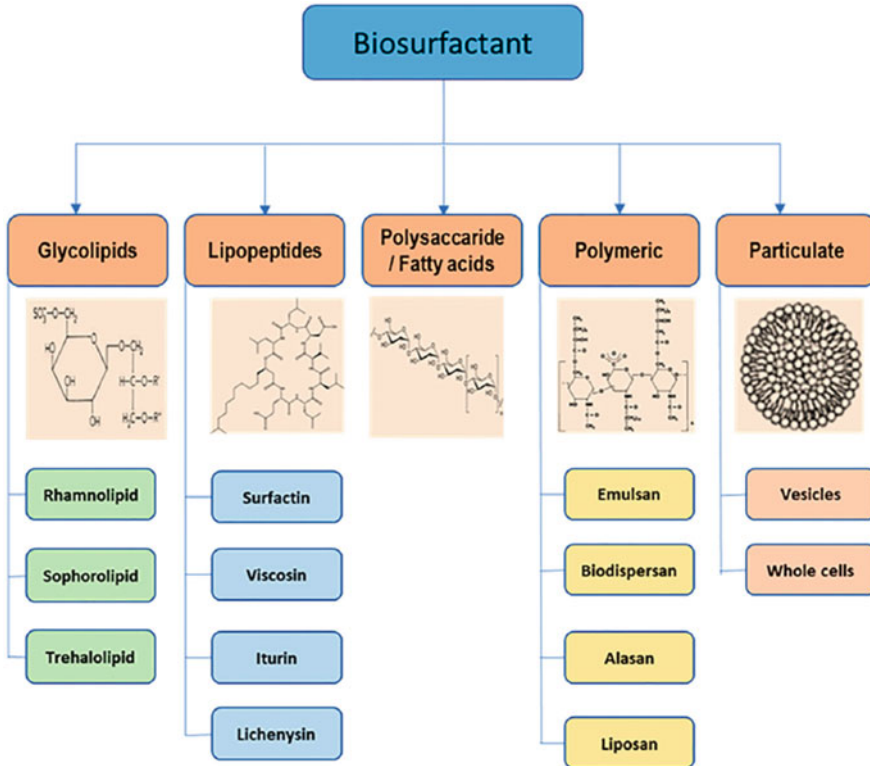


Fig. 1 Main classes of biosurfactants and their structures (Sharma et al. 2021)

molecules. The main rhamnolipids are produced by *Pseudomonas aeruginosa*. Hydrocarbons can be emulsified by them, and reports of their antibacterial and antifungal properties have also been made (Rahman et al. 2002).

- (b) *Trehalolipids*: The majority of *Mycobacterium*, *Nocardia*, and *Corynebacterium* species have these features. Trehalose lipid particles from *Rhodococcus erythropolis* and *Arthrobacter* spp. have a decreased interfacial tension and surface area in culture media from 25–40 and 1–5 mN/m, respectively (Bages-Estopa et al. 2018), which can be used for their anticancer and immunomodulatory activities. They are also used as emulsifiers and solubilizers in various food, cosmetic microbial-enhanced oil recovery, and bioremediation applications (Paściak et al. 2010).
- (c) *Sophorolipids*: These are yeast-produced glycolipids that have a long-chain hydroxyl fatty acid attached to a dimeric carbohydrate called sophorose via a glycosidic bond. For many applications, the lactone form of the sophorolipid, which is often a combination of at least six to

nine distinct hydrophobic sophorolipids, is preferred. They are employed in medicine for their antibacterial, anti-inflammatory, and immune system regulating activities, in cosmetics for their moisturizing and wetting qualities, and in stabilizing oil/water emulsions (Asmer et al. 1988; Gaur et al. 2019).

(ii) *Lipopeptides or lipoprotein*

A lipid and a polypeptide chain are combined to form a type of protein known as a lipopeptide. These molecules can reduce interfacial and surface tension and are characterized by their structural variety. Two primary types of molecules are involved in this process: acyl tails and linear oligopeptide sequences with an amide bond (Cochrane and Vederas 2016). The biosurfactant's hydrophobic tail and hydrophilic head are made up of a combination of components, including a peptide sequence and a hydrocarbon chain. The peptide component is also equipped with anionic and cationic residues. The lipopeptide's potential as an anticancer and antibacterial agent has been studied in various studies. Due to their unique structural and functional characteristics, these molecules are commonly used in various sectors. Based on their structural differences, the various groups of lipopeptide surfactants include isoforms with various D and L amino acids, as shown in Fig. 1. Some of these include the viscosine and iturin from *Bacillus subtilis*, the serrawettin from *B. licheniformis*, the gramicidin from *B. fluorescens*, and the polymyxin from *B. polymyxa* (Carrillo et al. 2003).

Types of lipopeptides are discussed below:

- (a) *Surfactin*: It belongs to the family of cyclic lipopeptides and is made up of a loop of seven amino acids, including L-asparagine (Asn), L-leucine (Leu), glutamic acid (Glu), Leucine (Leu), and L-valine (Val), as well as two D-leucines linked together by a lactone linkage. More than 30 different varieties of surfactin have been identified so far, each with a unique combination of fatty and amino acid residues. *Bacillus* sp. most likely produce this sort of surfactant, which has numerous beneficial qualities (Sajid et al. 2020). Surfactin is widely employed in many applications due to its antibacterial, antiviral, antifungal, and anti-mycoplasma properties. In the food business, it was also effective as a stabilizer, emulsifier, and surface modifier (Salek and Euston 2019). With a concentration of less than 5% by volume (Datta et al. 2020), the surface tension may be decreased from 72 to 27 mN m⁻¹, and it demonstrated low critical micelle concentration (CMC), therefore its use is being investigated in various applications (Datta et al. 2018).
- (b) *Iturins*: Iturins, a family of nonribosomal cyclic lipopeptides having seven residues of α and one β -amino acid, are not known to differ from other lipopeptide antibiotics in any way. Due to their hemolytic

and antifungal capabilities, they are mostly generated by *Bacillus* sp. and have the potential for use in biomedicine and biocontrol.

- (c) *Fengycin*: An antifungal lipopeptide complex called fengycin is produced by the *Bacillus subtilis* strain F-29-3. Leguminous plants' roots are a habitat for synergistic bacterial growth that shields the plants from phytopathogens. Fengycin functions as a fungicide and is effective against several plant diseases, including clubroot disease (*Plasmodiophora moniliforme*), maize rot (*Fusarium moniliforme*), barley head blight (*Fusarium graminearum*), and cucurbit powdery (*Podosphaera fusca*).
- (d) *Kurstakin and locillomycin*: A new class of lipopeptides called Kurstakin was identified in 2000. The partial heptapeptide kurstakin, produced by *B. thuringiensis*, is made up of several fatty acids joined by an amino acid. Thr (Threonine), Gly (Glycine), Ala (Alanine), Ser (Serine), His (Histidine), and Gln (Glutamine) are the residues found in Kurstakin. Nonapeptides called locillomycin are produced by the bacteria *B. subtilis* and contain the amino acids Thr, Gln, Asp (aspartic acid), Gly, Asn (asparagine), Asp, Gly, Tyr (tyrosine), and Val (Valine). *Bacillus subtilis* 916 produces the majority of the cyclic lipopeptides found in locillomycin.
- (e) *Lichenysin*: *Bacillus licheniformis* produces lichenysins, which are the most effective anionic cyclic lipoheptapeptide biosurfactants, in a hydrocarbon-free medium with glucose as the main carbon source. They can reduce water's surface tension from 72 to 27 mN/m. They are referred to as lichenysin A, B, C, D, G, and surfactant BL86 based on species-specific variants. With acid precipitated lichenysin B, the lowest interfacial tension against decane ever measured at 0.006 mN/m is achieved. Under ideal circumstances, the lowest CMC of any surfactant was achieved by lichenysin B and surfactant BL86, which both measured 10 mg/L. Seven amino acids are present, including L-Gln-L-Leu-D-Leu-L-Val-L-Asp-D-Leu-L-Ile (Isoleucine) in the peptide of lichenysins.
- (f) *Viscosin*: A lipopeptide of 9 amino acids, including L-Leu-D-Gln-D-Thr-D-Val-L-Leu-D-Ser-L-Ile, linked with the -hydroxydecanoyl C10-C12 fatty acid is called viscosin (Janek et al. 2020). With the aid of nonribosomal peptide-synthetase (NRPS) enzymes, (f) viscosin is produced by marine and soil bacteria, including *Pseudomonas* sp. Viscosinamides, pseudodesmins, and massetolides (Geudens et al. 2017).
- (g) *Amphisin*: A cyclic decapeptide called amphisin was found in *Pseudomonas* sp. DSS7 in 2001. It is biodegradable and less poisonous, and the β -hydroxydecanoyl fatty acid is joined by amino acids. It is made up of 11 amino acids in its peptide moiety (D-Leu-D-Asp-D-Thr-D-Leu-D-Leu-D-Ser-L-Leu-D-Gln-L-Leu-L-Ile-L-Asp) (Janek et al. 2020). Amphisin's CMC in water is 0.075 mmol L⁻¹ (Janek et al. 2018).

(iii) *Phospholipids and fatty acids (mycolic acids)*

Byproducts of microbial oxidation from alkanes include fatty acids and phospholipids, which are regarded as biosurfactants (Rehm and Reiff 1981). While fatty acids are often employed in the food sector, gene carrier systems have found usage for phospholipids because of their membrane nature. Phospholipid biosurfactants include, for example, lecithin and lysolecithin (McClements and Gumus 2016; Rehn and Reiff 1981). Microorganisms also produce complex fatty acids with OH groups and alkyl branching in addition to straight-chain acids. Corynomycolic acids, which are surfactants as well, are one example of such complex acids (Kretschner et al. 1982). The ratio of fatty acids that are hydrophilic or lipophilic is directly correlated with the length of the hydrocarbon chain. The most effective saturated fatty acids for reducing surface and interfacial tensions are in the C12–C14 range (Rosenberg et al. 1979).

(iv) *Polymeric surfactants*

The best-studied polysaccharide-protein biosurfactants are emulsan, Alasan, and lipomannan. Emulsan is an effective water-soluble emulsifier that can be used as an emulsifying agent for hydrocarbons in water. Liposan is produced by *Candida lipolytica* and is composed of 83% carbohydrate and 17% protein. In the cosmetics and food industries, the polymeric biosurfactant is most commonly used.

(v) *The particulate type*

The extracellular membrane is composed of a variety of lipid and protein structures that are designed to provide a microemulsion for the uptake of alkanes by microbes. Vesicles of *Acinetobacter* species strain HO1-N are 20–50 nm thick and have a buoyant density of 1.158 g/cm³.

2. Classification based on chemical composition and microbial origin

- (i) Hydrophilic moiety containing amino acids or polypeptides anions or cations (mono, di, or polysaccharides)
- (ii) Hydrophobic moiety containing unsaturated, saturated fatty acids
 - (a) Lipopeptides
 - (b) Gramicidins (*B. brevis*)
 - (c) Fatty acid, phospholipids, and
 - (d) Polymeric

3. Classification based on producing source:

- (i) Microbial biosurfactants and
- (ii) Enzyme synthesized surfactants

4. Classification based on the type of substrate used:

- (i) Biosurfactants that are produced using alkanes as carbon sources (*Corynebacterium* sp. and *Arthrobacter* sp.)
- (ii) Biosurfactants that are produced using water-soluble substrates as carbon sources (*Bacillus* sp.)
- (iii) Biosurfactants that are produced using alkanes and water-soluble substrates as carbon sources (*Pseudomonas* sp.)

5 Sources of Production of Biosurfactants

1. Microorganisms and growing media

According to the literature, various microorganisms, such as *Candida*, *Corynebacterium*, and *Bacillus*, can produce biosurfactants. One of the most common types of biosurfactant is *P. aeruginosa*, which is used to produce rhamnolipids. Due to the high demand for biosurfactants and the technological tools needed to produce them, the market for biosurfactants is expected to continue growing. Besides these, microorganisms can also use various compounds to grow (Ejike Ogbonna et al. 2021a). One of the most common carbon sources for these organisms is glucose and glycerol. Since glucose is a common industrial feedstock, it can increase the cost of biosurfactant production (Varjani and Upasani 2016; Wongsirichot et al. 2021).

2. Fermentation process

Solid-state fermentation (SSF) and submerged fermentation are the main bioprocessing methods used in the production of biosurfactant products. Compared with conventional fermentation, SSF offers various advantages, such as its ability to avoid inhibiting the substrates, low energy consumption, and the ability to use agro-industrial waste and industrial residues which makes the processes rentable (Eras-Muñoz et al. 2022). However, it has drawbacks, such as the complexity of the downstream processing and operational monitoring (Borah et al. 2019; Sánchez et al. 2015).

3. Low-cost byproducts and waste as feedstock

Besides basic raw materials, biosurfactants are also made from a variety of industrial waste such as molasses, corn steep liquor, whey, animal fat, vegetable fat, soap stocks, starch substrate, and oil effluents. These materials are typically cheaper than the raw materials used to produce other biosurfactants (Jimoh and Lin 2019). The growth of microbes on low-cost substrates such as industrial waste is facilitated using nitrogen, carbon, and energy. The use of glucose, starch, and agro-industrial products such as those used for food production can help increase the concentration of biosurfactants in the cell (Akbari et al. 2021). L-amino acids, which include β -alanine, glutamic acid, and l-valine, were selected as the ideal nitrogen source for biosurfactant production (Vallejo et al. 2021). Bacteria, yeast, and fungi can also produce biosurfactants, and the most common

producers are the *Candida* species and the *Pseudomonas* species. Biosurfactants are mainly synthesized during the stationary or exponential phases.

Due to the increasing demand for biosurfactant products, researchers are looking for new materials that can be used as substrates for their production. These low-cost materials can be used to reduce manufacturing costs and provide waste management services. This section aims to provide a comprehensive overview of the various feedstocks that can be used in the production of biosurfactants. They include municipal solid waste, which can be a sustainable and cost-effective alternative to traditional waste treatment. It also includes information about the advantages of using this resource in the biosurfactant production process. Unfortunately, there has been a lack of comprehensive coverage of this subject in the literature. It also discusses a wide range of other problems connected to the processing of biosurfactants, as well as the approaches taken to solve these problems and the perspectives that will lead society toward cleaner manufacturing. There are still significant difficulties in producing biosurfactants at an industrial scale, including excessive foaming during batch processing, decreased yield, the cost of downstream processing and purification, and the availability of reasonably priced raw ingredients (Cruz et al. 2018; Schultz and Rosado 2020). Processes could relate to the utilization of waste as substrates to address this issue, minimizing pollution, and balancing overall costs (Ejike Ogbonna et al. 2021b; Martins and Martins 2018). With this approach, you can profit from the sale of the biosurfactant while also lowering the cost of waste treatment. To remedy this condition, industrial, agricultural, food waste, and other inexpensive substrates could be used (Fontes et al. 2012; Marques et al. 2020).

Most biotechnological processes require high monetary inputs and a low-cost material to achieve an optimal yield. However, due to the lack of surface-active agents, most biotechnological products are produced by microorganisms. The downstream processing of these products usually costs around 60%–80% of the total cost. Most biosurfactants and bio emulsifiers that are used in the production of various products are very expensive. Therefore, the cost of production must be reduced using renewable and inexpensive substrates. To produce BS, a variety of carbon (water soluble and water insoluble) and nitrogen sources have been employed. As a result, the structure or location of production within the cell (intra or extracellular, cell-associated) may vary depending on the composition of the substrate, particularly the carbon source. Various types of carbon sources, such as blended gasoline, ethanol, and hexadecane, have been utilized as standard water-soluble compounds.

Table 1 lists various inexpensive substrates and the microorganisms used (Gaur et al. 2019b; Bezerra et al. 2019; Reddy et al. 2016; Sahebnazar et al. 2018; Patowary et al. 2016, 2018; Sharma et al. 2018a; Tomar et al. 2019; Pi et al. 2017; Lee et al. 2018; Huang et al. 2020; Silva et al. 2018; Sun et al. 2019; Gaur et al. 2019a; Rodríguez et al. 2021; Luna et al. 2015; Da Rocha Junior et al. 2019; Sajna et al. 2015; Jakinala et al. 2019; Long et al. 2017; Machado et al. 2020; Ayed et al. 2015; Liu et al. 2021; Pradhan et al. 2018; Sharma et al. 2018b; Bezza and Chirwa 2015, 2016, 2017; Prakash et al. 2021; Durval et al. 2020; Tian et al. 2016; Zouari et al.

Table 1 Summary of various renewable substrates used to produce biosurfactants (Eras-Munoz et al. 2022)

Biosurfactant	Producer microbe	Carbon Source	Operating conditions					References	
			Medium and supplement	Volume (mL)	T (°C)	pH	Time (h)		Speed (rpm)
<i>Glycolipid</i>									
Rhamnolipid	<i>Lysinibacillus sphaericus</i> IITR51	Glycerol 1.5% (w/v)	Basal salt medium	8	30	7	72	160	Gaur et al. (2019b)
Rhamnolipid	<i>Pseudomonas aeruginosa</i> TGC01	Glycerol 4% (w/v)	Mineral salt medium and (g L ⁻¹): 4.00 NaNO ₃	500	30	–	96	150	Bezerra et al. (2019)
Rhamnolipid	<i>Pseudomonas aeruginosa</i> DR1	Mango oil and glucose 1% (w/v) Mango oil 1% (w/v)	Mineral salt medium	250	30	–	96	–	Reddy et al. (2016)
Rhamnolipid	<i>Pseudomonas aeruginosa</i>	Glucose 6% (w/v)	Mineral salt medium	250	37	–	168	330	Sahebazar et al. (2018)
Rhamnolipid	<i>Pseudomonas aeruginosa</i> SR17	Glucose 2% (w/v) and paneer whey waste Crude oil 2% (w/v)	Mineral salt medium	500	35	7	48	150	Patowary et al. (2016)
Rhamnolipid	<i>Pseudomonas aeruginosa</i>	Glycerol, glucose, mannitol, molasses, and n-hexadecane at 2% (w/v)	Mineral salt medium	–	35	–	120	150	Patowary et al. (2018)
Rhamnolipid	<i>Pseudomonas aeruginosa</i> PBS	Glucose 5% (w/v) and kerosene 3% (w/v)	(g L ⁻¹): 5.00 KNO ₃ , 1.00 KH ₂ PO ₄ , 2H ₂ O 1.00 K ₂ HPO ₄ , 2H ₂ O 0.20 MgSO ₄ ·7H ₂ O 0.02 CaCl ₂ ·2H ₂ O	100	37	7	72	150	Sharma et al. (2018a)
Rhamnolipid	<i>Pseudomonas aeruginosa</i> PA1	Glycerol 2% (w/v)	Nutrient broth	–	28	–	288	180	Tomar et al. (2019)

Rhamnolipid	Pseudomonas LSH-7	Crude oil 0.5% (w/v)	Mineral salt medium and (g L ⁻¹): 3.00 (NH ₄) ₂ SO ₄ 2.00 K ₂ HPO ₄	250	25	7.5	168	120	Pi et al. (2017)
Mono- and di-rhamnolipid	<i>Bacillus aligicola</i> 003-Phe1, <i>Rhodococcus soli</i> 102-Nas, <i>Isoperitcola chitayensis</i> 103-Na4, and <i>Pseudoalteromonas agarivorans</i> SDRB-Py1	Mannitol 2%, glucose, glycerol, starch, and crude oil 1% (w/v)	Mineral salt medium and (g L ⁻¹): 10.00 (NH ₄) ₂ SO ₄ 0.50 yeast extract	100	28	7	72168	180	Lee et al. (2018)
Rhamnolipid	<i>Pseudomonas aeruginosa</i>	Glycerol 2% (v/v)	Mineral salt medium	250	30	7	192	180	Huang et al. (2020)
Rhamnolipid	<i>Pseudomonas aeruginosa</i> UCP 0992	Corn steep liquor 0.5% and vegetable oil residue 4% (v/v)	–	1200	28	7	120	225	Silva et al. (2018)
Rhamnolipid	<i>Pseudomonas aeruginosa</i>	Glucose 1% (w/v)	Mineral salt medium	250	30	7	168	180	Sun et al. (2019)
Sophorolipid	<i>Candida albicans</i> and <i>Candida glabrata</i>	Glucose 2% (w/v)	Synthetic defined medium with yeast nitrogen base 0.67%	200	30	–	72	150	Gaur et al. (2019a)
Sophorolipid	<i>Starmerella bombicola</i> ATCC 22214	Winterization oil cake (WOC) and molasses	–	500 22 000 100 000	30	5.6	168	–	Rodriguez et al. (2021)
Sophorolipid	<i>Candida sphaerica</i> UCP 0995	Corn steep liquor 9% and ground-nut oil refinery residue 9% (v/v)	Basal medium	25050 000	27	–	144	150	Luna et al. (2015)
Sophorolipid	<i>Candida tropicalis</i>	Corn liqueur 4%, molasses 2.5%, and canola frying oil 2.5% (v/v)	–	500 300050 000	28	6	144	200	Da Rocha Junior et al. (2019)

(continued)

Table 1 (continued)

	Producer microbe	Carbon Source	Operating conditions						References
			Medium and supplement	Volume (mL)	T (°C)	pH	Time (h)	Speed (rpm)	
Biosurfactant MELS	<i>Pseudozyma</i> sp. NII 08165	Soybean oil, diesel, kerosene, and petroleum	Bushnell Haas broth	–	30	7	216	200	Sajna et al. (2015)
<i>Lipopeptide</i>									
Surfactin	<i>Bacillus velezensis</i> MHNK1	Residual frying oil 2% (v/v)	Mineral salt medium	500	37	–	48	150	Jakimala et al. (2019)
Surfactin	<i>Bacillus subtilis</i> ATCC 21332	Glucose 4% (w/v)	Mineral salt medium	250	30	7	72	200	Long et al. (2017)
Surfactin	<i>Bacillus methylotrophicus</i>	Soybean oil 2% (v/v) and whey	g L ⁻¹ : 1.00 (NH ₄) ₂ SO ₄ 0.03 NaBr 1.00 CuSO ₄ ·5H ₂ O 0.81 MgSO ₄ ·7H ₂ O 0.31 ZnSO ₄ ·7H ₂ O	250	30	6.7	120	200	Machado et al. (2020)
Surfactin	<i>Bacillus amyloliquefaciens</i> SAS-1 and <i>Bacillus subtilis</i> BR-15	Glycerol 15% (w/v) and glucose 2.17% (w/v)	Mineral salt medium with yeast extract 0.5% (w/v)	–	37.5	7	72	–	Ayed et al. (2015)
Surfactin and others	<i>Bacillus licheniformis</i> L20	Glucose, sucrose, and lactose 10 g L ⁻¹ – 1	Mineral salt medium	3000	37	–	168	120	Liu et al. (2021)
Cyclic lipopeptide	<i>Bacillus tequilensis</i>	–	Mineral salt medium	–	35	–	168	150	Pradhan et al. (2018)
Crude lipopeptide	<i>Bacillus amyloliquefaciens</i> An6	Glucose 20 g L ⁻¹	Landy medium	100	30	7	72	160	Sharma et al. (2018b)

Crude lipopeptide	<i>Bacillus subtilis</i> CN2	Glycerol 4% (v/v)	Mineral salt medium	500	37	7	96	150	Bezza and Chirwa (2015)
Crude lipopeptide	<i>Paenibacillus dendritiformis</i>	Sunflower oil 3% (v/v) and anthracene 0.01% (w/v)	Mineral salt medium	1000	37	–	120	150	Bezza and Chirwa (2017)
Crude lipopeptide	<i>Bacillus subtilis</i> AS2, <i>Bacillus licheniformis</i> AS3, and <i>Bacillus velezensis</i> AS4	Crude oil 2% (w/v)	Zobell marine medium	–	37	7	120	150	Prakash et al. (2021)
Crude lipopeptide	<i>Bacillus cereus</i> UCP 1615	Waste frying soy-bean oil 2% (w/v)	Mineral salt medium with: (g L ⁻¹): 1.00 yeast extract	2501 2003 00050 000	28	7	48	250	Durval et al. (2020)
Crude lipopeptide	<i>Bacillus subtilis</i>	Glucose 15 g L ⁻¹ and crude oil 1% (w/v)	(g L ⁻¹): 0.40 MgSO4 1.07 NH4Cl 1.49 KCl 18.90 Tris-HCl 10.00 peptone	–	30	–	72	160	Bezza and Chirwa (2016)
Crude lipopeptide	<i>Pseudomonas aeruginosa</i>	Glycerol 60 g L ⁻¹	Mineral salt medium	1000	37	7	144	150	Tian et al. (2016)
Syringafactin	<i>Pseudomonas putida</i>	Glucose 2.5 g L ⁻¹	Tryptic soy broth	100	30	–	48	160	Zouari et al. (2019)
<i>Other biosurfactants</i>									
Biosurfactant extract	<i>Serratia marcescens</i> and <i>Serratia nematodiphila</i>	Kerosene 2% (v/v)	Bushnell Haas broth	–	37	7	120	135	Borah et al. (2019)
Biosurfactant extract	Enterobacteriaceae, Pseudomonas, Microbacterium, and Rhodanobacteraceae	Colza oil and glucose 20 g L ⁻¹	(g L ⁻¹): 0.10 NH ₄ NO ₃ 0.25 K ₂ HPO ₄ 0.25 Na ₂ HPO ₄ 0.25 NaCl	100	20	–	168	180	Cazals et al. (2020)
Biosurfactant extract	<i>Rhizopus arrhizus</i> UCP1607	Crude glycerol and corn steep liquor	(g L ⁻¹): 0.20 KH ₂ PO ₄ 0.20 MgSO ₄ ·7H ₂ O	100	28	5.5	96	150	Pele et al. (2019)

(continued)

Table 1 (continued)

		Operating conditions							
Biosurfactant	Producer microbe	Carbon Source	Medium and supplement	Volume (mL)	T (°C)	pH	Time (h)	Speed (rpm)	References
Biosurfactant extract	<i>Wickerhamomyces anomalous</i> CCMA 0358	Glucose 2 g L ⁻¹ and olive oil 20 g L ⁻¹	(g L ⁻¹); 4.00 yeast extract 4.00 (NH ₄) ₂ SO ₄	500	28	–	24	200	Teixeira-Souza et al. (2018)

2019; Borah et al. 2019; Cazals et al. 2020; Pele et al. 2019; Teixeira-Souza et al. 2018) to produce biosurfactants (Eras-Muñoz et al. 2022).

6 Factors Affecting Biosurfactants Production

The various factors that affect the production of biosurfactants include a carbon source, glucose, mannitol, and oil. Other factors such as pH, nitrogen source, agitation speed, and the presence of a lipophilic group can also affect the process (De et al. 2015).

Carbon Sources The three classes of carbon sources for the formation of biosurfactants include vegetable oils (sunflower oil, soybean oil, and olive oil), hydrocarbons (n-hexadecane, n-hexane, and octadecane), and carbohydrates (glucose, sucrose, fructose, mannitol, and lactose) (Varjani and Upasani 2017; Nurfarahin et al. 2018). The production of biosurfactants from various sources, such as waste frying oils, fruit and vegetable leftovers, and molasses, has gained widespread attention (Domínguez Rivera et al. 2019).

Nitrogen Source The type of nitrogen that can be used by microorganisms to produce biosurfactants has a significant impact on their development. Some of the organic nitrogen sources that can be used by microorganisms include urea, peptones, and yeast. On the other hand, inorganic nitrogen sources include potassium nitrate, ammonium chloride, and sodium nitrate. Complex organic nitrogen molecules are selected because they do not cause a significant pH shift. Utilizing inorganic salts might reduce the effectiveness of fermentation because they can hydrolyze cations or anions, altering the pH of the culture medium (Santos et al. 2016). However, *P. aeruginosa* prefers nitrates, ammonia, and amino acids as nitrogen sources (Wu et al. 2008). In addition to these nitrogen sources, waste materials can be used in place of expensive commercial nitrogen sources to lower manufacturing costs.

The Carbon/Nitrogen (C/N) Ratio In addition to the type of nitrogen that can be used by microorganisms, the ratio of carbon to nitrogen (C/N) in fermentation also affects the development of biosurfactants (Gurkok 2021). Usually, when the nitrogen source is exhausted during the cell growth phase, biosurfactant synthesis can occur. However, this process can only take place if the culture medium has a high C/N ratio (10–40).

Salts and Trace Elements Biosurfactants are commonly made using various metal supplements, such as calcium, magnesium, iron, and trace metals. To maintain the proper pH levels during fermentation, potassium dihydrogen phosphate and dipotassium hydrogen phosphate are added to the production medium. CaCl_2 is a common mediator used by microorganisms to transfer signals from the cell's surface to intracellular activities. The balance of the osmotic pressure and the cell membrane potential is controlled by the presence of potassium and calcium. The osmotic

pressure and the cell membrane potential are balanced, and these two factors regulate the cell membrane's potential, and potassium and calcium ions prevent the cell from lysis. The amount of magnesium ion (Mg^{2+}) required to produce biosurfactants is commonly given as magnesium sulfate ($MgSO_4$), which has a Ca^{2+} content that is roughly 50 times greater (Thavasi et al. 2011). A crucial component in the metabolism of many bacteria is iron (Fe). The microorganism itself determines the precise requirements for trace elements, although the most crucial trace elements utilized in the synthesis of biosurfactants are zinc (Zn), copper (Cu), boron (B), molybdenum (Mo), and cobalt (Co). Chemical elements known as "trace elements" are needed by microbes in concentrations that do not exceed 0.1% of their working volume.

Fermentation Conditions Important factors influencing the synthesis of biosurfactants include temperature, pH, agitation speed, and oxygen content. However, the optimal conditions for microbe growth frequently affect how much biosurfactant is produced. *Acinetobacter* M6 was used in the investigation, and the ideal temperature and pH were found to be 37 °C and 7, respectively (Peele et al. 2016). Depending on the strain employed, maximum rhamnolipid synthesis in several *P. aeruginosa* species occurs at pH values between 6 and 8 (Zhu et al. 2012). Rhamnolipid was generated in solid-state fermentation using the gamma-ray-derived mutant *P. aeruginosa* 15GR at pH 8, 30 °C, and 1% bacterial inoculum concentration (El-Housseiny et al. 2019).

7 Challenges and Future Research Directions

Despite their many benefits, biosurfactants also have certain drawbacks, such as high production costs. Pure biosurfactants are difficult to obtain, which is crucial for applications in the food, cosmetic, and pharmaceutical industries. This is due to the possibility that the diluted broths involved require many sequential stages during downstream processing. Bacterial strains that overproduce are uncommon, and those that are detected typically have low productivity. O_2 -limitation has been mentioned as one important criterion to control the generation of biosurfactants. The significant foam development prevents an increase in production yield. By integrating waste substrates into the process and countering their polluting effects while doing so, the difficulties associated with producing biosurfactants on a wide scale could be overcome (Tripathy and Mishra 2011). An economic study that considers the purification procedure and potential uses for biosurfactants should be conducted in addition to the manufacturing of biosurfactants. Scaling up the synthesis of biosurfactants for industrial use remains difficult. Finding the appropriate surfactant for industrial scale-up is obvious given that nutritional, micronutrient, and environmental factors all have an impact on the end products' composition. The purity of biosurfactants is necessary depending on the use; for instance, surfactants used in environmental remediation should not contain any microbiological loading, otherwise, the product's quality may suffer. Owing to the increased cost of microbial

cultivation to biosurfactant recovery (cultivation, manufacturing, purification, and recovery), industrial biosurfactant production is still in its infancy now. Because employing pure substrates and media replenishment has an expense, it has also been investigated to use low-cost byproducts and waste as a feedstock to produce sophorolipids.

8 Conclusion

Besides their biodegradability, biosurfactants also have low toxicity and stability under various conditions, such as pH, salinity, and temperature. These are safer and more eco-friendly than chemical or synthetic chemicals due to their low toxicity and biodegradability. They can be produced from a variety of bioresources, which can lower their production costs. Unfortunately, their low insolubility and biodegradability can prevent them from being utilized in various environmental and biotechnological applications. Therefore, the process must be performed properly and efficiently. This can be achieved using renewable substrates. This process can make it easier for biosurfactants to be produced, which is beneficial for both the economy and the environment. More research is needed to determine the optimal conditions for their production.

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Innovative and Sustainable Production Processes for Biosurfactants



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1 Introduction

The concept of sustainability is emerging and is based on the intersection of three main pillars: economic, social, and environmental factors. This concept aims the reconciliation between social equality and environmental problems with economic growth. In the current scenario, the search for sustainable technologies has intensified due to the necessity for processes that do not harm the environment and that are also efficient as traditional ones. In addition, stricter environmental protection legislation has been promulgated, since the growing global awareness regarding environmental safety has caused an increase in the demand for “green products” (Purvis et al. 2018; Manga et al. 2021). Among these green products are bioproducts that are bio-based materials and chemicals, produced by biocatalysts such as bacteria, yeast, and fungi (Kopsahelis et al. 2018).

Bioproducts are biodegradable and present low toxicity, which makes them very useful and attractive products in biomedical, cosmetics, and food applications and substitute bioproducts for those already available on the market. Among the products of great commercial interest, surfactants have useful characteristics for the industry due to their amphiphilic behavior, which allows these molecules to interact in media

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with different polarities. Thus, surfactants are widely used in the production of paints, agrochemicals, detergents, and some industrialized products (Baskaran et al. 2021).

Surfactants can be generated by one of the three raw materials: petroleum products (petrochemicals), plant oils (oleochemicals), or plant or microbial (biosurfactants) (Rebello et al. 2020). Currently, the most viable way to obtain surfactants is from petroleum derivatives through the chemical route mostly due to its economic feasibility. However, synthetic surfactants cause environmental damage associated with their production and disposal in the sewage system or straight into surface-water bodies. The mass usage of surfactants in both households and industries can cause eutrophication and high toxicity to mammals and bacteria (Rebello et al. 2020; Baskaran et al. 2021). On the other hand, biosurfactants (BS) have significant advantages when compared to their counterparts since they can be produced from renewable feedstock, present excellent surface activity, have less toxicity and high selectivity, are more durable and biodegradable, can be recycled, have extensive foaming activities, are biocompatible and ecologically acceptable, and are effective at extreme environments (Jimoh and Lin 2019a; Olasanmi and Thring 2018).

Such advantages have made the world market for BS to grow exponentially in recent years and the tendency is that this market keep growing in the next decades. For instance, the 2019 report of the Global Market Insights projected that the world BS market is going to exceed 2.4 billion US dollars by 2025 (Rawat et al. 2020). Thus, current research efforts in scientific and industrial communities are centered on improving the cost-effectiveness and sustainability of BS production. That improvement has been made by using renewable and low-cost substrates, reusable processes, engineered microorganisms, co-production practices, optimization of fermentative and productive processes using statistical procedures, and novel separation technologies. However, some strengths, weaknesses, opportunities, and threats need to be carefully analyzed to make sure that BS are sustainable biotechnology product (Manga et al. 2021), as shown in Fig. 1. This chapter discusses processes, strategies, and methodologies applied to obtain high yield and lower the costs of BS production within the context of sustainability.

2 Sustainable Approaches to Biosurfactant Production in Submerged Fermentation Using Low-Cost Substrates

Recently, the UN put the concept of sustainability in evidence again through the elaboration of the Sustainable Development Goals (SDGs), which seek to guide development in nations. A key theme in the SDGs is to substitute non-renewable resources with renewable ones for all inputs along a product's value chain. Because of this, biosurfactants (BS) become a promising alternative for the replacement of chemical surfactants (Purvis et al. 2018; Manga et al. 2021). In the literature, most of



Fig. 1 The SWOT (strengths, weaknesses, opportunities, and threats) of BS applications as sustainable products. Adapted from: Manga et al. (2021), Jimoh and Lin (2019a)

the articles report the production of BS in a submerged medium with a pure or mixed culture of microorganisms under a wide range of growth and environmental conditions (Brumano et al. 2017; Das and Kumar 2018; Jimoh and Lin 2020; Nazareth et al. 2021). The processes, which can be aerobic or anaerobic, have as their main focus the optimization of process parameters (i.e., temperature, agitation speed, oxygen flow, and pH) and culture medium. Many statistical approaches have been successfully used to optimize BS production, where factorial designs (i.e., full factorial, fractional factorial, and response surface designs) have been widely used. According to Manga et al. (2021), artificial intelligence combined with genetic algorithm approaches has recently been used to provide optimization conditions in less time.

Although they present several advantages over chemical-based surfactants, such as low toxicity and greater surface and interfacial activity, BS production on an industrial scale presents several challenges, mainly concerning low yield and high costs of production and purification. Thus, researchers from all over the world have sought to enable the production of BS through the development of low-cost processes using agro-industrial residues, in submerged and solid media, in the presence of different types of microorganisms (bacteria and fungi). The use of agro-industrial residues is an interesting alternative due to its rich amount of organic matter, which contains macronutrients (proteins and carbohydrates) and micronutrients (minerals)

(Zanotto et al. 2019). The bioconversion to BS of wastes from food, oil, and agriculture industries is an eco-friendly and economic approach, once solves the problem of waste management, and provides a low-cost production of value-added products (Gaur et al. 2022a, b).

According to their structure and molecular weight, BS are classified into lipopeptides, glycolipids, phospholipids, lipopolysaccharides-protein complexes, neutral lipids, and long and short-chain fatty acids (Ahmad et al. 2021). For Manga et al. (2021), the low molecular weight BS groups are better at reducing the surface tension between liquid surfaces and interfaces, while high molecular weight groups present better emulsification properties and oil–water emulsion stabilization.

2.1 *The Use of Renewable Resources for Glycolipids Production*

Glycolipids are low molecular weight BS, where the rhamnolipids, sophorolipids, and recently mannosylerythritol lipids (MEL) are the major groups, being the only BS produced on an industrial scale (Henkel et al. 2017), and therefore will be detailed in this section. The glycolipids present applications in agriculture as biopesticides and, due to their antimicrobial property, in food additives and food preservatives (Mnif and Ghribi 2016). According to Mohanty et al. (2021), rhamnolipids have great potential to become the next generation of BS, being also the only BS approved by the US Environmental Protection Agency for use in pharmaceuticals, cosmetics, and food products (Zhu et al. 2022).

Rhamnolipids

Rhamnolipids are secondary metabolites produced by microorganisms, being composed of rhamnose fractions (glycone part) and lipid fractions (aglycone part) linked through an O-glycosidic bond (Chebbi et al. 2021). These compounds have a diverse group of molecules with more than 60 congeners reported and they are the most popular BS due to their physicochemical properties, being excellent natural emulsifiers (Varjani et al. 2021). Rhamnolipids are produced mainly by the pathogen *Pseudomonas aeruginosa*. However, depending on their application, nonpathogenic microorganisms, such as *Burkholderia* spp., have been used in the production of rhamnolipids, particularly in biomedical applications (Chebbi et al. 2021), as well as genetically modified microbes. Nevertheless, the yields achieved for both approaches were significantly lower than those obtained by *P. aeruginosa* strains (Baskaran et al. 2021).

The production of bio-based rhamnolipids from renewable resources (see Table 1) has been reported in the literature in the last two decades, where olive oil residues, crude glycerol, winery residues, residual cooking oil, cassava residues, lignocellulose residues, and oil residues (petroleum) are some examples (Chebbi et al. 2021; Varjani et al. 2021). According to Nazareth et al. (2018), glycerol is the

Table 1 Glycolipids production from renewable resources

BS (BS)	Low-cost substrate	Microorganism	Fermentation conditions	BS concentration	Productivity	References
Rhamnolipid	Distillery wastewater (20% v/v)	<i>Pseudomonas aeruginosa</i> SRRBL1	Batch, 37 °C, 120 rpm, 120 h	2.90 g L ⁻¹	0.040 g L ⁻¹ h ⁻¹	Ratna and Kumar (2022)
	Crude glycerol (60 g L ⁻¹)	<i>Pseudomonas aeruginosa</i> PrhlAB	Batch, 37 °C, 200 rpm, pH 6.8 (initial), 168 h	2.87 g L ⁻¹	0.0239 g L ⁻¹ h ⁻¹	Zhao et al. (2019)
	Petroleum oil waste (2% oil)	<i>Pseudomonas aeruginosa</i>	Batch (bioreactor) 10 L (working volume: 5 L), 37 °C, pH 7 (initial), 0.5 vvm, 360 h	2.68 g L ⁻¹	0.019 g L ⁻¹ h ⁻¹	Mostafa et al. (2019)
Sophorolipid	Cooking oil fume condensates	<i>Pseudomonas aeruginosa</i> AB93066	Batch 7-L bioreactor pH 7.0 (initial), 110 h	12.3 g L ⁻¹ CMC: 45.0 mg L ⁻¹	0.157 g L ⁻¹ h ⁻¹	Wu et al. (2019a, b)
	Waste syrup (7% w/v); oleic acid (2% w/v)	<i>Starmerella bombicola</i> ATCC 22214	Batch, 30 °C, pH 5.0, 240 h	42.9 g L ⁻¹ CMC: 76.12 mg L ⁻¹	0.255 g L ⁻¹ h ⁻¹	Pratap et al. (2021)
	Sugarcane molasses (100 g L ⁻¹); chicken fat (100 g L ⁻¹)	<i>Starmerella bombicola</i> ATCC 22214	Batch, 30 °C, 150 rpm, 120 h	9.78 g L ⁻¹	0.0815 g L ⁻¹ h ⁻¹	Minucelli et al. (2017)
	Sugarcane molasses (250 mg L ⁻¹ , 137.5 g L ⁻¹ sucrose) ^a	<i>Starmerella bombicola</i> ATCC 22214	Batch, 25 °C, 250 rpm, pH 6 (uncontrolled), 120 h	14.4 g L ⁻¹	0.120 g L ⁻¹ h ⁻¹	Takahashi et al. (2011)

^a No additional hydrophobic carbon substrate

main residue obtained from biodiesel production, in which approximately 10% (wt) of glycerol is formed. Due to its impure nature by the presence of salts, alcohol, esters, and residual oil, glycerol purification is not feasible. However, glycerol is successfully used in biological processes as a water-soluble carbon source.

Baskaran et al. (2021) have explored the valorization of glycerol for rhamnolipids production by *P. aeruginosa* RS6. Different compositions of the mono-RLs and di-RLs were observed when the effects of fermentation conditions (temperature, initial medium pH, glycerol concentration, nitrogen sources, and their concentrations) were investigated to optimize the rhamnolipids production. The highest rhamnolipids production (2.73 g L^{-1}) was achieved for a medium supplemented with 1% waste glycerol and 0.2 M sodium nitrate when incubated at $35 \text{ }^\circ\text{C}$ and pH 6.5. The BS was able to reduce the water surface tension from 72.13 to $29.4\text{--}30.4 \text{ mN m}^{-1}$.

In another recent study, the use of hydrolyzed pineapple skin, corncob, and glycerol as the sole carbon source for rhamnolipids production by *Planomicrobium okeanokoites* IITR52 was investigated (Gaur et al. 2022a, b). The authors evaluated different concentrations of low-cost substrates (1, 3, and 5%) in a 72 h batch at $30 \text{ }^\circ\text{C}$ and 150 rpm. As expected, the best substrate concentration for BS production was 5%, since a higher content of simple sugars was available. The highest concentration of BS was achieved for glycerol (1500 mg L^{-1}), followed by corncob (568 mg L^{-1}) and pineapple shell syrup (304 mg L^{-1}). According to the authors, approximately 144 million years⁻¹ tons of corncob are produced, generating toxic fumes due to the burning carried out at its disposal. However, corncob is rich in lignocellulose biomass, being suitable for microbial production of value-added products.

Farmers, suppliers, manufacturers, retailers, and consumers generate huge amounts of food waste every day. These residues pose dangerous environmental problems such as soil pollution, odor production, and pest attraction. In addition, their biological treatment is difficult due to the presence of high amounts of oil, which reduces composting efficiency due to oxygen diffusion limitations (Shi et al. 2021). Annually, between 41 and 67 million tons of waste cooking oil are generated in the world (Kim et al. 2021). The oily waste, particularly cooking oil, presents a high concentration of polar hydrocarbons, monoglycerides, diglycerides, triglycerides, and free fatty acids, which make it an alternative carbon source in biological process. In addition, oil waste has been proven a good substrate for BS production (Mohanty et al. 2021).

Pathania and Jana (2020) reported an optimized rhamnolipid production by an indigenous *P. aeruginosa* using waste frying oils and/or glucose as a substrate. Several factors, such as frying oil ($0\text{--}20 \text{ g L}^{-1}$), glucose ($0\text{--}20 \text{ g L}^{-1}$), and ammonium nitrate ($0\text{--}2.5 \text{ g L}^{-1}$), were evaluated by a central composite design (CCD) with six axial points and four central points. The experiments were conducted for 96 h at $30 \text{ }^\circ\text{C}$ and 150 rpm, where the highest rhamnolipids production (6.2 g L^{-1}) was achieved from the interactive effect between the frying oil at above 18 g L^{-1} and glucose $9\text{--}12 \text{ g L}^{-1}$ at constant ammonium nitrate at 1.25 g L^{-1} . The same rhamnolipid concentration (6.2 g L^{-1}) was obtained at a high concentration of frying

oil from the interaction between frying oil and ammonium nitrate when glucose was constant at 10 g L^{-1} . The authors also observed that the highest rhamnolipid production was lower (5.5 g L^{-1}) when frying oil was constant at 10 g L^{-1} . The BS was able to reduce the water surface tension to 30 mN m^{-1} at CMC 150 mg L^{-1} . According to the authors, co-substrate utilization presented effects on quorum sensing, cell growth, and changed biosynthetic pathways to improve rhamnolipid production.

Shi et al. (2021) evaluated rhamnolipids production by *P. aeruginosa* M4 in a low-cost medium, where different concentrations of waste cooking oil (5, 10, and 25 g L^{-1}) were used as the sole carbon source. $1119.87 \text{ mg L}^{-1}$ of rhamnolipids was produced after 216 h of incubation at $35 \text{ }^\circ\text{C}$ and 180 rpm, when using 25 g L^{-1} of waste cooking oil (WCO). According to the authors, rhamnose precursors were synthesized from a product of the hydrolysis of WCO (glycerol). The precursors of R-3 hydroxyalkanoate were synthesized de novo using acetyl-CoA produced from the β -oxidation of fatty acids.

Sophorolipids

Another prominent glycolipid is sophorolipid, which is a BS produced by nonpathogenic yeast strains (i.e., *Starmerella bombicola*, *Candida batistae*, *Candida apicola*, *Candida bogoriensis*, and *Wickerhamiella domercqiae*, among others) at high amounts (over 400 g L^{-1} by *Starmerella bombicola*) (Bogaert et al. 2011; Konishi et al. 2015). According to Wang et al. (2019), *Starmerella bombicola* is known as the most productive strain in the synthesis of sophorolipids, reaching volumetric productivity of up to $3.7 \text{ g L}^{-1} \text{ h}^{-1}$.

The sophorolipids are composed of a disaccharide sophorose linked by a β -glycosidic bond to a long fatty acid chain. When extracellularly secreted into the culture medium as secondary metabolites, sophorolipids appear as a mixture of little different molecules with three main points of lactonization variation, acetylation pattern, and fatty acid part (chain length, saturation, and hydroxylation position), which give them the variation in biological and physical–chemical properties. Hydrophobic and hydrophilic carbon sources are required to achieve high levels of sophorolipid production, once the yield is low if only one type of substrate is present in the culture medium (Ma et al. 2020).

Currently, purified glucose and oleic acids/food-grade oils are used as substrates for industrial sophorolipid production, which contributes to high production costs. Thus, much effort has been made to reduce costs by using low-cost substrates (see Table 1), such as food waste, molasses, corncob, sweet sorghum bagasse, rice straw, and waste glycerol as hydrophilic carbon sources, and waste frying oil, coconut fatty acid residue, and tallow fatty acid residue as hydrophobic carbon sources (Wongsirichot et al. 2021).

Kaur et al. (2019) reported the production of sophorolipids by *S. bombicola* in the presence of hydrophobic (corn oil, hydrolyzed food waste-derived lipids, and fat) and hydrophilic (textile waste, bakery waste, and mixed food waste) substrates. A first screening of the hydrolysates generated from the tested substrates indicated that food waste hydrolysate was the most suitable substrate to produce sophorolipids.

Fed-batch fermentation was performed in a 2 L bioreactor (1.15 L working volume) at 30 °C and pH 3.5. The food residue hydrolysate (100–120 g L⁻¹ of glucose) was the only compound present in the culture medium (i.e., without the addition of nitrogen, salts, vitamins, and phosphate) and the aeration was maintained at 3.48 vvm, as well as the agitation speed at 1200–1600 rpm, to the maintenance of aerobic conditions. 115.2 g L⁻¹ of sophorolipid was obtained after 92 h, and a productivity of 1.25 g L⁻¹ h⁻¹ was successfully achieved, which is comparable to results based on first-generation feedstocks.

Sophorolipids production by *S. bombicola* using waste cooking oil 10% (w/v), as a hydrophobic substrate, was studied by Kim et al. (2021). Fed-batch fermentation was carried out at 25 °C and pH 3.5 in a 5 L bioreactor (2 L of working volume). Waste cooking oil and 500 g L⁻¹ of glucose were fed at one-day intervals after the first 48 h. The authors reported a high concentration of BS (315.6 g L⁻¹) after approximately 240 h of the process with an aeration rate of 1 vvm and 400 rpm.

Food residues, waste glycerol, and waste frying oil have shown great potential to produce sophorolipids since a considerable number of works in the literature have reported successful production of BS in batch and fed-batch fermentations (Maddikeri et al. 2015; Konishi et al. 2018; Wang et al. 2020; To et al. 2022). However, little has been addressed concerning the life cycle assessment (LCA) of the production of sophorolipids from low-cost substrates, although it is essential to quantify the environmental impacts regarding this bioconversion when an industrial scale is considered. In this sense, Hu et al. (2021a, b) explored the production of sophorolipids from food waste considering the life cycle assessment (LCA), which is an innovative approach based on the evaluation and quantification of possible environmental impacts associated with a product or process. Previously to works done by Hu and collaborators, only Baccile et al. (2017) evaluated the life cycle environmental impacts of sophorolipid production from first-generation raw materials (rapeseed oil and glucose). To the best of our knowledge, no other work has been reported since then.

In the first stage of the study, Hu et al. (2021a, b) aimed to identify the most appropriate raw material to produce sophorolipids, where first-generation (glucose solution, canola, and corn oils) and second-generation (textile, bakery, and food wastes) feedstock were evaluated individually, and then compared to each other. After identifying the best substrate, the authors evaluated the steps with the highest energy cost concerning the selection of feedstock, hydrolysis, and pre-culture, in which the first one showed a higher energy cost due to the use of non-renewable energy (electrical energy). Another important observation is that the effort to increase the concentration of sophorolipids in the process resulted in a decrease in energy demand in production. Among the second-generation substrates evaluated, food waste resulted in the lowest environmental impact, measured by the parameters of cumulative energy demand (CED) and global warming potential (GWP), which presented values of 273,719 MJ and 23,115 kgCO₂eq., respectively. In addition, the authors found that the yields obtained in the production of sophorolipids in the batch were very low, but with a high environmental impact. In conclusion, the food waste hydrolysate in a fed-batch fermentation coupled with in situ separation

(semi-continuous separation) was the most environmentally sustainable proposal to produce BS on a laboratory scale and from a scale-up perspective.

Mannosylerythritol lipids

Mannosylerythritol lipids (MELs) are a glycolipid class of BS with remarkable antimicrobial and biomedical properties as well as excellent interfacial activity. This BS is mainly produced by amorphous basidiomycetous yeasts, *Pseudozyma* spp., and fungi *Ustilago maydis*, which was the first discovered microorganism capable to produce MEL (Niu et al. 2019; Liepins et al. 2021). MEL is a nonionic BS, consisting of the polar 4-O- β -D-mannopyranosyl-D-erythritol and two nonpolar fatty acid acyl chains. In general, MEL is classified as MEL-A (diacetylated at O-4 and O-6), MEL-B (monoacetylated at O-6), MEL-C (monoacetylated at O-4), and MEL-D (deacetylated) according to the number of mannose acetylation groups and their positions on the mannose (Fan et al. 2014; Liepins et al. 2021).

Little has been reported in the literature about mannosylerythritol lipids production from renewable sources. More studies regarding the optimization of process parameters and the identification and potential use of renewable sources as substrate as well as the evaluation of possible environmental impacts considering the life cycle assessment (LCA) approach are necessary. To the best of our knowledge, Morita et al. (2007) reported one of the first studies using a low-cost substrate for MEL production.

Morita et al. (2007) aimed to explore the potential of MELs production by several strains from *Pseudozyma* genus (*P. antarctica*, *P. aphidis*, *P. flocculosa*, *P. fusiformata*, *P. parantarctica*, *P. prolifica*, *P. rugulosa*, *P. thailandica*, and *P. tsukubaensis*) in a low-cost medium contained glycerol. After identifying the best strain, which was *P. antarctica*, the influence of temperature (20, 25, 30, and 35 °C), initial glycerol concentration (6, 8, 10, 12, and 14%), and concentrations of additional carbon sources (mannose, erythritol, and glucose, all at 1 and 2%) were also investigated, one factor at a time. The best conditions for temperature and initial glycerol concentration were 30 °C and 10%, respectively. Higher MELs concentrations were obtained when mannose and erythritol were added to the culture medium, however, the maximum concentration was achieved by adding 2% of mannose. A time-course at the best conditions was carried out, in fed-batch mode, where after 7 days of incubation, glycerol was added to the flask for 3 weeks. The authors observed that MEL concentration increased with cultivation time, achieving 12.6 g L⁻¹ after 3 weeks, without the presence of additional carbon sources. The maximum MEL production (16.3 g L⁻¹) occurred in the presence of mannose (2%), which improved the BS production by more than 30%.

Cassava wastewater was used for mannosylerythritol lipids (MEL) production in a work done by Andrade et al. (2017), where a novel bioprocess for the production of mannosylerythritol lipids (MEL) by *Pseudozyma tsukubaensis*, a microorganism capable of producing only MEL-B homologous was studied. The low-cost substrate was used in the BS production in a bioreactor (3.0 L working volume), where agitation speed and aeration rate varied from 100 to 150 rpm, and 0.4 to 0.8 vvm, respectively. *P. tsukubaensis* was capable to produce MEL-B in the presence of

cassava wastewater, achieving 1.26 g L^{-1} , and after 24 h of fermentation, the surface tension of the culture medium decreased from about 50 to 26 mN m^{-1} . A successful purification process by fractionation and foam ultrafiltration was carried out for the first time, where 80% of the purified MEL-B was recovered in a single step, with more than 95% of the proteins contained in the permeate.

Niu et al. (2019) investigated MELs production by *Pseudozyma aphidis* ZJUDM34 using waste cooking oil as a sole carbon source. The BS production was optimized by a central composite design (CCD), where four factors (waste cooking oil amount, inoculum size, medium volume, and initial pH) were investigated. The fermentation lasted 10 days, at $28 \text{ }^\circ\text{C}$ and 180 rpm, and the BS concentration achieved was 61.50 g L^{-1} , which was six times higher than in the non-optimized medium. The authors also mentioned that MELs exhibited good surface activity and better performance in contrast to MELs grown on soybean oil, and 55–60% of the oil was converted into MELs for both feedstocks.

2.2 The Use of Renewable Resources for Lipopeptide Production

Lipopeptides BS are a class of microbial metabolites, mainly synthesized by *Bacillus* and *Pseudomonas* genera, which can produce them in the presence of hydrophilic carbon sources (carbohydrates) and hydrophobic ones (hydrocarbons and oils). This class of BS is composed of a fatty acid (between C12 and C18) linked to a linear or cyclic oligopeptide moiety (from 4 to 12 amino acids). Surfactin, iturin, and fengycin are the most prominent lipopeptides, where many researchers (Table 2) have sought production optimization strategies and production cost reduction through the use of renewable substrates (Inès and Dhouha 2015). Surfactin plays an important role in bioremediation due to its foaming and emulsifying activity, and iturin has considerable antimicrobial activity in vitro and in vivo against diverse fungi (Paraszkiwicz et al. 2018).

Surfactin

Surfactin is a BS with remarkable surface properties and biological activities, acting as antimicrobial, antifungal, and anticancer agents, as well as having promising applications in bioremediation, food, cosmetic, and pharmaceutical industries. Generally, surfactin is a cyclic heptapeptide (L-Glu-L-Leu-D-Leu L-Val-L-Asp-D-Leu-L-Leu) attached to a β -OH (lactone) fatty acid chain (12–16 carbons). The properties of surfactin are strongly affected by the physical state of the two carboxyl groups present in the amino acid residues (Glu1 and Asp5) in the peptide portion of the molecule, which represents the hydrophilic group of surfactin (Gang et al. 2015; Ding et al. 2022).

Verma et al. (2020) evaluated lipopeptide (surfactin) production by an isolated *Bacillus subtilis* RSL-2 using sugarcane molasses as a sole nutrient source, without any pre-treatment and supplement additions. Molasses is a by-product generated

Table 2 Lipopeptides production from renewable resources in submerged fermentation

Biosurfactant (Bs)	Low-cost substrate	Microorganism	Fermentation conditions	Bs concentration	Productivity	References
Surfactin	Waste glycerol (40 g L ⁻¹)	<i>Bacillus subtilis</i> #309	Batch, 37 °C, 160 rpm pH 7.0 (initial), 96 h	2.8 g L ⁻¹ , CMC: 15 mg L ⁻¹	0.029 g L ⁻¹ h ⁻¹	Janek et al. (2021)
	Orange peel extract (10% v/v)	<i>Bacillus haynesii</i> E1	Batch, 35 °C, 130 rpm, pH 6.0, 120 h	3.7 g L ⁻¹ , CMC: 50 mg L ⁻¹	0.030 g L ⁻¹ h ⁻¹	Rastogi et al. (2021)
	Hemicellulosic corn cob liquor (32.16%)	<i>Bacillus subtilis</i> ICF-PC	Batch, 30 °C, 120 rpm, pH 6.85, 72 h	3.95 g L ⁻¹ , CMC: 100 mg L ⁻¹	0.054 g L ⁻¹ h ⁻¹	Prado et al. (2019)
	Distillers' grains (200 g L ⁻¹)	Co-culture of <i>Bacillus amyloliquefaciens</i> (MT45 and X82)	Batch, 30 °C, 160 rpm pH 7.0 (initial), 96 h	3.4 g L ⁻¹	0.035 g L ⁻¹ h ⁻¹	Zhi et al. (2017)
Fengycins	Kitchen waste (10% w/v)	Co-culture of <i>B. amyloliquefaciens</i> HM618 and <i>Pichia pastoris</i>	Batch, 30 °C, 220 rpm, 144 h	21.2 mg L ⁻¹	0.145 mg L ⁻¹ h ⁻¹	Wang et al. (2022)
Iturin	Rapeseed meal ^a (50 g L ⁻¹); glucose (80 g L ⁻¹)	<i>Bacillus amyloliquefaciens</i> CX-20	Batch, 28 °C, 220 rpm, pH 7.0 (initial), 72 h	1.64 g L ⁻¹	0.022 g L ⁻¹ h ⁻¹	Chen et al. (2019)
	Rapeseed meal ^a (90 g L ⁻¹); glucose (20 g L ⁻¹)	<i>Bacillus subtilis</i>	Batch (bioreactor), 7 L (3.0 L working volume), 28 °C, 600 rpm, 2 vvm, pH 7.0 (controlled), 80 h	600 mg L ⁻¹	8.571 mg L ⁻¹ h ⁻¹	Jin et al. (2014)
	Malt residue (250 g L ⁻¹)	<i>B. subtilis</i> RB14	Batch, 30 °C, 120 rpm, pH 6.3 (initial), 144 h	170 mg L ⁻¹	3.541 mg L ⁻¹ h ⁻¹	Khan et al. (2009)

^a As alternative nitrogen source

from sugarcane industries, with rich composition in carbohydrates (30–50%), vitamins, and minerals. The BS production was optimized by a central composite design, where the impact of molasses (1–5% w/v), temperature (25–45 °C), and pH (4–8) was investigated. The authors found that the best conditions for surfactin production were at pH 6.6, the temperature of 41 °C, and 5% (w/v) molasses concentration, achieving the highest surfactin concentration (12.34 g L⁻¹) after 9 days. In addition, surfactin presented high thermostability at 160 °C, good ability to lower the surface tension of water (24.09 mN m⁻¹), and a low CMC of 80 mg L⁻¹.

Ostendorf et al. (2019) investigated BS production by selecting the best producing strains (*Pseudomonas cepacia*, *Bacillus methylotrophicus*, and *Bacillus cereus*), carbon sources (glucose, sucrose, molasses, and waste frying oil), and nitrogen sources (NH₄NO₃, (NH₄)₂SO₄, peptone, yeast extract, and corn steep liquor). Firstly, the authors tested all the carbon sources at 2.0% and, after identifying the best substrate, several concentrations (1.0 to 7.0%) were tested for it. A similar procedure was performed for the nitrogen source. The microorganism with the best performance in the production of surfactin (2.05 g L⁻¹) was *B. cereus* incubated at 28 °C and 200 rpm for 48 h in 2.0% molasses and 1.0% corn steep. In addition, the BS presented high stability under a wide range of pH, temperature, and salt concentrations, being also capable to reduce the culture medium surface tension to 26.2 mN/m.

Das and Kumar (2019) evaluated surfactin production by *Bacillus safensis* incubated in an agro-industrial waste (bagasse) as a sole carbon source for 72 h. Bagasse is composed of 50% cellulose, 25% lignin, 25% hemicellulose, and 1–5% sucrose, being a potential cost-effective substrate for microbial conversion due to its high carbon content. Hence, the authors investigated three concentrations of bagasse (10, 15, and 20 g L⁻¹), finding that the highest BS concentration (920 mg L⁻¹) was produced by the strain when using 15 g L⁻¹ of bagasse in a bioprocess at 35 °C, pH 7.0, and 180 rpm.

Iturin Iturins are molecules similar to surfactin, being cyclic heptapeptides linked to a fatty acid (β -amino) chain that can vary from C-14 to C-17 carbon molecules. Cyclization occurs through an amide bond between the first and last amino acid, having fatty acid attached to the first amino acid. The iturin amino acids sequence is composed of three D-amino acids (Tyr, Asn, and Asn) and the four L-amino acids (Pro, Ser, Asn, and Gln) (Khem Raj and Kanwar Shamsher 2015; Geissler et al. 2019). The production of iturin from renewable resources (i.e., soybean curd residue—okara) occurs mainly by solid-state fermentation (Ohno et al. 1996; Mizumoto et al. 2006; Khan et al. 2012), with few works reporting production in submerged fermentation using low-cost substrates (see Table 2).

Paraszkiewicz et al. (2018) studied surfactin and iturin production by two *Bacillus subtilis* using renewable resources (brewery wastewaters, beet molasses, apple peels extract, and carrot peels extract, both supplemented with yeast extract or peptone). The authors found that both strains synthesized surfactin and iturin and that each strain preferentially produced surfactin (*B. subtilis* KP7) or iturin (*B. subtilis* IO-1), regardless of the culture media used. *B. subtilis* IO-1 strongly

stimulated iturin production in all culture mediums, except for brewery waste. The highest iturin concentration (428.7 mg L^{-1}) was achieved for the production with carrot peels extract supplemented with peptone, which was also seven times higher than iturin concentration obtained for the standard medium (Luria-Bertani). 269.5 mg L^{-1} of iturin was obtained for the apple peels extract medium supplemented with peptone. In addition, *B. subtilis* KP7 produced surfactin (140.6 mg L^{-1}) using carrot peels extract supplemented with yeast extract (0.5%), being higher than surfactin concentration (100.3 mg L^{-1}) obtained from standard medium (Luria-Bertani).

Narendra Kumar et al. (2017) screened 100 bacterial isolates for iturin production from various rhizosphere soil samples. Twenty isolates were selected for BS production according to their superficial/interfacial and emulsification activities, where *B. subtilis* RHNK22 showed the best potential for BS production. Furthermore, the authors used a Plackett–Burman (PB) design to evaluate sixteen different agro-industrial wastes, they found out that only eight of them were suitable for BS production (sunflower oil cake, cottonseed oil cake, coconut oil cake, Pongamia seed cake, jatropa seed cake, cheese whey permeate, dry yeast cells, and groundnut oil cake). From the PB, only sunflower oil cake, cheese whey permeates, and dry yeast cells were statistically significant on iturin production, where the first one presented the highest impact. A second design was employed to optimize iturin production by *B. subtilis* RHNK22 using sunflower oil cake (SOC) as a carbon source. A central composite rotatable design (CCRD) with five factors (pH, temperature, inoculum size, incubation time, and Sunflower oil cake concentration) was performed, where 832.8 mg L^{-1} was produced at the optimum conditions of SOC (4%), pH 6.0, inoculum size (1%), at $37 \text{ }^\circ\text{C}$ for 48 h.

2.3 Research Needs and Future Directions to Sustainable BS Production in Submerged Bioprocesses

A lot of progress has already been made regarding process optimization and the use of several low-cost substrates for BS production once the substrate represents a large part of the production costs (30–50%) (Zanotto et al. 2019). However, other relevant factors such as waste type, purity, stability, availability, need for pre-treatment, and storage conditions must be considered in the cost–benefit analysis, in addition to possible further downstream processing (i.e., purification) to make it a suitable product for its applications (Manga et al. 2021).

Attempts need to be made to further research progress concerning three main points: an increase in production yields through genetic engineering toward the more efficient use of renewable substrates and the robustness against culture medium inhibitors (Dierickx et al. 2022), an increase in production scale, and in purification techniques, which represent the highest cost in the process (~60%) (Zanotto et al. 2019).

A lack of studies regarding environmental life cycle assessment (LCA) of BS production from waste is another upcoming approach, once the industrial sector has

been progressively moving toward biotechnology and the circular bioeconomy, where this requires the mitigation of issues such as climate change, resource depletion, and environmental degradation (Paraszkiewicz et al. 2018; Wongsirichot et al. 2021; Hu et al. 2021a, b).

3 Solid-State Fermentation as a Sustainable Technology for Biosurfactant Production

In recent years, the increasing concern about the development of sustainable processes and green products motivated the interest in BS (BS) production. BS are widely known as surface-active compounds which can be used for several applications. Microorganisms that are known as BS producers can be also used in microbial-enhanced oil recovery (MEOR) (Borah and Yadav 2016; Marchant and Banat 2012) as well as in a synergic oily wastewater biosorption–biodegradation process (Wang et al. 2015). Despite the wide range of applications and the various advantages, the low yields and production costs associated with BS, especially regarding the downstream processes, are still the main technological limitation for industrial exploitation (Banat et al. 2021). To overcome the economic issues of BS production, the development of bioprocess has been switched to the utilization of low-cost substrates such as food and agro-industrial waste. These residues, usually poorly explored, are organic biomaterials that can be used as renewable substrates in bioprocess and are primarily composed of carbohydrates and other micronutrients. The bioconversion of agro-industrial wastes into valuable products reduces both bioprocess costs and the disposal of these residues in landfills (Gaur et al. 2022a, b).

It is widely known that BS can be produced by both submerged fermentation (SmF) and solid-state fermentation (SSF). SmF is a very known technique applied to produce several biomolecules by different microorganisms like enzymes, antibiotics, and other metabolites with biologic activity. In this case, the culture media is mainly composed of water-soluble carbon and nitrogen sources, and it is possible to have more precise control over the process parameters (e.g., pH and temperature). However, the production of BS in submerged conditions poses problems associated with severe foaming and the low yields usually obtained (Thomas et al. 2013). On the other hand, the SSF technique is known to offer many advantages over SmF like low energy consumption and higher yield. SSF is defined as a bioprocess carried out on a solid substrate in absence of free water and it is considered an important sustainable processing approach for bioconversion of agro-industrial wastes into high-value add products (Yafetto 2022). The water content of the substrate has hence to be kept low, creating a porous medium, which allows the microbial growth under the solid particles. The air-filled space in the solid medium also maintains the oxygen supply and the heat and mass transfer, crucial for microbial activity. The solid matrix could be either the source of carbon and nitrogen and other micronutrients, or it could be an inert material, just supporting the growth of the microorganisms on it, impregnated with the substrate solution (Thomas et al. 2013). Despite the several advantages mentioned, the downstream process from the crude fermented products is a key factor in SSF.

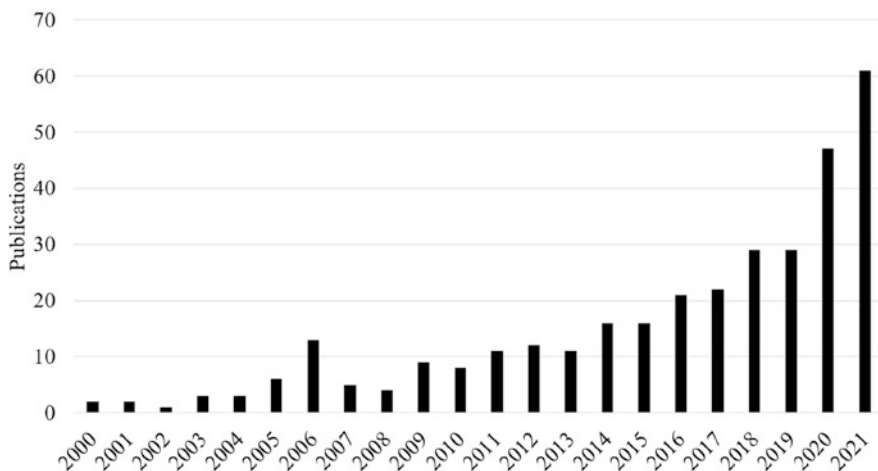


Fig. 2 Total number of publications per year found on the Science Direct website using both “solid-state fermentation” and “biosurfactant” keywords

SSF is considered to be a sustainable technology for converting agro-industrial wastes and by-products into biomolecules with industrial application, and it has received significant attention in recent years, especially due to its advantages in terms of solid waste management. In addition, the lowered water content within the SSF process supports a greener manufacturing activity with lower wastewater levels and water consumption (Chilakamarry et al. 2022). In the case of BS production, the use of SSF has been raised in recent years (Fig. 2), mainly due to the investigation of the use of alternative low-cost substrates to reduce production costs. Despite the production of BS is mainly related to bacteria, the development of SSF process has attracted attention to the potential production of BS by filamentous fungi. Filamentous fungi are well known for their ability to grow at lower water activity on different solid surfaces and to promote the deterioration of a wide variety of materials, such as fuels, cellulose, agro-industrial waste, and industrial effluents (Banat et al. 2021). The use of fungi for BS production is still incipient and few researchers have attracted their attention to these microorganisms. It is known that different fungi can synthesize secondary metabolites with excellent surface-active properties (Colla et al. 2010; Sajna et al. 2015; Velioglu et al. 2015), however, the identification of the genes responsible for the production of fungal BS is still unknown (Das et al. 2008).

3.1 The Use of Food and Agro-industrial Wastes for Biosurfactant Production by SSF

Food and agro-industrial activities produce a large amount of waste generally disposed of in landfills or used to obtain energy, contributing to environmental

pollution. It is estimated that 1.3 billion tons of food and agriculture are wasted per year (Dahiya et al. 2018) which is poorly explored in the recycling and reuse process. In this context, to circumvent the drawbacks and contribute to the circular economy, the valorization of several by-products and wastes, such as pomaces, seeds, and peels, as substrates to produce bioactive compounds, including BS, has been increased (Greses et al. 2020).

Several studies had be conducted to evaluate the influence of carbon sources on the production of BS, and most of them pointed out that BS biosynthesis can occur through two different pathways. When it is used water-soluble substrates, like carbohydrates, the carbon flow is regulated in such a way that both hydrophobic (lipogenic pathway) and hydrophilic (glycolytic pathway) portions formation are specially supplied by microbial metabolism. On the other hand, the use of the hydrophobic substrate, such as lipids, induces microbial metabolism through several mechanisms, oxidizing the fatty acids by β -oxidation to acetyl-CoA, related to the synthesis of the hydrophilic portions of the BS (Fontes et al. 2008; Santos et al. 2016). Thus, the selection of the carbon source has an important role in BS production. Besides, the use of low-cost substrate is an interesting alternative, since the cost related to the raw material represents 10–30% of the total cost of production (Mulligan et al. 2014).

As mentioned before, SSF is suggested as a potential waste recycling method using solid wastes as substrates with microorganisms to convert them into BS in a sustainable approach (Chilakamarry et al. 2022). The production of BS by SSF using a low-cost substrate has been studied, and oily residues are already beginning to be recognized as potentially recyclable and can be investigated as substrates in the most diverse biotechnological processes to produce BS (see Table 3). The use of oily

Table 3 Biosurfactant production from alternative hydrophobic substrates by solid-state fermentation

Microorganism	Substrate	BS	BS concentration	References
<i>Bacillus subtilis</i>	Olive leaf residue and olive cake	Lipopeptide	30.67 mg/g of dry substrate	Zouari et al. (2014)
<i>Bacillus pumilus</i>	Okara and sugar-cane bagasse	Surfactin	3.3 g/kg of dry substrate	Slivinski et al. (2012)
<i>Pleurotus djamor</i>	Sunflower seed shell	n.d. ^a	10.205 \pm 0.5 g/L	Velioglu and Urek (2015)
<i>Serratia rubidaea</i>	<i>Madhuca indica</i> oil cake	Rhamnolipid	n.d. ^a	Nalini and Parthasarathi (2014)
<i>Trametes versicolor</i>	Two-phase olive mill waste	n.d. ^a	373.6 \pm 19.4 mg in 100 g of culture medium	Lourenço et al. (2017)
<i>Pleurotus ostreatus</i>	Oil cakes of coconut and sesame	n.d. ^a	3.85 mg/g of biomass	Kulkarni et al. (2020)

^a n.d. = not determined

residues is quite relevant due to their high contaminating capacity. It is estimated that each liter of oil can pollute about one million liters of water (Ferreira and Fernandes 2011). Moreover, every day large amounts of frying residual oils from homes, industries, and commercial establishments are improperly discarded, ending up in sewage systems, causing disturbances in the sanitation network and pollution of water resources. In water bodies, these compounds remain on the water surface, compromising the photosynthetic function of plants and the base of the food chain (Jamaly et al. 2015). It evidences the importance of developing a suitable and sustainable process to use food and agro-industrial wastes, avoiding their disposal.

Das and Mukherjee (2007) studied the production of lipopeptide by *B. subtilis* in SSF using potato peels as the main carbon source. The production of BS was observed post 48 h, and thereafter production remained almost constant up to 96 h. SSF is governed by many factors, each of which is critical for the technical and economic feasibility of the process development. According to the results, the initial moisture content of the solid substrate is a crucial factor for SSF process. Therefore, it is recommended to optimize the moisture content that controls the water activity (a_w) of the fermenting substrate to achieve a maximum BS yield. In addition, the authors highlighted the less effort required for downstream processing, due to less requirement of water.

Jiménez-Peñalver et al. (2018) approach the production of sophorolipids by *Starmerella bombicola* using stearic acid derivate from the low-cost substrate in SSF. The choice of SSF was mainly due to the chemical properties of stearic acid, a low-cost carbon source that is difficult to work within submerged fermentation since it remains a solid due to its high melting temperature. The process was monitored for 16 days, obtaining the maximum yield on day 13 (0.211 g/g). Also, it was observed a gradual consumption of the substrate up to 30%. This result reinforces the use of hydrophobic substrates on SSF for BS production.

The production of lipopeptide from *B. cereus* in SSF was studied by Nalini et al. (2016). The authors explored different oily residues (coconut oil cake, gingelly oil cake, castor oilcake, palm oil cake, sunflower oil cake, and peanut oil cake) as substrates. All low-cost substrates were suitable for lipopeptide production in SSF, obtaining better results using peanut oil cake. In addition, the authors concluded that the substrate concentration plays an important role in the production of BS in SSF, being also important to consider the cost, availability, and therefore the selection of an appropriate solid substrate in the development of efficient and sustainable processes.

Zhu et al. (2013) produced surfactin by *Bacillus amyloliquefaciens* in SSF using rice straw and soybean flour as substrate. The authors studied the effect of additional carbon sources (e.g., glucose, sucrose, glycerol, and maltose) and nitrogen sources (e.g., tryptone, peptone, yeast extraction, and urea). The results indicate that no significant changes in surfactin production with the addition of various nitrogen sources, while the addition of 2.0% (w/w) of maltose and glycerol increased surfactin concentration. These results indicated the great potential of the use of agro-industrial wastes for biosynthesis in SSF, with no requirement of supplement using inorganic or synthetic sources of macronutrients and micronutrients. Using

these residues as a sole substrate for BS production in SSF would significantly reduce the cost of fermentation and prevent a potential environmental problem that mainly comes from improper disposal in the field.

3.2 Challenges and Perspectives

The production of BS is relatively low in the global market, primarily due to their high feedstock and processing cost, especially regarding the downstream processes, especially in SSF processes (Banat et al. 2021; Gaur et al. 2022a, b). In addition, biosynthesis is governed by many factors, each of which is critical for the technical and economic feasibility of the process development. These included the selection of microorganisms and substrate, optimum process parameters, and purification of the desired products (Thomas et al. 2013).

The use of crude BS extracts can be a viable solution to overcome the limitations in the downstream process, especially if the application is in an environmental context. BS, in these cases, do not need to present levels of purity and can be synthesized using a mixture of low-cost carbon sources, which would allow the creation of an economically viable technology for bioremediation processes (Marchant and Banat 2012).

4 Genetically Enhanced and Hyper-Producing Recombinant Strains

Some microorganisms are capable of synthesizing BS while degrading hydrocarbons and other carbon sources. This behavior may be ascribed to the induction of specific genes or enzymes activated in the presence of hydrocarbon compounds (Jimoh and Lin 2019a; Vieira et al. 2021). Since BS yield is affected by the genetics of the producing microorganism, the use of genetic engineering in these microorganisms is another alternative to promote higher yields and increase cost-effectiveness besides the modification of the fermentative parameters (Manga et al. 2021; Vieira et al. 2021).

Genetic engineering can be defined as the modification of microbial genetic materials aiming to obtain new or improved product capabilities of biotechnological and environmental importance (Jimoh et al. 2021). Usually, genetic engineering approaches used in BS production include substitution, replacement, and modifications of amino acids, in silico computation for the discovery of novel metabolic pathways, bioprospecting using high throughput screening, genome mining, metagenomic screening, gene/gene cluster knock-out, overexpression of extracellular peptides, recombinant DNA technology, and mutagenesis (Manga et al. 2021; Jimoh et al. 2021).

Genetically modified BS-producers need to present the capability to produce effective congeners, which are a combination of closely associated bioproducts,

Table 4 Microorganisms submitted to different genetic modification techniques for biosurfactant production

Microorganisms	Strategy	BS	References
<i>Acinetobacter calcoaceticus</i> A2 ^a	Mutagenesis	Biodispersan	Calvo et al. (2009)
<i>Bacillus subtilis</i> ^a	Metabolically engineered (to improve the synthesis of BS)	Surfactin	Wu et al. (2019a, b)
	Substitution of the native promoter		Jung et al. (2012)
	Overexpression of specific extracellular signaling peptides	Lipopeptide	Willenbacher et al. (2016)
<i>B. subtilis</i> SK320 ^a , <i>Paenibacillus</i> sp. D9 ^a	Cloning of biosurfactant genes	Lipopeptide	Jimoh and Lin (2019b), Sekhon et al. (2011)
<i>Bacillus licheniformis</i> ^a	Replacement of native promoter of BS synthesis operon	Lichenysin	Qiu et al. (2014)
	Heterologous expression of surfactin synthetase genes	Surfactin	Anburajan et al. (2015)
<i>Rhodococcus erythropolis</i> SB-1A ^a	Random mutagenesis with ultraviolet radiation	Glycolipids and lipopeptides	Cai et al. (2016)
<i>Paenibacillus</i> sp. ^a	Cloning of BS genes to produce hyper-producing recombinant strain	Lipopeptide	Jimoh and Lin (2019a)
<i>Pseudozyma hubeiensis</i> ^b	Mutagenesis	Mannosylerythritol lipid (MEL-D)	Konishi and Makino (2018)

^a Bacteria; ^b Yeast

Adapted from: Jimoh and Lin (2019a), and Vieira et al. (2021)

the ability of enhanced BS production yields, and, in some cases, resistance to extreme process conditions. Although several hyper-BS producers have been reported in the literature, industrial and biotechnological applications of hyper-producing recombinant and genetically enhanced strains have not been suitably established yet (Manga et al. 2021; Jimoh and Lin 2019a). Several studies have been done aiming to overcome this bottleneck. Some of the strategies used to genetically modify the BS-producers' microorganisms are presented in Table 4.

The use of *in silico* methods and the wealth of information on metabolic processes are constantly increasing, helping to improve the development of BS-producers' strains. Moreover, the process of genetic engineering has been less time-consuming and more efficient owing to the identification of possible targets for overexpression through the analysis of whole genomes and transcriptional profiles (Manga et al. 2021). Table 5 brings some information about the functional characterization of different BS biosynthetic genes in microorganisms.

Table 5 Functional characterization of different biosurfactant biosynthetic genes in microorganisms

Type of BS biosynthetic genes	Description and function
Alasan synthetase genes	The alasan of <i>Acinetobacter radioresistens</i> KA53 is composed of covalently bound alanine (apoalasan) and complex anionic polysaccharide (containing AlnA, AlnB, and AlnC proteins). AlnA protein has a similar amino acid sequence to the recombinant protein <i>Escherichia coli</i> OmpA. AlnB amino acid sequence is homologous to peroxiredoxins.
Arthrofactin synthetase gene cluster	The arthrofactin synthetase gene cluster modular architecture obeys the collinearity rule. The three genes of arthrofactin operon (<i>arfA</i> , <i>arfB</i> , and <i>arfC</i>) are responsible for encoding ArfA, ArfB, and ArfC with two, four, and five functional modules representing cyclic lipoundecapeptide BS.
Emulsan synthetase genes	It was confirmed in the emulsan synthetase cluster that the biosynthesis of emulsan by <i>Acinetobacter lwoffii</i> RAG-1 demands five different emulsan synthetase genes (<i>wza</i> , <i>wzb</i> , <i>wzc</i> , <i>wzx</i> , and <i>wzy</i>).
Iturin synthetase genes	The iturin synthetase operon is significantly comprised of four open reading frames (<i>ituD</i> , <i>ituA</i> , <i>ituB</i> , and <i>ituC</i>). The <i>ituD</i> gene is responsible for encoding the putative malonyl coenzyme A transacylase, however, the disruption of this coenzyme causes a specific deficiency in iturin A production. Three functional areas homologous to amino acid adenylation, aminotransferase, and β -ketoacyl synthetase are present in the <i>ituA</i> gene. The peptide cyclization and two adenylation domains are supported by peptide synthetase, which is encoded <i>ituC</i> and <i>ituB</i> genes.
Lichenysin synthetase operon	The synthesis of lichenysin occurs through the action of non-ribosomal peptide synthetases (NRPS) group, also known as multimodular peptide synthetases.
Non-ribosomal peptide synthetase	NRPS enzymes are responsible for the assembly of the non-ribosomal peptides. Thus, the sequential selection, activation, and condensation of precursor amino acids, α -hydroxy acids, fatty acids, α -keto acids, as well as polyketide-derived units depend on modules present in the non-ribosomal peptides.
Rhamnosyl-synthetase genes	The mono- and di-rhamnolipid biosynthesis are accomplished by three main enzymatic reactions with β -oxidation playing a significant role in rhamnolipid production. Phosphomannomutase enzyme AlgC is responsible for catalyzing the conversion of typical D-glucose molecule to D-glucose-1-phosphate. The process then follows the RmlBCAD pathway, which involves the enzymes RmlA, RmlB, RmlC, and RmlD, converting D-glucose-1-phosphate into dTDP-L-rhamnose. Finally, the rhamnosyl-transferases RhIB and RhIC are responsible for catalyzing the synthesis of dTDP-L-rhamnose into mono- and di-rhamnolipid. When fatty acids are used as a substrate, the rhamnolipid pathway is predominant, in which RhIG enzyme functions by relaying fatty acid synthesis intermediates.
Surfactin synthetase genes	NRPS are used to promote molecular characterization and biosynthetic regulation of surfactin, including a multienzyme peptide synthase complex comprised of four enzymatic subunits SrfA, SrfB, SrfC, and SrfD. These enzymes are responsible for the conversion of

(continued)

Table 5 (continued)

Type of BS biosynthetic genes	Description and function
	the substrate into surfactin. Moreover, the surfactin synthetase activation is accomplished by the phosphopantetheinyl transferase, which is encoded by the <i>sfp</i> gene (<i>SrfA</i> operon). Finally, the <i>srf</i> operon encodes surfactin synthetases needed for surfactin biosynthesis.

Adapted from: Jimoh et al. (2021)

One of the main concerns about using engineered organisms is their high regulation, however, that will not be a deterrent since the live microorganisms are usually eliminated during the downstream purification steps of BS production. Genetic engineering strategies are always advancing, and the current researchers are centered on diminishing the industrial difficulties through the development of strains that will produce high BS yields at minimal costs. Thus, these strategies offer enormous opportunities for making enhanced BS production a success story (Jimoh and Lin 2019a; Manga et al. 2021).

5 Biosurfactant Co-production

Co-production process can be defined as the simultaneous production of more than one product. Thus, one of the major advantages of this type of process is to obtain several products using the same resources and in less time (Manga et al. 2021; Vieira et al. 2021).

Although microorganisms typically produce several metabolites during fermentation processes, the industrial focus is usually centered on just one of them. This can end up being a waste of high-value-added products. Since the same thing occurs during BS production, the development of processes capable to produce BS jointly with other economically beneficial products such as lipases or pectinases can be a promising tendency (Manga et al. 2021; Singh et al. 2019).

According to Kumar and Kim (2018), and Manga and collaborators (2021), other metabolites that can be co-produced simultaneously with biosurfactants include pigments and carotenoids, amino acids and derivatives, hydrogen, alcohols, organic acids, ectoines, and bioelectricity. Some examples of studies concerning the co-production of BS and other metabolites are presented in Table 6.

Table 6 Co-production of biosurfactant concomitantly with other products

Type of product	BS	Microorganism	References
Alkaline amylase	Lipopeptides	<i>B. methylotrophicus</i> ^a	Hmidet et al. (2019)
Bacteriocin-like inhibitory substances	Glycolipopeptide	<i>Lactococcus lactis</i> ^a	Vera et al. (2018)
Keratinolytic protease and amylase	Undetermined	<i>B. subtilis</i> PFI ^a	Bhange et al. (2016)
Lipase	Undetermined	<i>Aspergillus niger</i> ^b	Sperb et al. (2018)
Pectinase	Undetermined	<i>B. subtilis</i> ^a	
Polyhydroxyalkanoates (PHA)	Rhamnolipid	<i>Enterobacter aerogenes</i> ^a	Arumugam and Furhana Shereen (2020)
		<i>Burkholderia thailandensis</i> ^a	Kourmentza et al. (2018)
Triacylglycerols	Rhamnolipid	<i>Rhodotorula babjevae</i> ^c	Guerfali et al. (2019)
2,3-butanediol	Surfactin	<i>B. subtilis</i> ^a	Kavuthodi et al. (2015)

^a Bacteria; ^b Filamentous fungus; ^c Yeast

Adapted from: Manga et al. (2021) and Vieira et al. (2021)

6 Final Considerations

Surfactant compounds of synthetic or biological origin are used in several products and processes in the most diverse sectors (Fig. 3), being of great importance to society. Although surfactants of biological origin are far from commercially surpassing those of synthetic origin and they are not perfect molecules, their ecologically correct characteristics make them targets of great technological and industrial interest. Thus, this chapter aimed to contribute to the necessary dissemination and understanding of the mechanisms of action, characteristics, fermentation parameters, applications, and market perspectives of BS.

The development of commercial production of biological surfactants and their applications must necessarily be based on the three pillars of sustainability so that we can build a future based on added values of social and environmental responsibilities. Figure 4 summarizes the different renewable feedstocks used, benefits, strategies of improvement, applications, and drawbacks of BS.

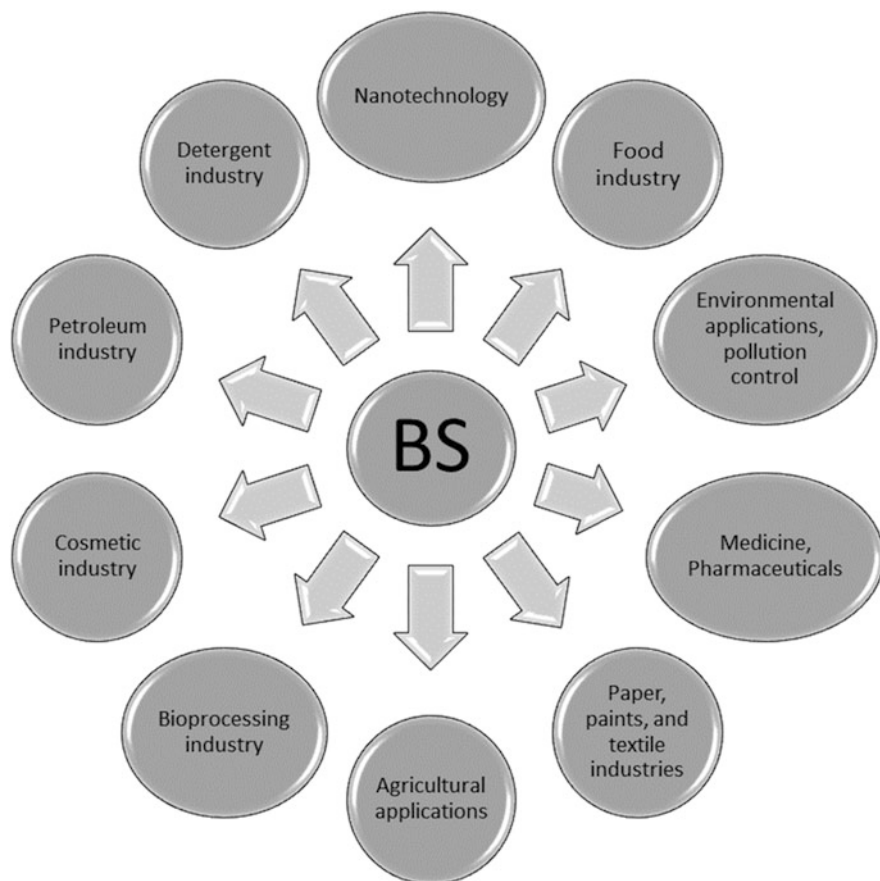


Fig. 3 The summary of diverse applications of biosurfactant. Adapted from: Jimoh and Lin (2019a)

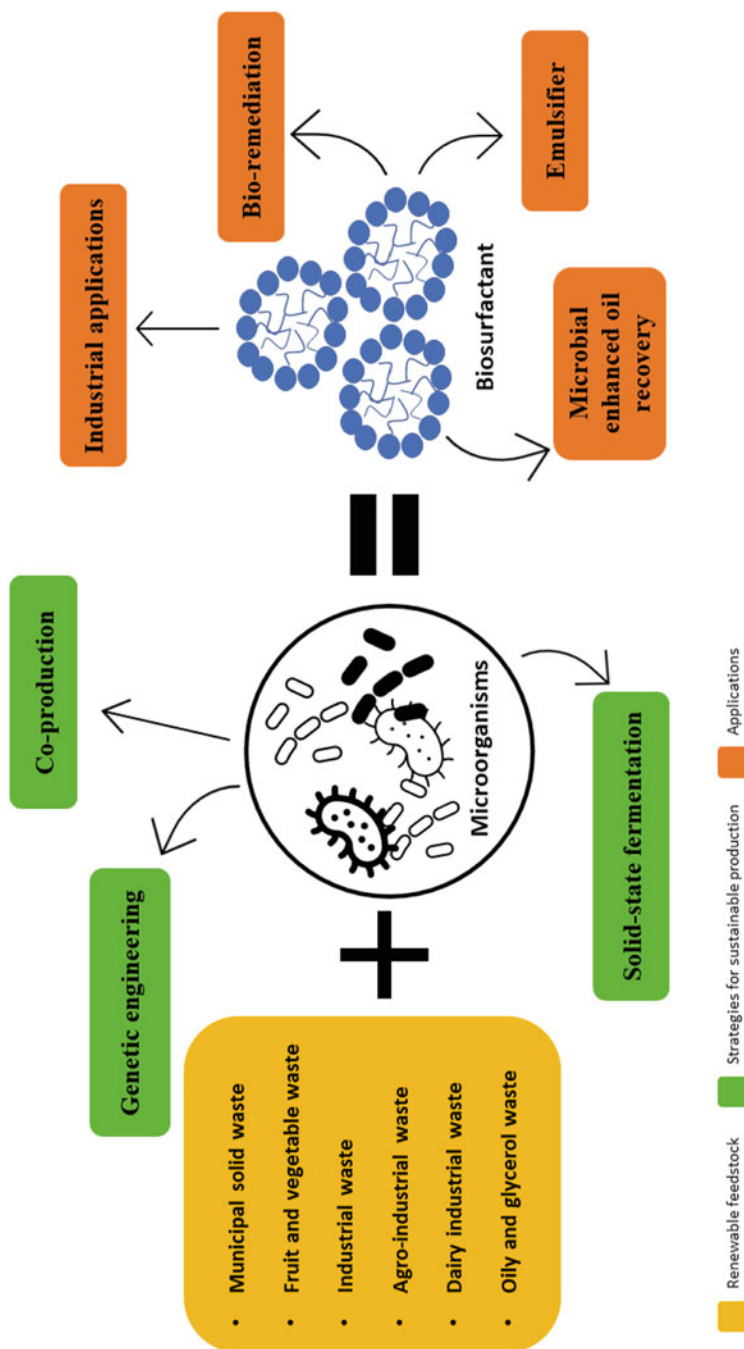


Fig. 4 Biosurfactants: renewable feedstocks, strategies for sustainable production, and applications. Adapted from: Mohanty et al. (2021)

Acknowledgements Danielle Maass is thankful to São Paulo Research Foundation (FAPESP) for the research grant #2019/07659-4.

Declaration of competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this chapter.

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Sustainable Production of Biosurfactants Using Waste Substrates



Catherine N. Mulligan

1 Introduction

Pollutants can be released into the air, water, and soil environment and then transported, transformed, or accumulated in organisms or in the environment (Yong et al. 2014). Some sources of contaminants include petroleum hydrocarbons, dry cleaning solvents, metals, wastewater treatment plant biosolids, wastewater, and industrial wastes. Contamination of soil is due to accidental spills, leaks, cleaning of equipment, inadequate storage of wastes, and improperly managed landfills.

In particular, various techniques must be considered for the remediation of soils and sediments. These remediation techniques can include natural attenuation or biological, chemical, and/or physical based options (Yong et al. 2014). Sustainable management options for contaminated soils are required (Mulligan 2019). Development of cost-effective solutions that require less resources is a specific objective for achieving sustainable remediation.

Some guidelines for site remediation exist to reduce environmental impacts (ASTM 2013, 2016). An ASTM guide includes environmental, social, and economic aspects (ASTM E2876-13). Reduction in the amounts of materials used, wastes generated, and water impact are key elements of best management practices. Figure 1 shows the process of sustainable remediation.

Biosurfactants have shown potential for environmental applications (Mulligan 2014). Production of biosurfactants via renewable or waste substrates can enhance the sustainability of the production process and lower costs to compete with synthetic surfactants. Therefore, the objectives of this chapter are to review and evaluate the use of waste materials and to identify future research directions.

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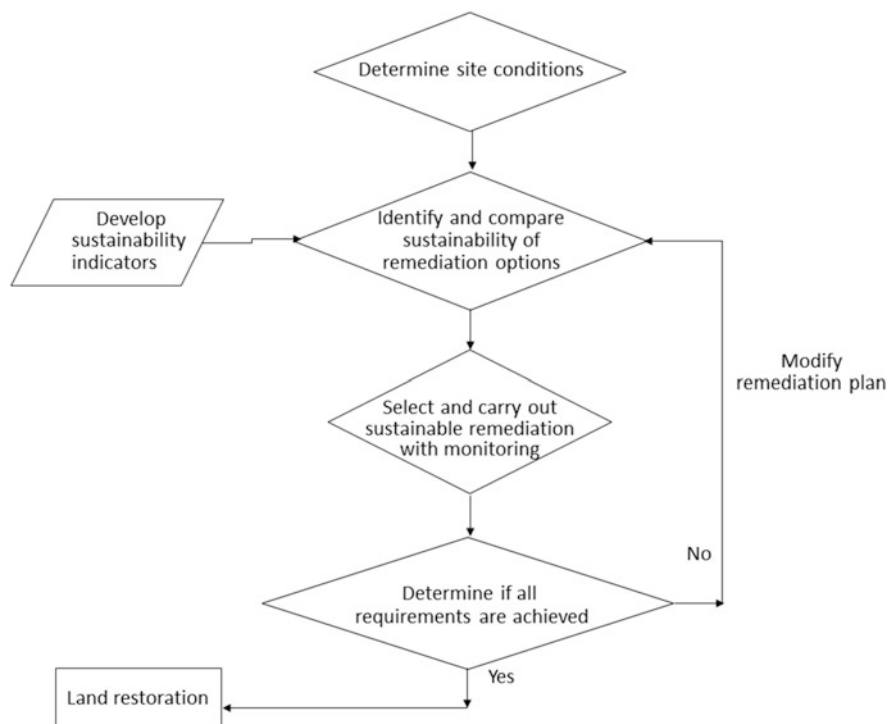


Fig. 1 Steps in a sustainable remediation process (adapted from Mulligan 2019)

2 Biosurfactant Production from Wastes

Some of the major classifications of biosurfactants from bacteria or yeast are rhamnolipids, sophorolipids, and lipopeptides (Biermann et al. 1987). Anionic or neutral biosurfactant compounds are most common. Critical micelle concentrations (CMCs) of these compounds typically vary between 1 and 200 mg/L and from 500 to 1500 daltons in molecular mass (Lang and Wagner 1987).

Biosurfactants can be produced from soluble carbohydrates, or hydrophobic, insoluble substrates. Compared to the highly employed synthetic surfactants, biosurfactants can potentially have some distinct advantages. Among these are biodegradability, biocompatibility, and high surface activity (Cooper 1986). To enhance sustainability, waste materials have been evaluated for reduction of costs and disposal requirements. Some of these include glycerol or sugar molasses, and various residues from fruit or vegetables, coffee, tea, dairy, or cooking oil wastes (Mulligan et al. 2014). In this chapter, three of the most well-known biosurfactants (rhamnolipids, sophorolipids, and surfactin) will be examined.

2.1 Rhamnolipids

Rhamnolipids produced by *Pseudomonas aeruginosa* have been studied extensively (Guerra-Santos et al. 1984). Various homologues consisting of rhamnose(s) and fatty acids (Abdel-Mawgoud et al. 2010) with surface tensions of 29 mN/m have been produced. Substrate type, nutrient and media composition, fermentor configuration, oxygen level, pH, and temperature used affect the composition and yields (Mulligan and Gibbs 1993).

A variety of soluble sugars, hydrocarbons, and vegetable oils have been studied as substrates (Liu et al. 2017). Recently, lipid-rich wastes such as coconut oil cake, rice husk, and used cooking oil were evaluated as biosurfactant substrate by Suryawanshi et al. (2021). *P. aeruginosa* was found to be an effective biosurfactant producer from coconut oil cake with the highest yield of 19 mg/ml. Biosurfactant extraction was also optimized. It was determined that maximum yields were obtained using chloroform and methanol (2:1).

Paneer whey waste was employed by Patowary et al. (2016) to produce biosurfactant by a strain of *Pseudomonas aeruginosa* isolated from contaminated soil. Concentrations of 2.7 g/L were obtained from the waste alone. The rhamnolipids showed emulsification ability against various hydrocarbons, a CMC of 110 mg/L, surface tension of 26.5 mN/m, and low toxicity against a mouse fibroblastic cell line. Partovi et al. (2013) showed 18 g/L concentration of rhamnolipid from soybean oil waste. *P. aeruginosa* PG1 has been shown to produce rhamnolipid from bakery waste (an excellent source of starch, carbohydrates, proteins, and lipids) (Patowary et al. 2019). A production rate of 11.6 g/L-day of a crude biosurfactant was obtained (surface tension of 25.8 mN/m at a CMC of 100 mg/L). No cytotoxicity against the mouse cell line of L292 fibroblasts was found.

Jimoh and Lin (2020) showed that biosurfactant yields by *Paenibacillus sp.* D9 could be doubled from 2.11 to 5.31 g/L using waste frying oils. Another study by Sharma et al. (2022) showed that a concentration of 16 g/L of biosurfactant at a rate of 5.7 g/L-h was produced from waste cooking oil (WCO) via a fed-batch feeding strategy. Biodegradation of 94% of the WCO was achieved. Bioremediation of motor and diesel oils was demonstrated. Kitchen waste oil yielded up to 6 g/L of biosurfactant by *P. aeruginosa* (Chen et al. 2019). The biosurfactant could be applied for recovery of up to 70% of the oil from drilling cuttings.

Fifty L bioreactors were used to produce biosurfactant by *P. cepacia* from an industrial waste (Soares da Silva et al. 2019). Levels of 40.5 g/L of the biosurfactant were reached. The surface tension was lowered to 29 mN/m. The cost was estimated at \$0.02/g of the biosurfactant that was applicable for treating up to 100% of the effluents containing oil in a thermoelectric plant. Sari et al. (2019) showed that ozonation of a biodiesel waste was required to produce biosurfactant by *P. aeruginosa*.

Oil mill waste (OMW, 25% v/v) with molasses (10% w/v) and corn steep liquor (10% v/v) enabled up to 5 g/L of rhamnolipid to be produced by *P. aeruginosa* #112 with a very low CMC of 13 mg/L. This is the first report of all three wastes together.

The study was of particular interest as OMW is a hazardous waste and thus production of biosurfactants from this waste helps to solve an environmental issue (Gudiña et al. 2016).

The bacterium *Planomicrobium okeanokoites* IITR52 produced rhamnolipid from corncob and pineapple waste at levels of 568 mg/L and 304 mg/L, respectively (Gaur et al. 2022). The biosurfactant could increase petroleum hydrocarbon solubility by a factor of 2.34-fold, showed bactericidal potential, and was thermal stable and halo-tolerant.

A life cycle assessment (LCA) was performed on glycolipid biosurfactant production on waste oils (Kopsahelis et al. 2018). Rhamnolipid and sophorolipid production were compared. The environmental impact of rhamnolipids was 22.7% lower than sophorolipids due to lower energy requirements. The highest impact was due to the fermentation process. Further analysis of this type could be used to reduce environmental impacts as indicated by Marchant (2019) to reduce energy and other requirements during the entire fermentation process.

2.2 Lipopeptides

Bacillus subtilis produces surfactin, a lipopeptide composed of seven amino acids with a β -hydroxy fatty acid in a lactone ring (Kakinuma et al. 1969). Surface tensions can be reduced to 27 mN/m at surfactin concentrations of 0.005%. Bonmatin et al. (1995) discovered the three-dimensional structure. Mass spectrometry of a mixture of surfactins showed that the length of the acyl chain was between 12 and 15 carbons (Hue et al. 2001). Yields of surfactin have been low. Various food wastes have been used as substrates as shown in Table 1. For example, an agro-industrial waste, potato peel powder, was studied by Das and Kumar (2018) as a substrate for lipopeptide production by an indigenous *Bacillus licheniformis* strain for remediation of a petroleum-contaminated soil.

Felix et al. (2019) examined another food substrate, cashew apple juice, for lipopeptide production. The biosurfactant has a CMC of 12.5 mg/L and could reduce the water surface tension to 31.8 mN/m. Under various environmental conditions, the biosurfactant was stable and effective for oil remediation.

The head and liver wastes from cod were hydrolyzed enzymatically (Zhu et al. 2020). The biosurfactant produced has a surface tension of 27.9 mN/m with a CMC of 180 mg/L. Up to 55 critical micelle dilutions (CMD) of the lipopeptides could be produced from the waste. The biosurfactant was capable of effectively dispersing Alaska North Slope oil in a 80/20 ratio with dioctyl sulfosuccinate sodium (DOSS), a component of the dispersant Corexit 9500. The costs of the substrates are typically 10 to 30% of the total costs of production according to Kosaric and Sukan (2014). Zhu et al. (2020) thus estimated that a crude form of the biosurfactant could cost about \$277/kg which is similar to the synthetic DOSS. Higher levels of surfactin (Hu et al. 2021) were produced by *B. subtilis* from enzyme hydrolysis of fish waste (274 mg/L). A 100 L pilot scale fermentation was performed.

Table 1 Biosurfactant production from various renewable and waste substrates and species (adapted from Rivera et al. 2019)

Biosurfactant	Substrate	Species	Maximum concentration (g/L)	Reference
Rhamnolipid	Vegetable oil waste	<i>P. aeruginosa</i> LBI	11.7	Nitschke et al. (2005)
		<i>P. aeruginosa</i> AB 4	40	Hazra et al. (2011)
		<i>P. aeruginosa</i> 47 T4	2.7	Haba et al. (2000)
	Frying oil waste	<i>P. aeruginosa</i> zju. ul M	20	Zhu et al. (2007)
		<i>P. aeruginosa</i> DG30	15.6	Zheng et al. (2011)
		<i>B. thailandensis</i> E264	2.2	Kourmentza et al. (2018)
		<i>P. aeruginosa</i> SWP-4	13.93	Lan et al. (2015)
	Cashew apple juice	<i>P. aeruginosa</i> ATCC 10145	3.8	Da Rocha et al. (2007)
	Bagasse and potato peels	<i>P. azotoformans</i> AJ15	1.16	Das and Kumar (2018)
	Orange peel	<i>P. aeruginosa</i> MTCC 2297	9.2	George and Jayachandran (2009)
Animal fat/waste	<i>P. aeruginosa</i> 101045	3.84	Da Silva Borges et al. (2012)	
Sugar cane molasses, corn steep liquor, and oil mill wastewater	<i>P. aeruginosa</i>	5.1	Gudiña et al. (2016)	
Lipopeptides	Vegetable oil waste	<i>B. subtilis</i> K1	0.011	Jajor et al. (2016)
	Frying oil waste	<i>B. stratosphericus</i> FLU5	0.05	Hentati et al. (2019)
	Palm oil effluent and crude glycerol	<i>B. subtilis</i> TD4	1.18	Louhasakul et al. (2020)
	Tuna fish cooking residue and sesame peel flour	<i>B. subtilis</i> SPB1	4.5	Mnif et al. (2013)
	Cashew apple juice	<i>B. subtilis</i> LAM1005	0.319	de Oliveira et al. (2013)
	Cashew apple juice	<i>B. subtilis</i>	0.123	Giro et al. (2009)
	Molasses	<i>B. subtilis</i> RSL-2	12.34	Verma et al. (2020)
	Banana peel	<i>H. archaeon</i> AS65	5.3	Chooklin et al. (2014)

(continued)

Table 1 (continued)

Biosurfactant	Substrate	Species	Maximum concentration (g/L)	Reference
	Orange peel	<i>B. licheniformis</i> KC710973	1.8	Kumar et al. (2016)
	Potato processing effluent	<i>B. subtilis</i> 21,332	0.9	Noah et al. (2005)
	Potato peel powder	<i>Klebsiella</i> sp RJ-03	15.4	Jain et al. (2013)
	Cassava flour	<i>B. subtilis</i> LB5a	5.0	Nitschke and Pastore (2006)
	Hydrolyzed olive oil mill	<i>B. subtilis</i> N1	5.1–13.7 g/L	Ramírez et al. (2016)
Sophorolipids	Vegetable oil waste	<i>S. bombicola</i> MTCC1910	51.5	Jadhav et al. (2019)
	Frying oil waste	<i>C. bombicola</i>	34	Shah et al. (2007)
	Lignocellulosic hydrolysates	<i>C. bombicola</i> ATCC 22214	120	Deshpande and Daniels (1995)
		<i>C. bombicola</i> ATCC 22214	3.6–84.6	Samad et al. (2015)
		<i>C. bombicola</i> ATCC 22214	52.1	Samad et al. (2017)
		<i>S. bombicola</i> NBRC 10243	49.2	Konishi et al. (2015)
		<i>C. bombicola</i> ATCC 22214	49.2	Minucelli et al. (2017)
	Soy molasses	<i>C. bombicola</i>	55	Solaiman et al. (2007)
	Soybean and sunflower oil	<i>C. bombicola</i>	41.2	Rashad et al. (2014a)
	Waste motor oil and sunflower oil cake	<i>C. bombicola</i> NRRL Y-17069	26.6	Rashad et al. (2014b)
	Soybean dark oil	<i>C. bombicola</i> ATCC 22214	90	Kim et al. (2005)
	Sweet sorghum bagasse with soybean oil	<i>C. bombicola</i> ATCC 22214	84.6	Samad et al. (2015)
	Catfish fat residue	<i>C. bombicola</i>	21.8	Hoa et al. (2017)
	Corn cob hydrolysate and waste oil	<i>S. bombicola</i>	33.8	Konishi et al. (2015)

Santos et al. (2014) studied the growth of *B. subtilis* on various wastes including corn steep liquor, beet peel, and glycerin from biodiesel production. A statistical model would be used to optimize the biosurfactant production. Ostendorf et al.

(2019) used agro-industrial wastes, waste frying oil, and molasses in addition to nitrogen sources such as corn steep liquor to produce a lipopeptide biosurfactant. The biosurfactant was produced in concentrations up to 2.05 g/L with 2% molasses and 1% corn steep liquor by *B. cereus*. The toxicity against the marine bioindicator *Artemia salina* was low and potential for motor oil desorption and dispersion was exhibited. *B. subtilis* biosurfactant production from a high glucose industrial wastewater from a candy factory was evaluated by Secato et al. (2016). Surface tension was reduced by 36% and emulsification of soybean oil and motor oil was obtained.

Dos Santos et al. (2016) used a surface response methodology to optimize biosurfactant production by *B. subtilis* from glycerin, potato processing waste, corn steep liquor, and frying oil. Highest emulsification indices were obtained using 9% glycerin and 1% potato peels. Potato waste was suitable as a low-cost substrate. Grossi et al. (2018) developed a neural model with good correlations to predict the concentrations of *B. subtilis* biomass using waste substrates such as glycerol from biodiesel production and beet peel. The economics of lipopeptide production by *B. mojavensis* A21 was determined as a fungicide against *Fusarium* sp.

Brewery waste has also been evaluated for surfactant production by *B. subtilis* (Moshtagh et al. 2019). A response surface methodology was employed to optimize the production according to the variables, carbon/nitrogen ratio, agitation speed, initial pH, and temperature. The lowest surface tension was 27.3 mN/m and the CMC was 107 mg/L. At 6.22 g/L of waste, the optimal concentration of 0.66 mg/L of biosurfactant was reached. Paraszkiwicz et al. (2018) studied the production of a lipopeptide produced by *B. subtilis* from renewable substrates. Molasses derived from beets, peel extract from apples or carrots, and two types of brewery wastewater were compared. Surfactin and iturin were produced at yields dependent on the media composition.

Zhang et al. (2016) reviewed the use of cassava wastes for biorefinery purposes. Biosurfactants are one of the by-products that can be considered. Agro-industrial wastes have the appropriate balance of carbohydrates/lipids and nutrients. Cassava flour wastewater showed potential for growth of *B. subtilis* LB5a for biosurfactant production (Nitschke and Pastore 2006).

The production of the lipopeptide was reviewed by Zanotto et al. (2019). They indicated that production is still expensive and thus methods to reduce the costs must be done. Agro-industrial wastes are proposed as the substrate can represent 30–50% of the total production cost. Waste reduction can also be achieved. This type of waste also contains substantial amounts of organics, macro and micronutrients. They classified the wastes as starch-rich, vegetable oil, whey and protein substrates, and others. Cassava wastewater, in particular, seemed to be particularly advantageous due to the high nutrient content and thus yields up to 3 g/L were achieved. Another advantage is that seasonal conditions do not affect the waste composition. Other substrates such as corn steep liquor needed to be supplemented with micronutrients. Hydrolyzed olive oil mill waste yielded 13.7 g/L while okara was the most promising protein substrate, yielding 359 mg/L. Among the other wastes, rice mill residues showed the highest yield of 4.17 mg/kg residue.

2.3 Sophorolipids

A sophorolipid biosurfactant is produced by the yeast *Candida (Starmerella) bombicola* (formerly known as *Torulopsis bombicola*) (Cooper and Paddock 1984). High concentrations of this biosurfactant have been achieved from a mixture of a sugar and an oil (lactose and canola oil) (Zhou and Kosaric 1995). Sophorose lipids at concentrations of 10 mg/L reduce the water surface tension to 33 mN/m and interfacial tension of n-hexadecane and water to 5 mN/m (Cooper and Paddock 1984). The sophorolipids are stable from pH 6 to 9, at a range of salt concentrations and from 20 to 90 °C.

Few applications for sophorolipids have been reported concerning environmental remediation. One of the first studies was to remove hydrocarbons from oil sands (Cooper and Paddock 1984). The high yields of the sophorolipid are highly attractive. In addition, the crude sophorolipids have potential for treatment of metal-contaminated media (Mulligan et al. 1999, 2001; Arab and Mulligan 2018).

Wang et al. (2019) reviewed the sophorolipid production by *Starmerella bombicola* from waste streams. Volumetric productivities of 3.7 g/L-h and concentrations up to 477 g/L have been obtained, among the highest known. Waste streams, particularly from food which have high levels of nutrients, have been proposed as a more sustainable approach and waste management strategy.

Deproteinized whey concentrate (DWC), sunflower oil cake, soybean dark oil, and sweet sorghum bagasse have been evaluated as substrates. Food wastes containing carbohydrates, lipids, and proteins have excellent potential. Catfish fat residue, corn cobs, waste oils, and biomass hydrolysates have been used. Table 1 shows a summary of various substrates used and biosurfactant concentrations obtained.

Several species can be produced biosurfactants from waste cooking oils, including *P. aeruginosa*, *Bacillus sp.*, and *Streptomyces sp.* Yeasts such as *Starmerella* species usually require a hydrophilic co-substrate with hydrophobic waste oil (Liu et al. 2017). Concentrations of up to 67 g/L have been obtained from waste cooking oils up to 100 g/L. Variations were significant between species and growth conditions. Pure olive, coconut and soybean oils seemed to provide higher biosurfactant yields. Nitrogen type and levels, and reactor operation and volume can highly influence yields. Pretreatment of the waste oils with activated carbon also enhanced yields as peroxides formed during the frying process were removed.

Kaur et al. (2019) studied hydrolyzed restaurant food waste that was as a substrate for *S. bombicola*. After a 92 h fermentation, 115.2 g/L of the sophorolipid were obtained at a productivity of 1.25 g/L-h. Jadhav et al. (2019) determined that a sunflower oil waste for sophorolipid production by *S. bombicola* could yield up to 51.5 g/L. The surface tension was reduced to 35.5 mN/m.

2.4 Other Biosurfactants

Various other species have also been studied for their abilities to produce biosurfactants from waste materials. Hasananizadeh et al. (2017) were able to produce biosurfactants by *Mucor circinelloides* from waste frying oil leading to a surface tension of 26 mN/m. 87% of the crude oil was degraded and hence these biosurfactants could be feasible for remediation.

Martins and Martins (2018) studied various industrial wastes for production of biosurfactants by *Corynebacterium aquaticum* and *Corynebacterium spp.* CCT 1968. The former produced biosurfactants from fish and bagasse wastes, whereas the latter produced them only on fish wastes. The products were capable of paint removal. A weed *Parthenium hysterophorus* was subsequently used for biosurfactant production by *Pseudomonas mosselii* (Deveraj et al. 2019).

Pele et al. (2019) evaluated agro-industrial wastes (crude glycerol (3%) and corn steep liquor (5%)) for biosurfactant production by *Rhizopus arrhizus* UCP 1607. Yields of 1.74 g/L could be achieved from these wastes. The glycoprotein biosurfactant lowered surface tension to 28.8 mN/m and removed 79.4% of the diesel oil from polluted soil. For the first time, the white rot fungus *Trametes versicolor* was capable of producing a biosurfactant from two-phase olive oil mill waste (TPOMW) via a solid-state fermentation (Lourenço et al. 2018). Yields were up to 376 mg per 100 g of media from 35% TPOMW. The minimum surface tension was 34.5 mN/m.

A native marine bacteria (*Acinetobacter calcoaceticus* P1-1A) used waste cooking oil and crude glycerol for producing a biosurfactant (Moshtagh et al. 2021). Up to 862 mg/L could be produced after using a response surface method for condition optimization. The culture has potential for treating oil spills in a harsh environment of high salinity and low temperatures.

Vera et al. (2018) studied whey and vinasse as agro-industrial waste substrates for biosurfactant production by *Lactobacillus lactis* CECT 4434. Optimization was performed via a 2^4 central composite design (CCD). Economic viability was enhanced by the coproduction of a bacteriocin-like inhibitory substance. Another *Lactobacillus* species (*Lactobacillus plantarum* MGL-8) was evaluated for biosurfactant production from mango juice (consisting of water, glycerol, sucrose, and mango paste) (Sittisart and Gasaluck 2022). A concentration of up to 4.22 g/L of a glycolipoprotein biosurfactant was obtained. The surface tension was 36.6 mN/m of the biosurfactant which also exhibited bactericidal activity, indicating potential for use as a food sanitizer. Previous work (Gasaluck 2020) showed that production with a 700 L fermentor was possible and that costs for the materials during production were reduced to 0.51 US\$/g.

Biosurfactant can be produced from used vegetable oil (10 mL/L) at concentrations of up to 3.05 g/L (Liu et al. 2019). A surface tension of 25.2 mN/m was obtained. The culture *Serratia sp.* was grown in a microbial electrosynthesizing fuel cell anode chamber and was able to produce 1.13 mW/m² of energy simultaneously.

Another approach for production of biosurfactants includes extracting biosurfactants from waste materials. Vargas et al. (2014) extracted soluble bio-based organic substances (SBO) from urban and gardening wastes. The wastes were aerobically or anaerobically digested and then chemically extracted. The ability to form O/W emulsions was also evaluated. Composted biowastes showed higher performance and composition varied depending on the biowaste composition. The SBO could be an ecofriendly substrate alternative.

Tabasso et al. (2020) examined municipal biowaste (MBW) as a source of various chemicals such as polymeric biosurfactants (BPS). The products could be used to wash polluted soil. The leachate was then treated by acid addition and membrane filtration to separate the pollutants to enable solution reuse.

3 Discussion

The concept of industrial ecology is based on protection of the environment and resource conservation (Mulligan 2019). The overall process of production from wastes can be seen in Fig. 2. Sustainable production of biosurfactants and their application for remediation should be considered this way. The approach of LCA as previously mentioned can be utilized to quantify emissions and wastes throughout the production process as seen in Fig. 3 and to determine where the impacts can be minimized.

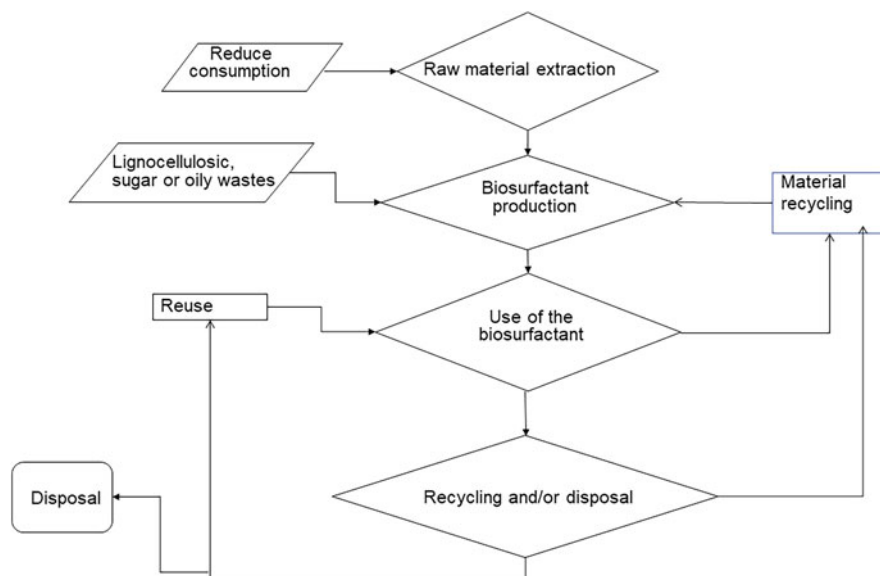


Fig. 2 Biosurfactant production used waste materials

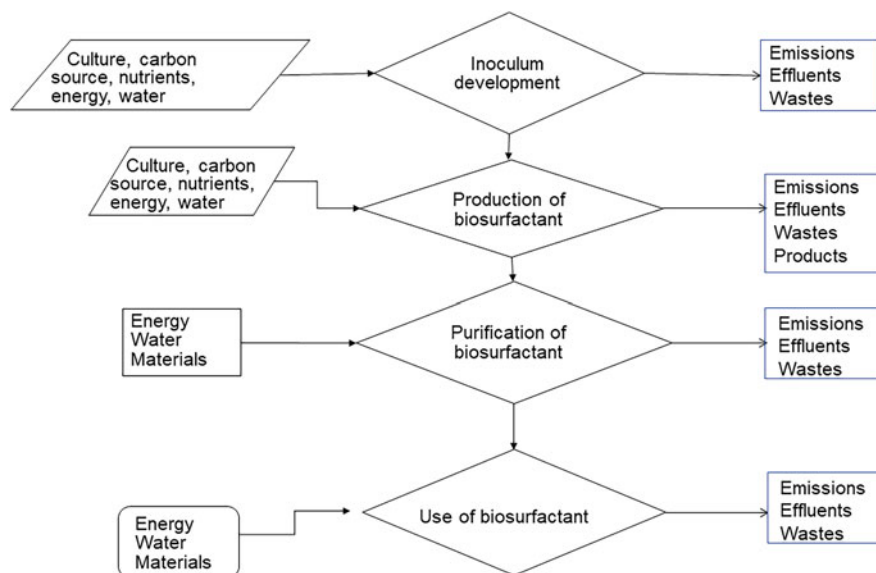


Fig. 3 Life cycle assessment of biosurfactant production process

Olasanmi and Thring (2018) reviewed the role of biosurfactants toward environmental sustainability. Potential avenues of using renewable by-products or waste materials have been identified to reduce costs and waste that would otherwise need further management. Banat et al. (2014) and Makkar and Cameotra (2002) have indicated that agro-industrial by-products, agricultural residues, and food by-products and wastes can be used as substrates for biosurfactant production. Another advantage of using wastes is that they do not compete with food (Henkel et al. 2012). High carbohydrate and lipid content wastes with other nutrients are particularly useful (Joshi et al. 2008) to obtain the desired biosurfactant production level and quality. The microbial strain, pH, and temperature are particularly important. For environmental applications, cost reduction is highly important due to the requirements for large amounts of biosurfactants. Thus, inexpensive substrates and high yields for biosurfactant production are very important. Singh et al. (2019) have predicted that the market for biosurfactants will be up to 5.5 billion US\$ by 2023. Low-cost substrates can reduce the costs by up to 30%. Saisa-Ard et al. (2013) indicated that the raw materials account for 30 to 50% of the final product cost.

Availability and the costs of the substrates are very country dependent. Jimoh and Lin (2020) indicated that corn, for example, is much less expensive where it is produced. They summarized various renewable and industrial wastes that could be used as substrates for biosurfactant. The oil industry represented the largest proportion (35%), followed by agro-industrial wastes (20%), dairy products (18%), the food industry (15%) and then industrial wastes (12%).

Waste cooking oils have been examined for biosurfactant and other products (Lopes et al. 2020). A variety of vegetable oils are used in households and

restaurants which cannot be reused as the composition changes during frying. Millions of tonnes are produced worldwide yearly including, for example, 50 million tonnes of fruit and vegetable wastes each year in India, Mexico City, and China, in addition to another 11.2 million m³ of dairy waste, 350.9 million tons of edible oil processing wastes, and up to 8 million tonnes of shellfish waste (Sharma et al. 2020). Discharges of the oils can cause many problems of odor, drain blockages, increased organic loads and foaming, and inhibition of microbial communities in wastewater treatment. Enhanced management of these wastes is highly desirable.

Rivera et al. (2019) have also reviewed the use of inexpensive agro-industrial wastes. These included those from oil-processing, starchy and sugary materials, fruit and vegetable processing, distillery waste, and animal fats. Petroleum wastes were not discussed. Glycolipids, sophorolipids, rhamnolipids, lipopeptides, manosynthritol lipids- β were included in the review. Soares da Silva et al. (2019) indicated that glycolipids could be produced up to 40.5 g/L from canola frying oil by *P. cepacia* at a cost of 20 US\$/kg. No additional substrates were required and have been estimated that the cost with the same culture and substrate would be \$0.11/L. The same authors have also indicated that the cost of production of sophorolipid (Saraya Co.) and glycolipid (Soliance) can be estimated at 2.5 to 6.3 US\$/kg from palm oil and rapeseed, respectively. As surfactin is produced at a high purity (98%), production costs are very high (13.94 US\$/mg, Sigma-Aldrich). Freitas et al. (2016) determined that sophorolipids could be produced at 0.1 to 0.22 US\$/L from waste frying oil, molasses, with corn steep liquor. Sekhon Randhawa and Rahman (2014) have indicated that Citrosolv (Cleveland Biotech Ltd.) could be produced at \$0.014/L from citrus peel. Commercial biosurfactant applications have been limited by their high cost. Yields, production rates, and recoveries need to be increased.

Synthetic surfactants, in comparison, can be obtained for 1–3 US\$ per kg (Hazra et al. 2011). If crude biosurfactants and wastes can be used, it has been estimated by da Rocha et al. (2019) that sophorolipid costs could be reduced by 70% to 0.6 to 1.5 US\$ per kg. Glycolipids and rhamnolipids can be produced in much higher yields than surfactin and thus could be produced at much lower costs.

The waste material, however, must be consistent in composition to ensure a consistent production process. In addition, there is a lack of information on the effects of various components of waste on the biosurfactant composition and yields. In addition, there is a lack of information on the effect of the various components in the waste substrate on the purification (downstream) process which can make up 60% of the final product cost (Chen et al. 2021). Various membrane, solvent extraction, foam collection, and precipitation processes have been employed, either singly or in combination. An overall process for evaluating and designing a biosurfactant production process can be seen in Fig. 4. Large amounts of biosurfactant would be needed for environmental remediation. Crude formulations of the biosurfactant could be employed or other low-cost purification processes to decrease downstream costs. For example, micellar-enhanced ultrafiltration (El Zeftawy and Mulligan 2011; Abbasi-Garravand and Mulligan 2014) can be used to recover the biosurfactant for subsequent reuse in the process, thus reducing

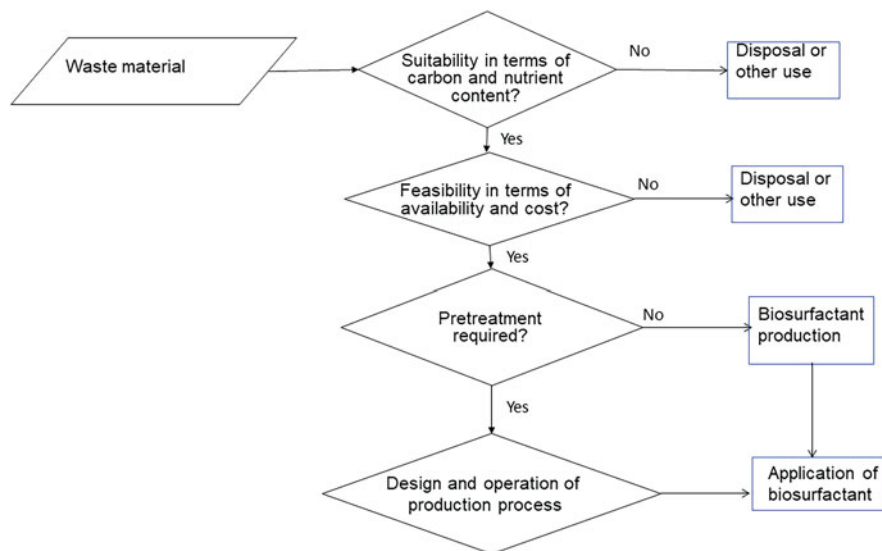


Fig. 4 Process for development of a biosurfactant process using waste materials

the biosurfactant requirements. In addition, metals and other contaminants can be recovered via precipitation for further reuse.

Another potential route is in situ production of biosurfactants to reduce costs due to material, energy, and transport reduced requirements. Understanding how to stimulate the in situ production process to increase yields would be required (Jalali and Mulligan 2008). While introduction of genetically modified organisms will likely not be acceptable to both regulatory authorities and the public, isolation and reintroduction of high yield biosurfactant producers could be highly advantageous over the addition of synthetic surfactants. This has been shown by the growth of anaerobic indigenous bacteria in oil sands tailings (Rezaeitamijani and Mulligan 2021). Further study on the identification and stimulation of these strains, however, is needed.

4 Conclusions and Future Directions

In conclusion, although biosurfactants are potentially applicable for remediation of various media, the costs of biosurfactants need to be reduced to compete with synthetic surfactants (1–3 US\$/kg). However, as substrates contribute substantial to the total production cost, a potential avenue for cost reduction is to use waste materials. Renewable and waste materials are substrates of high interest and potential for biosurfactant cost reduction due to their low costs. The use of waste materials also will enhance the sustainability of biosurfactant use by reducing required

resources and providing a means of waste management. Availability, transportation costs, and substrate pretreatment requirements are all considerations when evaluating feasibility as a substrate.

Waste pretreatment should be avoided if possible as this step can add extra costs for materials and processing (e.g., particle size reduction, ozonation, acid or enzyme hydrolysis of lignocellulosic waste). A variety of substrates have been studied, including lignocellulosic, oily, and food processing wastes. Food waste substrates are of particular interest due to their low costs and thus there is potential for increasing the economic viability of biosurfactants. Up to 40 g/L of rhamnolipids, and 51.5 g/L of sophorolipids from vegetable oil processing wastes, and 13.7 g/L of surfactin from hydrolyzed oil mill oil wastes have been produced. To minimize costs, the selected wastes must contain adequate nutrient and mineral contents to avoid the need for supplementation. Utilization of wastes also is more sustainable and in alignment with a circular economy concept.

The entire life cycle of the biosurfactant production needs to be taken into consideration, and material, energy, and cost requirements must be minimized. Research is needed to isolate microbial strains through recent developments in molecular techniques for those that can produce biosurfactants from agro-industrial and other wastes at high yields. More research is also needed to understand waste composition and its effect on biosurfactant yields. Recovery and purification steps must also be improved. A greater understanding of the effect of product purity on biosurfactant application would enable the appropriate purity level to be matched with the application. Scale-up studies are needed for future full-scale production. In addition, further studies on in situ biosurfactant production are needed to facilitate contaminant degradation and avoid the need for surfactant addition.

Web sites of companies producing biosurfactants

www.jeneilbiotech.com/biosurfactants

corporate.evonik.com/en/company

www.rhamnolipids.com

www.agaetech.com/pages/rhamnolipids

locusbioenergy.com/biosurfactants

www.sophorolipid.com/about-us

TensioGreen Technology Corp.

Advanced BioCatalytics—Green Biotechnology for a Better Environment (abioecat.com)

www.glycosurf.com

www.saraya.com

www.soliance.com

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Characterization and Purification of Biosurfactants



Mridul Kumar Medhi, Shweta Ambust, Rajesh Kumar, and Amar Jyoti Das

1 Introduction

Recently, biosurfactants have attracted enormous attention due to their high biodegradability, low toxicity, great selectivity, and specific activity under harsh temperature, pH, and salinity conditions (Das and Kumar 2018; Felix et al. 2019). These advantages make biosurfactant one of the best surfactants with a variety of applications like households, medicinal formulations, food industry, bioremediation of the environment, crude oil degradation, microbial enhanced oil recovery, cosmetics, etc. (Gaur et al. 2019; Das et al. 2021). A surfactant derived from microorganisms, such as filamentous fungi, yeast, and bacteria, contains metabolic by-products (Ostendorf et al. 2019). Being secondary metabolites it is produced in the stationary phase of microbial growth and contains amphipathic compounds with both (non-polar) hydrophobic as tails and (polar) hydrophilic as the head (Banat et al. 2000; Ambust et al. 2021). The hydrophobic group is known as lyophobic and the hydrophilic group as lyophilic in aqueous media (Akbari et al. 2018). Lipophilic groups can be proteins or peptides with hydrophobic parts made up of 10 to 18 carbon chains of fatty acids. Amino acids, monosaccharides, disaccharides, and polysaccharides are all examples of hydrophilic groups (Singh et al. 2019; Bjerk et al. 2021). Microbes' hydrophobicity can be modulated by biosurfactants by modifying their cell surface

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structures. This increases the availability of hydrocarbons to microbial cells (Desai and Banat 1997). By increasing hydrophobic pollutants' solubility, biosurfactants facilitate biodegradation (Boopathy 2000; Das and Mukherjee 2007; Das et al. 2020). A compound annual growth rate of 5.6% is expected to push the global biosurfactant market to around \$5.52 billion by 2022 from around \$4.20 billion in 2017 (Ambaye et al. 2021). Eco-friendly nature makes biosurfactants stand out from the box and increases awareness of various industries to use microbial surfactants as an alternative to chemical surfactants (Singh et al. 2019).

2 Properties of Biosurfactants

2.1 Critical Micelle Concentration (CMC)

CMC stands for the concentration of biosurfactant requisite for micelle formation and is an important characteristic of a biosurfactant. Surface tension was found to be correlated with concentration of biosurfactant in order to determine the CMC of the pure biosurfactant isolated. The concentrations of freeze-dried purified biosurfactant in distilled water ranged from 0.01 to 8 mg mL⁻¹. Using surface tension, the CMC was determined at the point of intersection of pre- and post-CMC curves, which are related to biosurfactant concentration. At room temperature (approximately 23 °C), the surface tension of each sample was evaluated using the Ring technique (Gudina et al. 2010; Hanano et al. 2017) (Fig. 1).

2.2 Temperature and pH Tolerance

Biosurfactant manufacturing from extremophiles acquired economic interest over a decade ago. Temperature and pH are natural elements that have no effect on the surface activity of biosurfactants. According to McInerney et al., lichenysin from *Bacillus licheniformis* can withstand temperatures of up to 50 °C, pH levels ranging from 4.5 to 9.0, and NaCl and Ca concentrations of up to 50 and 25 g L⁻¹, respectively. *Arthrobacter protophormiae* produced biosurfactant was perceived to be thermostable (30–100 °C) and pH stable (2–12). Because industrial operations entail pH, temperature, and weight extremes, innovative microbial things must be isolated that can function in these conditions (Das and Mukherjee 2007; Roy 2017).

2.3 Biodegradability

Microbially generated chemicals are easily destroyed and bioremediation/biosorption applications are appropriate as compared to manufactured surfactants

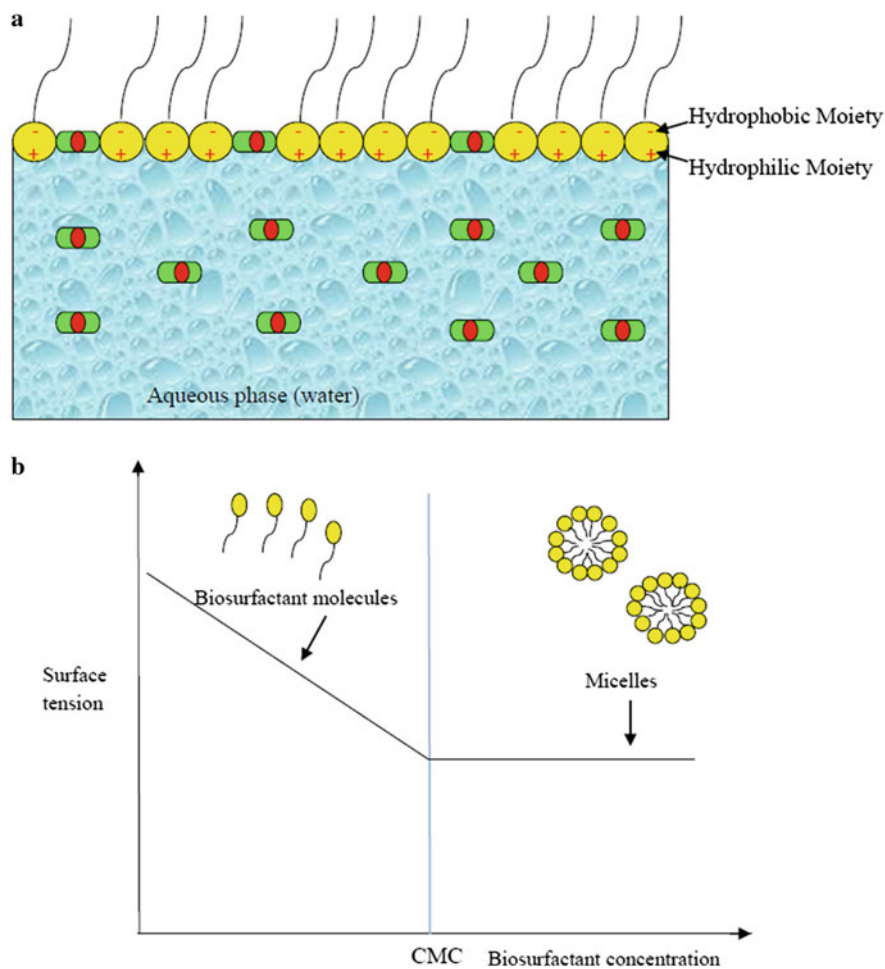


Fig. 1 (a) Biosurfactants accumulation at the interface between air and liquid. (b) Reduction of surface tension and formation of micelles with biosurfactant concentration (Source: Das et al. 2017)

(Desai and Banat 1997; Edwards and Hayashi 1965; Roy 2017). Due to the environmental damage caused by synthetic chemical surfactants, aquatic surfaces were biosorbed with biodegradable biosurfactants originally made from marine microbes, which are ineffectively solvent polycyclic sweet-smelling hydrocarbons (Gharai-Fathabad 2011; Roy 2017).

2.4 Specificity

Since biosurfactants contain distinct functional groups, the compound's properties are complex, they typically have specialized activities (Santos et al. 2016).

2.5 Emulsion Forming/Breaking

The “Biosurfactant” means that it is either an emulsifier or de-emulsifier. Emulsions contain two immiscible liquids that cannot mix; they are neither homogeneous nor homogeneous and distributed as droplets with sizes larger than 0.1 mm in another liquid. Emulsions are classified as either oil-in-water (o/w) or water-in-water (w/w) as well as w/o (water-in-oil). Emulsions have limited stability, however, by adding biosurfactants, an emulsion can be stable for months or even years (Velikonja and Kosaric 1993; Santos et al. 2016). As beads, an emulsion is a mixture of two immiscible liquids with diameters greater than 0.1 mm.

2.6 Antiadhesive Agents

A biofilm is a collection of microorganisms or other organic materials that have accumulated on any surface. The formation of a biofilm begins with bacterial adhesion to a surface, and it is influenced by a number of factors, including number of factors that affect how cells adhere to surfaces, including the type of microbe, the hydrophobicity, and electrical charges of the surface, environmental factors, and the ability of microbes to produce extracellular polymers (Kachholz 1987). In order to improve the hydrophobicity of a surface, biosurfactants can be used, impacting microorganism binding on the surface. It has been shown that a surfactant produced by *Streptococcus thermophilus* prevents other thermophilic strains of *Streptococcus* from colonizing steel and fouling it. An anti-stick biosurfactant derived from *Pseudomonas fluorescens* was also found to inhibit the growth of *Listeria monocytogenes* on steel surfaces (Konishi et al. 2008).

3 Classification of Microbial Biosurfactant

Chemical structure and microbiological source determine the classification of biosurfactants. In general, biosurfactants fall into two categories: those with higher molecular weights (HMC) and those with lower molecular weights (LMW) (Singh et al. 2019). Surfactants with an extremely low mass, such as glycolipids, lipopeptides, and phospholipids, are much easier to dissolve than those with a high mass, such as polymers and particulates (Shoeb et al. 2013; Ambust et al. 2022).

3.1 *Glycolipids*

The majority of known biosurfactants produce glycolipids. Hydrocarbons, frying oil waste, and olive oil wastes are the most researched low-molecular weight biosurfactants (Inès and Dhouha 2015; Thakur et al. 2021). The hydrophilic moiety of glycolipid biosurfactants is made up of carbohydrate molecules including glucose, mannose, galactose, trehalose, rhamnose, and sophorose, whereas the hydrophobic moiety is made up of a long fatty acid chain (Müller and Hausmann 2011; Mukherjee and Das 2010; Thakur et al. 2021).

3.2 *Lipopeptide*

Surfactins are lipopeptides. Many cyclic lipopeptides (polymyxins) are anti-toxins based on decapeptides (gramicidins) and lipopeptides (lipopeptides). These are made up of a lipid chain that is connected to a polypeptide chain. *Bacillus subtilis* lipopeptides have antibacterial, antifungal, antimycoplasmal, and antiviral activities. Numerous microorganisms, most notably *Bacillus* sp., *Lactobacillus* sp., and some actinomycetes, secrete them extracellularly into the production medium (Sharma 2016). Since lipopeptides do not pollute the environment, they are less prone to causing bacteria-resistant and have a larger bacteriostatic spectrum than standard antibacterial agents (Banat et al. 2000; Chen et al. 2017).

3.3 *Neutral Lipids, Fatty Acids, and Phospholipids*

During n-alkane growth, a substantial number of fatty acid and phospholipid bacteria and yeast both produce surfactants (Asselineau and Asselineau 1978). To produce such biosurfactants, a number of strains of *Acinetobacter* sp. and *Aspergillus* sp. have been reported from various sources. Phospholipids are known to be important components of microbial cell membranes. Cultures of separate microorganisms with hydrocarbon-degrading abilities on hydrophobic substrates result in an increase in phospholipid production (Sharma 2016).

Polymeric Biosurfactant Most polymeric biosurfactants have been studied in detail, including emulsan, lipomanan, alasan, and liposan. Emulsan, at concentrations ranging from 0.001% to 0.10%, is an emulsifier for hydrocarbons in water (Lang 2002; DKF Santos et al. 2016). It is known that *Acinetobacter calcoaceticus* produces a polymeric biosurfactant with a spine made of fatty acids covalently bonded to polymer (Sharma 2016).

4 Factors Affecting Biosurfactants

It is not only the maker strain that impacts biosurfactant production, but it is also the way of life conditions, for example, the carbon source, nitrogen source, and also carbon source: nitrogen proportion, nutritional restrictions, concoctions, and physical parameters, such as temperature, pH, air circulation, divalent cations, and saltiness, influence the volume and type of biosurfactant produced (Roy 2017).

4.1 Carbon Source in Biosurfactant Production

The carbon supply in the culture medium is crucial for bacteria generating biosurfactants. Carbohydrates (a), hydrocarbons (b), and vegetable oils are the three groups (c). It has been reported that Rhamnolipids can be synthesized by several *Pseudomonas* species from glucose, fructose, ethanol, mannitol, and glycerol (Gudiña et al. 2016; Varjani and Upasani 2017). Carbon substrates clearly play a key part in biosurfactant synthesis, but their significance varies with the kind of life form. In a study with *Pseudomonas* sp., different carbon sources had an effect on biosurfactant production, but different chain lengths in the substrate did not have an impact on unsaturated fats in glycolipids (Roy 2017).

For example, *B. licheniformis* grew better on a mineral salt medium enriched with glucose and yeast extract. A surface tension reduction of 28 mN/m was achieved as a result of rhamnolipid synthesis (Robertson et al. 1994; Varjani and Upasani).

4.2 Nitrogen Source in Biosurfactant Production

Nitrogen is essential in order to produce biosurfactants. As protein and chemical blends rely on nitrogen-containing medium, it plays a key role in microbial development. In the synthesis of biosurfactants there have been various nitrogen sources used in the process, such as ammonium sulfate, yeast extract, sodium nitrate, beef concentrate, ammonium nitrate, and malt extricates (Roy 2017). Ammonium salts and urea aid in the synthesis of biosurfactants by *Arthrobacter paraffineus*. *Rhodococcus* sp., and *Pseudomonas aeruginosa* increasing surfactant synthesis with nitrate, however, may be less effective. Maximal rhamnolipid synthesis after nitrogen restriction occurred in surfactants are produced in a carbon/nitrogen (C/N) ratio of 11:1, with a range of 16:1 to 18:1 (Jahan et al. 2020).

4.3 pH

Carboxyl groups that contribute to the anionic characteristics of rhamnolipid molecules heavily depend on the pH of aqueous solutions. pH 6.8 results in a majority of carboxyl groups dissolving into pH 5 which is associated with carboxylate groups resulting in solution, their protonated form dominates (Özdemir et al. 2004). The chemical structure of biosurfactants changes when pH fluctuates, resulting in varied behavior. In the presence of rhamnolipids, waste-activated sludge (WAS) is hydrolyzed and acidified and it was demonstrated to be more successful at alkaline pH levels than at acidic or near-neutral pH levels. Rhamnolipid biosurfactant may be more solubilized under alkaline conditions, proteins and carbohydrates are released in excess, rising cell permeability (Luo et al. 2013).

4.4 Aeration and Agitation

In addition to facilitating oxygen exchange between gas and fluid, aeration and agitation play a key role in the production of biosurfactants. A bio-emulsifier could be used to enhance the solubility of water-insoluble substrates, increasing the likelihood that a microorganism can utilize that supplement. The optimum surfactant concentration (45.5 g/l) was obtained when partial immersion oxygen was held at a wind current of 1 vvm. A broken-up oxygen focus was also in motion (Muthusamy et al. 2008; Roy 2017).

4.5 Salinity

The salinity of a particular medium is also significant in the synthesis of biosurfactants. In any case, contradictory impressions were seen for a number of biosurfactant products that were unaffected by fixing up to 10% (weight/volume), despite slight declines in CMC.

5 Characterization of Biosurfactant

5.1 Biochemical Assays

By the biochemical assay it may be determined that the strain's biosurfactants are rhamnolipid in origin. The absence of Ruhemann's purple complex formation in the ninhydrin test, for example, shows the absence of protein or amino acid in the extracted biosurfactant. Blue-green color development was seen in the anthrone

test, suggesting the presence of carbohydrates in the biosurfactant. The saponification of the lipid portion by NaOH demonstrates the existence of lipids in the biosurfactant. The rhamnolipid assay, which was used to determine the concentration of rhamnolipid in biosurfactant samples, in comparison with rhamnolipid, 1 g L^{-1} of crude biosurfactant produced by *P. aeruginosa* equated to 0.67 g L^{-1} of crude biosurfactant (Patowary et al. 2017).

5.2 *Thin-Layer Chromatography/Purified Biosurfactant Fractions*

Thin-Layer Chromatography (TLC) was used to examine the partly purified microbial derived surfactant, which was diluted in methanol and analyzed on a silica gel-covered aluminum sheet (Silica gel G, Fluka, Germany). The solvent system chloroform:methanol:acetic acid (65:25:4, v/v/v) was used to create thin-layer chromatograms. The chromatogram was not sprayed with developing reagents. To retrieve the discrete products, the non-sprayed plate parts corresponding to the appropriate predicted locations were scratched off. Scrapings were obtained separately from horizontally aligned areas with equal R_f values and re-extracted using a chloroform: methanol combination (2:1, v/v). Centrifuged at 10,000 rpm for 10 min, the solvent fraction was microfiltered (pore size: 3 μm), then air-dried.

5.3 *Product Analysis by Liquid Chromatography-Mass Spectrometry (LC-MS)*

Positive ion mode was used in liquid chromatography-mass spectrometry to determine the structural constituents of the column purified biosurfactant (Pantazaki et al. 2011). A methanol solution of the biosurfactant product was diluted with 1 mg/mL of methanol and filtered. An ultra-high performance liquid chromatography system (UHPLC, Ultimate 3000, Dionex) and the TSQ Endura™ Triple Quadrupole Mass Spectrometer were used to investigate the electrospray ionization (ESI) mass spectrum of the product (Thermo Scientific, USA). For the separation, a 1.9 m, 1002.1 mm Hypersil Gold C18 column was employed. Water, 1% formic acid, and acetonitrile were used to make up the mobile phase. To linear gradient system: 60–93% acetonitrile for 0–3.5 min; 93% acetonitrile and 7% water (1% formic acid) for 3–20 min; injection volume 5 L; flow rate 0.300 ml/min. The UHPLC–ESI–MS were employed in positive modes, with full scans covering m/z 200 to 2000.

5.4 *Fourier Transform Infrared Spectroscopy (FTIR)*

An organic or inorganic chemical compound in the waste can be identified using FT-IR (Ratna and Kumar 2022). By recognizing distinct types of chemical linkages, Fourier transform infrared spectroscopy (FT-IR) analysis may be utilized to identify particular components of an unknown mixture (functional groups) (Elazzazy et al. 2015). Wastes that have been untreated and bacteria-treated were centrifuged for 10 min at 4 °C (rpm: 10000) and kept for 48 h at 50 °C in a closed oven. The dried sample was mixed with 100 mg of KBr and then the spectrum was examined using an FTIR (Nicolet™ 6700, Thermo Scientific, USA) instrument, which produced a range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using potassium bromide (KBr) pellet (Ratna and Kumar 2022).

5.5 *Gas Chromatography-Mass Spectrometry (GC-MS)*

The GC-MS machine was equipped with an Agilent Technology 5890 gas chromatograph, with a split detector and mass spectrometer detector (MSD). It was injected with a 1 μl solution of biosurfactant. A constant flow of 1 ml of Helium as a carrier gas was used with a volume injection of 1 μl of Helium, a temperature injector of 250 °C, and an ion source temperature of 280 °C. This total time for running the analysis was determined and programmed by the GC-MS analyst and was 90.67 min for the whole GC run. Combining mass spectra and the NIST08 mass spectral database, the peaks in the chromatograms of these analyses were identified (Anaukwu et al. 2020).

5.6 *Electrospray Ionization Mass Spectrometry (ESI-MS)*

The clinical laboratory's use of electrospray ionization mass spectrometry (ESI-MS) for structural analysis or quantitative assessment of metabolites in complicated biological samples is rising. It is particularly helpful for detecting inborn abnormalities in amino acid, fatty acid, purine, and pyrimidine metabolism, as well as diagnosing galactosemia and peroxisomal diseases, because it can test biomolecules with comparable molecular structures (Ho et al. 2003).

5.7 *High-Performance Liquid Chromatography (HPLC)*

C-18 reversed-phase HPLC instrument equipped with diode array detectors. To purify the water, a column was utilized. Before injection, a 0.2- μm membrane

filter was used to filter the methanol extract. A glass microsyringe was used to inject the filtrate (50 l each run) into the column. Solvent A and solvent B were used in the elution system. Prior to usage, the solvents were purged with dry nitrogen to degas them. Using the 210 nm wavelength, different biosurfactant fractions were eluted (Lin et al. 1998). The original approach employed a 60-min flow rate of 0.4 ml/min while linearly increasing the proportion of solvent A (10–90%) (Desai and Banat 1997). To lower contaminant levels in the methanol extract, the flow rate was increased to 0.7 ml/min during stage I, while the flow of solvent B was steadily increased from 0 to 100% for 50 min. Using biosurfactant peak profiles established in intermediate processes such as stages II, III, and IV, the flow rate and duration were adjusted to achieve the ideal approach. In the final operation, the flow rate was kept constant at 2 ml/min from 0 to 4 min, at this pace, solvent A accounted for 60% of the total, with solvent B accounting for the remaining 40%. Over 4 to 12 min, the flow rate was maintained at 0.7 ml/min by changing the concentration of solvent A by 40–10% and solvent B by 60–90%. After 12 min, the flow rate was reduced to 1 ml/min and maintained for 20 min with 100% solvent B. The chemicals were gathered manually through the device's waste pipe (Sivapathasekaran et al. 2009).

6 Extraction and Purification of Biosurfactant

During the 35-day culture period, crude oil breakdown produced microbial-derived surfactant which was recovered from the flasks. Where in gravimetric assay the highest TPH (Total petroleum hydrocarbons) degradation were observed. For biosurfactant extraction was obtained by centrifuging culture broth for 20 min at 10,000 rpm at 4 °C (George and Jayachandran 2009; Patowary et al. 2017). Various extraction methods are available, such as the following: (1) extraction with chloroform/methanol (2:1 v/v); (2) extraction with ethyl acetate; (3) acidic precipitation with methanol, and (4) Cell-free broth was acidified to pH 2.0 using 6.0 M HCl for acidic extractions (Ostendorf 2019). HCl 6 N was used to acidify the supernatant to pH 2 before it was stored at 4 °C for the next day. At room temperature, the biosurfactant was extracted continuously from the chilled supernatant using ethyl acetate. To separate phases, a 1:1 mixture of ethyl acetate and supernatant was vigorously shaken and left stationary (George and Jayachandran 2009). Following reduced pressure solvent evaporation at 40 °C, the organic layer was collected and transferred to a rotary evaporator, where a viscous substance with a dark honey hue was found. The crude biosurfactant was measured gravimetrically (George and Jayachandran 2009).

Reverse-phase chromatography was used to further purify the sample. The sample was dissolved in methyl alcohol and then passed through a 0.45 m membrane and an octadecyl silica cartridge (HyperSep C18, 10 g/75 ml, Thermo Fisher Scientific). Elution was carried out using a solvent mix of CH₃OH/H₂O (8:2), CH₃OH/H₂O (9:1), and CH₃OH. 5 mL, the fractions were collected in glass vials and visually inspected (Yeh et al. 2005; Felix et al. 2019). The crude surfactants were

then refined by column chromatography. The crude extracts were diluted in chloroform and passed through a 100–120 mesh silica gel mesh. To get rid of all the neutral lipids, 100 mL chloroform was used to wash the loaded column. The biosurfactant was extracted using the mobile phases of various chloroform-methanol ratios in order: The biosurfactant was recovered using 80:20 v/v (100 mL) and 35:65 v/v (100 mL) at 1 mL/min. The purified biosurfactant fractions were combined and dried by rotary evaporation at 40 °C (Guo et al. 2022).

1. Chloroform/methanol extraction process

After homogenizing the microalgal paste in a 2:1 chloroform:methanol (v/v) mixture, Folch filtering was used to separate the cell suspension. Following solubilization with a new solvent combination, the homogenizer and recovered cell debris were rinsed with a 0.73% NaCl solution, and the filtrate was pooled with the previous filter, yielding 2:1:0.8 chloroform, methanol, and water (Axelsson and Gentili 2014).

2. Ethyl acetate extraction

In ethyl acetate extraction 1:4 mixture of ethyl acetate and the cell-free broth with the not centrifuged medium has been taken and repeating it for two times. After centrifuging for 20 min at 4500 rpm, the organic phase was filtered. The residual aqueous phase must be separated, the filtered sample was poured into a separation funnel, and a saturated solution of sodium chloride (NaCl) was added. On a heated plate, the organic phase was transferred into a beaker and dried at 60 °C. For the dehydration of the water content which might be present at the time of extraction, we can follow this process: sodium hydroxide (NaOH) and acetone (C₃H₆O) were added until granules formed, followed by paper filtration and drying at 50 °C (Lira et al. 2020).

7 Purification

Two techniques were used to purify the isolates that produced biosurfactants: ammonium sulfate precipitation and ZnCl₂ precipitation.

Ammonium Sulfate Precipitation

There are four steps in the ammonium sulfate precipitation procedure: Fractionation of ammonium sulfate, treatment with cooled acetone and hexane, and chromatography on a silica gel column.

At 4 °C, centrifuge the culture broth at 10,000 rpm for 15 min. Take the supernatants and precipitate them with 40% (NH₄)₂SO₄. Centrifuge again at 10,000 rpm for 10 min to get Ppt. supernatant and floating material. The floating material was dissolved in water (200 µl) and ten chilled acetone was added at 4 °C. Once again, centrifuge at 10,000 rpm for 10 min and concentrate the supernatant in a vacuum. After extracting hexadecane with hexane (repeat three to four times), there is a layer of hexadecane and an aqueous layer. The aqueous layer is concentrated in a vacuum. Purify the biosurfactant using TLC.

ZnCl₂ Precipitation

10 mL of culture supernatants were concentrated with ZnCl₂ to a final concentration of 75 mM. The precipitated substance was dissolved in 10 mL of sodium phosphate buffer (pH 6.5), then extracted twice with diethyl ether in equal volumes. After the organic phase had been evaporated to dryness, the pellets were dissolved in 100 µl of methanol. To further purify the material, we utilized preparative TLC (Singh 2012).

8 Conclusion

Astounding research reports recommend that there are a variety of techniques for the characterization and purification of biosurfactants. Hence, wide application of such a technique could ultimately lead to the isolation of some new strains having the ability to produce some commercially important biosurfactant.

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Biodegradation and Cytotoxic Effects of Biosurfactants



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1 Introduction

Biosurfactants are extracellular, surface-active secondary metabolites formed by microbes (Salminen et al. 2020). Many studies reported that bacterial genera such as *Bacillus*, *Pseudomonas*, *Micrococcus*, *Lactobacillus*, *Rhodococcus*, *Staphylococcus*, *Microbacterium*, *Kocuria*, *Paenibacillus*, *Streptomyces*, *Bradyrhizobium*, *Curtobacterium*, *Enterobacter*, *Brevibacterium*, *Kineococcus*, *Stenotrophomonas*, and *Candida* are producing biosurfactants (Rani et al. 2020). Biosurfactants are amphiphilic substances comprising hydrophilic and hydrophobic moieties, thereby reducing the accessibility and interfacial tensions between aqueous and other immiscible liquids (Banat et al. 2010). Hydrophilic biosurfactants are mono-saccharides (rhamnose, mannose, glucose, and galactose), polysaccharides, and peptides. Hydrophobic biosurfactants are saturated, unsaturated, and hydroxylated fatty acids (Abbasi et al. 2012; Darvishi et al. 2011; Henkel et al. 2012). Biosurfactants' activities depend on their ionic properties and are classified into ionic and non-ionic surfactants. Anionic biosurfactants have a negative charge at the hydrophilic end. They contain anionic functional groups such as sulfate, sulfonate,

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phosphate, and carboxylates, E.g. Surfactin and Rhamnolipids. Cationic biosurfactants have a positive charge at the hydrophilic end, E.g. Alkyl polyglycoside and *Morus nigra* leaf extract. Some biosurfactants have both positive and negative charges (Zwitterionic) at their hydrophilic end of the same molecule, they are otherwise called as amphoteric surfactants. E.g. Betaines and Amino Oxides. Non-ionic surfactants are primarily neutral, and no charge is present at their hydrophilic end. E.g. Trehalose Corynomycolates and Ziziphus Spina Christi (Franzetti et al. 2010; Pasternak et al. 2020).

Biosurfactants are found to have improved properties than chemically synthesized surfactants. These include high tolerance to extreme temperature, pH, and salinity conditions, easily produced by culture, high-scale production, and eco-friendly nature making them ideal for usage in various fields (Bhadoriya and Madoriya 2013). Biosurfactants' versatile functionality and non-toxic, bio-degradable, and ecologically safe properties make them as an promising substitute to the chemically manufactured surfactants. They are also employed to recover crude oil, remove heavy metals in soil, pharmaceutical formulations, and food industries and also degrade hydrocarbons in soil and water. Biosurfactants are also reported for their antioxidant, antimicrobial, anti-aging, cytotoxic, and anti-inflammatory activities (Saravanakumari and Mani 2010; Sharma and Saharan 2016). The role of biosurfactants in biodegradation and their cytotoxic effects against different cancer cells are discussed in this chapter.

2 Molecular Weight-Based Categorization of Biosurfactants

Biosurfactants are categorized into low-molecular-weight biosurfactants and high-molecular-weight biosurfactants based on their molecular weight (Fig. 1). Low-molecular-weight biosurfactants potentially decrease surface and interfacial tensions, and high-molecular-weight biosurfactants hold firmly to surfaces. The additional categories of biosurfactants comprise polymeric and particulate surfactants (Rosenberg and Ron 1999).

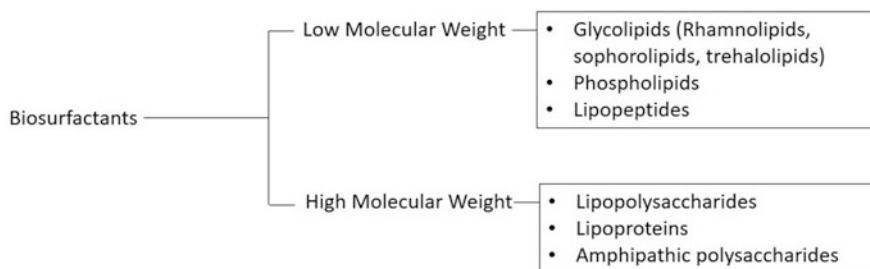


Fig. 1 Classification of biosurfactants by molecular weight

2.1 Low-Molecular-Weight Biosurfactants

2.1.1 Glycolipids

Glycolipids are carbohydrates linked with fatty acids and they are primarily formed by *Bacillus* sp. Most common glycolipids are rhamnolipids, sophorolipids, trehalolipids, fructose-lipids, and cellobiose. They have high potency to minimize the surface and interfacial tensions. Glycolipids have been described for their prospective antibacterial, antifungal, antiviral, anti-oncogenic, and antibiofilm properties (Inès and Dhouha 2015).

2.1.1.1 Rhamnolipids

Rhamnolipids are formed by combining molecules of rhamnose and b-hydroxy decanoic acid. *P. aeruginosa* produces two kinds of molecules such as rhamnolipid 1 and rhamnolipid 2 (Desai and Banat 1997). The rhamnolipids from *P. aeruginosa* L2-1 exhibit 100% emulsification toward soybean oil and 69% toward crude oil (Costa et al. 2010).

2.1.1.2 Sophorolipids

Disaccharide sophorose units are glycosidically linked to hydroxylated fatty acids to form sophorolipids (Gayathiri et al. 2022; Kim et al. 2002). A kind of sophorolipid formed by *C. bombicola* ATCC 22214 has an antagonistic effect toward the *B. subtilis*, *S. xylosus*, *S. mutans*, and *P. acnes* (Elshafie et al. 2015). Acidic sophorolipids possess good antimicrobial properties toward the nosocomial infectious agents, thus contributing to the production of antimicrobial cream (Lydon et al. 2017).

2.1.1.3 Trehalolipids

Arctic soil-isolated *Rhodococcus fascians* BD8 produced a trehalose lipid with a molecular weight of 848 g mol⁻¹. It has demonstrated anti-adhesive efficacy toward *C. albicans* and *E. coli* on polystyrene surfaces and silicone urethral catheters to a degree of 95% and 70%, respectively. Trehalose lipid can be utilized as a surface coating agent because it prevents microbes from colonizing silicone and polystyrene surfaces.

2.1.2 Phospholipids

Phospholipids are polar in nature and present in cell walls of animals, plants, and microorganisms. Phospholipids have glycerol backbone with two fatty acids and a phosphoric acid moiety. *K. pneumoniae* strain IVN51 obtained from hydrocarbon contaminated soil produced phospholipid biosurfactant that exhibits emulsifying action toward petrol, and similar by-products like kerosene and diesel (Nwaguma et al. 2016). The amount of phospholipid rises prominently during the growth of hydrocarbon-decomposing bacteria or yeasts on alkane substrates. *Acinetobacter* sp. HO1-N produce phospholipid (e.g., phosphatidylethanolamine) ionic vesicles (Youssef et al. 2005). *Rhodococcus erythropolis* from n-alkane environment produced phosphatidylethanolamine which reduces the interfacial tension developed in-between the water and hexadecane (de Carvalho et al. 2014).

2.1.3 Lipopeptides

Combinations of cyclic lipopeptides are developed from variants of heptapeptides and hydroxy fatty acid chains (surfactin/iturin/fengycin, viscosin, lichenysin, and serrawettin). Surfactin has a high surface activity and is successfully used to enhance the oil recovery process (Mnif and Ghribi 2015). A bacterial strain, *Bacillus siamensis* isolated from automobile waste contaminated soil, produced lipopeptide biosurfactant using a mineral salt medium supplemented with crude oil. The obtained biosurfactant was constant at very high/low temperature, pH, and salinity. Its application ability in Enhanced Oil Recovery (EOR) was assessed using a sand pack column that yielded up to 60% oil recovery at 70 °C.

2.2 High-Molecular-Weight Biosurfactants

High-molecular-weight biosurfactants are best studied for their bio-emulsifying capacity. They are highly efficient in stabilizing emulsions, e.g. lipopolysaccharides, amphipathic polysaccharides, combinations of heteropoly-saccharides, and proteins. They are also known as extracellular polysaccharides (EPSs), e.g. emulsan, alasan, etc.

2.2.1 Lipopolysaccharides and Amphipathic Polysaccharides

Amphiphilic extracellular polysaccharide producing *Halomonas* species TG39 isolated from the water surface polluted with the oil-spill has effectively increased the solubilization of aromatic hydrocarbons and enhanced their biodegradation (Rosenberg and Ron 1999). Amphipathic polysaccharides comprise hydrophobic and polar

groups. They are high-molecular-weight bioemulsifiers produced by microorganisms. They have high acceptance to extreme pH, temperature, and salinity and exhibiting low toxicity and biodegradability (Mnif and Ghribi 2015).

2.2.2 Lipoproteins

Lipoproteins are made up of lipid and protein molecules. They are more complex form than glycolipids, developing large particles with many lipids, and proteins. A *Streptomyces* sp. DPUA1566 obtained from lichens produced lipoprotein called Bioelan. It minimized the surface tension activity of water from 72 to 28 mN/m and showed activity toward high temperature, pH, and salt concentration (Santos et al. 2019). *Candida lipolytica* produced anionic lipopeptide that is comprised of 50%, 20%, and 8% of protein, lipid, and carbohydrate, respectively. This minimized the cell-free broth's surface tension by 25 from 55 mN/m (Diniz Rufino et al. 2014).

3 Biological Properties of Biosurfactants

Biosurfactants possess various biological properties such as antibacterial, antifungal, antiviral, and antibiofilm activities against pathogens, which make use of them in biomedical applications. Biosurfactant has been recognized as an ideal component, as additive in the pharmaceutical, cosmetic, and food industries, and as therapeutics for treating various diseases. Emulsifiers like lecithins and gum arabic produced by plants are used in food industries. Lecithins are mainly used along with cocoa powder to enhance their hydrophilic character since cocoa powder naturally has a hydrophobic character due to the presence of cocoa butter (Salminen et al. 2020). Bacteria such as *Klebsiella* sp. and *Acinetobacter calcoaceticus*, yeasts such as *Candida utilis*, *Candida valida*, *Hansenula anomala*, *Rhodospiridium diobovatum*, *Rhodotorula graminis*, and Red alga *Porphyridium cruentum* were identified in the formation of extracellular emulsifiers better than the stability produced by gum arabic and carboxymethyl cellulose (Shepherd et al. 1995). The discharge of biosurfactants by probiotic bacteria in vivo could be interfering with the colonization of pathogenic microorganisms in the urinary and gastrointestinal tracts due to their hydrophobicity nature (Rodrigues et al. 2004). Biosurfactants formed by lactobacilli exhibited minimal adhesion of harmful pathogens to glass (Rodrigues et al. 2006), silicone rubber (van Hoogmoed et al. 2004), and surgical implants (Velraeds et al. 2000). Hence, pre-adsorption of biosurfactants can be employed as a precautionary measure to postpone the start of harmful biofilm formation on catheters and other medical operational equipment, thereby minimizing the need for synthetic medications and chemicals (Falagas and Makris 2009; Mishra et al. 2020; Rodrigues et al. 2004). Various applications of biosurfactants are presented in Fig. 2.

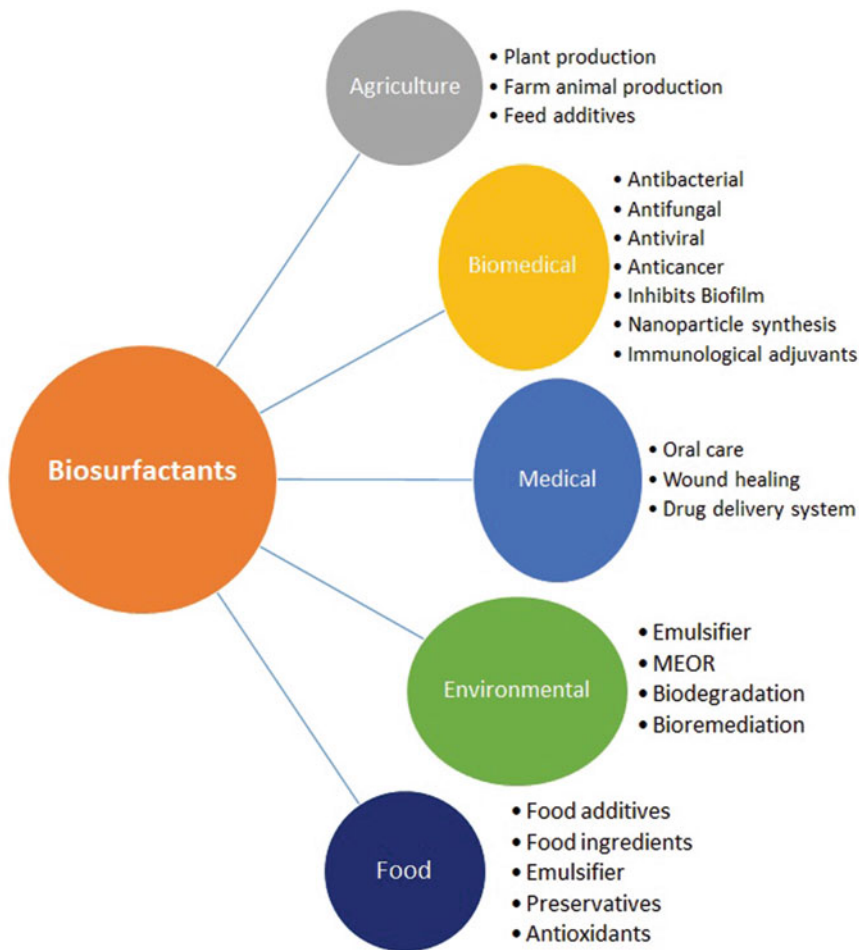


Fig. 2 Uses of biosurfactants in various fields

4 Role of Biosurfactants in Biodegradation

In bioremediation, biosurfactants work by expanding the contact area of substrates. Microorganisms that produce biosurfactants develop their own microenvironment and aid in emulsification by releasing specific chemicals via a variety of methods, including quorum sensing and cell membrane contact. Few biosurfactants have tendency of enhancing the availability of weakly soluble polycyclic aromatic hydrocarbons (Olivera et al. 2003) and resins (Venkateswaran et al. 1995). Biosurfactant-producing indigenous strain *Pseudomonas mendocina* ADY2B has been reported from the coastal water of Chennai harbor with potential in the petroleum hydrocarbon degradation (Balakrishnan et al. 2022). Consequently, the usage of

biosurfactants would be a hopeful means to emulsify the contaminated oils before biodegradation. Prime mechanism behind the concept is harnessing the ability of the native microbial population by enrichment, resulting in the biodegradation of hydrocarbons (Venkateswaran et al. 1995). In another study, PAHs were significantly degraded by a group of bacteria that produced glycolipids and sophorolipids. Biosurfactants produced by the microorganisms in the hydrocarbon contaminated sites have enhanced the biodegradation of 2,4-Dichlorophenolindophenol. Glycolipid biosurfactants produced by microorganisms completely degraded the PAH's within the period of 30 days (Chakrabarti 2012). Crude oil pollution in sea is mostly caused by stranding of tankers creating environmental issues over the world. Oil-containing bilge waste accumulated at the lower part of the ship's hull must be handled appropriately to evade environmental pollution (Olivera et al. 2003). Dispersion state of hydrocarbon decides the rate of biodegradation and it is maximized when the water-insoluble substrate is dissolved, solubilized, or emulsified. A large number of reports dealt with PAHs degradation, either by completely assimilating a defined range of compounds or carry out their transformation to different extents (Abdel-Shafy and Mansour 2016). Alasan, a bioemulsifier has improved the solubility of PAHs abundantly. *A. borkumensis* and *A. calcoaceticus* RAG-1 were found to synthesize many kinds of bioemulsifiers (Glória Pereira and Mudge 2004). Hydrocarbon degrading *P. aeruginosa* UKMP14T was observed to produce biosurfactant in mineral salt medium containing 1% (v/v) Tapis crude oil as carbon source at pH 9.0 and temperature 37 °C. The above conditions were found to be optimistic for all the qualitative analyses for biosurfactant production and reducing the medium's surface tension to 49.5 dynes/cm with emulsification index of 25.29%. The same strain UKMP14T when developed in mineral salt medium (MSM) containing 1% (v/v) glycerol and 1.3 g/L (NH₄)₂ SO₄ with C/N ratio 14:1 have shown decreased surface tension at 55% or 30.6 dynes/cm through emulsification index of 43% (Sabturani et al. 2016). The surface tension reduction of the above study indicates usage of biosurfactants in biodegradation of oil contaminated sites. Biosurfactants producing microorganisms isolated from various environmental sites are listed in Table 1.

5 In vitro Cytotoxic Effects of Biosurfactants

World Health Organization reported that cancer is the second foremost reason of death across the globe, and 9.6 million deaths occurred in 2018 (WHO 2018). The predominant cancers among male are lung, prostate, liver, stomach, and colorectal cancers and among female are breast, cervical, thyroid, colorectal, and lung cancers (WHO 2018). Currently, chemotherapy is an important therapeutic option for treating cancers (Alfarouk et al. 2015). Nearly 60% of anticancer drugs are derived from microorganisms, medicinal plants, and animals (Demain and Sanchez 2009). It has been experienced that chemotherapeutic drugs are extremely cytotoxic and targeting non-specifically to highly proliferative host cells (Dy and Adjei 2013;

Table 1 Biosurfactant-producing bacteria with biodegradation ability isolated from different environmental sites

Environmental site	Organism	Carbon source	Chemical composition of biosurfactant	References
Oil field	<i>Corynebacterium lepus</i>	Kerosene	Lipopeptide	Cooper et al. (1979)
Marine hydrothermal vent	<i>Alteromonas infernus</i>	Glucose	Acidic exopolysaccharide	Raguénès et al. (1997)
Marine	<i>Alcanivorax borkumensis</i>	Alkanes	Glycolipid	Yakimov et al. (1998)
Marine	<i>Halomonas eurihalina</i>	Glucose, polycyclic aromatic hydrocarbon	Sulfated heteropolysaccharide	Calvo et al. (2002)
Oiled sludge	<i>Brevibacillus sp. PDM-3</i>	Phenanthrene	Glycolipid	Reddy et al. (2010)
Marine	<i>Alcanivorax dieselolei</i>	Alkanes	Glycolipid	Hassanshahian et al. (2012)
Oil reservoir	<i>Pseudomonas aeruginosa</i> WJ-1	Alkanes, vegetable oils	Rhamnolipid	Xia et al. (2013)
Marine	<i>Cobetia sp. MMHDA2H-1</i>	Polycyclic aromatic hydrocarbon	Lipidic surfactant	Ibaache-Quiroga et al. (2013)
Oil reservoir	<i>Bacillus methylotrophicus</i> USTBa	Alkanes, polycyclic aromatic hydrocarbon	Glycolipid	Chandankere et al. (2014)
Marine sediment	<i>Pseudoalteromonas sp.</i> 93	Tetradecane	Glycolipid	Malavenda et al. (2015)
Marine	<i>Bacillus amyloliquefaciens</i> MB-101	Kerosene	Lipopeptide	Dhasayan et al. (2015)
Oiled soil	<i>Bacillus pumilus</i> 2IR	Crude oil/glucose	Lipopeptide	Fooladi et al. (2018)
Sea water contaminated with petroleum derivatives	<i>Bacillus cereus</i>	Motor oil	Biosurfactant	Durval et al. (2019)
Oiled soil/water	<i>Aneurinibacillus sp.</i>	Diesel	Lipopeptide	Wu et al. (2019)
Soils polluted with hydrocarbons near Gas Station	<i>Bacillus cereus</i>	Crude oil	Biosurfactant	Christova et al. (2019)
Oil contaminated sea water	<i>Paracoccus sp. MJ9</i>	Petroleum oil	Rhamnolipid	Xu et al. (2020)
Marine	<i>Planococcus sp. XW-1</i>	Diesel	Glycolipid-type biosurfactant	Guo et al. (2022)

Sak 2012). So, identifying the novel anticancer drugs that mainly act on the specific oncogenic cells and sensitize the chemo-resistant cancer cells are highly warranted (Gudiña et al. 2016). Recently, biosurfactants were reported for their cytotoxic activity against various cancer cells (breast, colon, leukemia, hepatic, and cervical cancers) (Wu et al. 2017). Glycolipids such as sophorolipids and rhamnolipids are microbially produced biosurfactants possessing cytotoxic effects (Adu et al. 2022). They kill the cancer cells by disturbing the cell membrane by lysis and increasing membrane permeability (Gudiña et al. 2013). Sophorolipids were reported for their cytotoxic effects against HPAC, H7402, A549, LN229, HNCG-2, KYSE109, KYSE450, MCF7, HeLa, HL60, K562, CT116, and CaCo-2 cancer cell lines (Chen et al. 2006a; Dhar et al. 2011; Fu et al. 2008; Ribeiro et al. 2015; Shao et al. 2012). Likewise, rhamnolipids were reported for their cytotoxic effects against human promyelocytic leukemia, breast cancer (MCF7), colon cancer (CaCo-2), and liver cancer (HepG2) cell lines (Christova et al. 2013; Thanomsab et al. 2006). Details of several types of biosurfactants obtained from microorganisms and their cytotoxic effects tested against various cancer cells are given in Table 2.

5.1 Breast Cancer

Breast cancer is highly affecting women. Over 2.3 million females were diagnosed with breast cancer, and 685,000 deaths occurred globally in 2020 (WHO 2021). Tamoxifen citrate, Cytoxan (cyclophosphamide), paclitaxel, and docetaxel are the most effective anticancer drugs in the chemotherapeutic treatments for breast cancer, but they show severe side effects (Marsh and McLeod 2007). Biosurfactants derived from microorganisms have been reported for their cytotoxic activity for breast cancer cells. A *Bacillus subtilis* CSY191 strain isolated from the soybean paste produced surfactin, and it exhibited cytotoxicity against breast cancer cell line MCF7 at an IC_{50} value of 9.65 μ M (Lee et al. 2012). Another strain *B. subtilis* Hs0121 produced surfactin that showed effective cytotoxicity on human B cap-37 breast cancer cells. It induced the apoptotic effect which was related with a considerable minimization of unsaturated cellular fatty acids in B cap-37 cells, thus improving the membrane fluidization (Liu et al. 2010). Trehalose lipid produced by *R. wratislaviensis* strain was found to be effectively inhibiting proliferation of MCF7 and MDA-MB231 cells (Nikolova et al. 2020).

Surfactin from *B. subtilis* 573 also considerably inhibited the human breast cancer cell lines T47D and MDA-MB-231 in a dose-dependent and time-dependent manner. Cell cycle arrest in G0/G1 phase was achieved with the concentration of 193 μ M of surfactin (Duarte et al. 2014). Likewise, surfactin obtained from *B. subtilis natto* TK-1 strain was reported for its cytotoxic activity against MCF-7 cells by arresting cells at the G2/M phase (Cao et al. 2009) and also by stimulating apoptosis by inducing ROS/ Ca^{2+} -mediated mitochondrial/caspase pathway (Cao et al. 2011). On the whole, surfactin-mediated inhibition of cell invasion and MMP-9 expression

Table 2 Cytotoxic activity of biosurfactants against various cancer cell lines

Type of cancer	Strain	Biosurfactant	Studied cancer cell line/s	Activity at concentration	References
Breast cancer	<i>Bacillus subtilis natto</i> TK-1	Surfactin	MCF7	IC ₅₀ : 86.2 µM	Cao et al. (2009)
	<i>Bacillus subtilis</i> Hs0121	C15 surfactin-like lipopeptide	Human Bcap-37	IC ₅₀ : 29 ± 2.4 µM	Liu et al. (2010)
	<i>Exophiala dermatitidis</i>	Biosurfactant	MCF7	IC ₅₀ : 71.64 µg/mL	Chiewpattanakul et al. (2010)
	<i>Bacillus subtilis</i> CSY 191	Surfactin	MCF-7	IC ₅₀ : 9.65 µM	Lee et al. (2012)
	<i>Lactobacillus paracasei</i> subsp. paracasei A20	Glycoprotein (BioEG)	T47D and MDA-MB-231, and non-tumor fibroblast cell line (MC-3 T3-E1)	IC ₅₀ : 0.15 g l ⁻¹	Duarte et al. (2014)
	<i>Bacillus subtilis</i> 573	Surfactin	T47D and MDA-MB-231	IC ₅₀ : 193 µM	Duarte et al. (2014)
	<i>Sargassum wightii</i>	Polysaccharides	MCF7	IC ₅₀ : 200 µg/ml	Vaikundamoorthy et al. (2018)
	<i>Sargassum wightii</i>	Polysaccharides	MDAMB-231	IC ₅₀ : 350 µg/ml	Vaikundamoorthy et al. (2018)
	<i>Micromonospora marina</i>	Surfactin	MCF7	IC50: 10 µg/ml	Ramalingam et al. (2019)
	<i>Pseudomonas aeruginosa</i> MRO1	Mono- and Di-rhamnolipids	MCF-7	IC ₅₀ : 25.87 µg/ml	Rahimi et al. (2019)
	<i>Bacillus safensis</i> F4	Biosurfactant	T47D	IC ₅₀ : 0.66 mg mL ⁻¹	Abdelli et al. (2019)
	<i>Rhodococcus wratislaviensis</i>	Trehalose lipid	MCF7	IC ₅₀ : 75 µM	Nikolova et al. (2020)
	<i>Planococcus maritimus</i> SAMP MCC 3013	Glycolipid	MCF7	IC ₅₀ : 42.79 ± 6.07 µg/mL	Waghmode et al. (2020)

	<i>Pseudomonas aeruginosa</i> BN10	Rhamnolipids	MCF-10-A MCF7	IC ₅₀ : 11.82 µg/ mL IC ₅₀ of 8.68 µg/ mL	Semkova et al. (2021)
	<i>Bacillus velezensis</i> strain T701	Lipopeptide	MCF-7	IC ₅₀ : 66.81 µg/ml	Jiang et al. (2021)
	<i>Bacillus velezensis</i> strain T701	Lipopeptide	BT474	IC ₅₀ : 95.04 µg/ml	Jiang et al. (2021)
Oral cancer	<i>Acinetobacter junii</i>	Lipopeptide	KB	IC ₅₀ : 2.4 ± 0.5 mg/ml	Ohadi et al. (2020)
Lung cancer	<i>Bacillus atrophaeus</i> strain AKLSR1	Cyclic Lipopeptides	A549	IC ₅₀ : 25.76 µg/ml	Routhu et al. (2019)
	<i>Acinetobacter indicus</i> M6	Biosurfactant	A541 lung cancer cell line	IC ₅₀ :100 µg/ml	Karlapudi et al. (2020)
Colon cancer	<i>Bacillus subtilis</i>	Surfactin	LoYo	IC ₅₀ : 26 µM	Kim et al. (2007)
	<i>Bacillus circulans</i> DMS-2	Surfactin-like lipopeptides	HCT-15 and HT-29 colon cancer cells	IC ₅₀ : 77 and 116 µM	Sivapathasekaran et al. (2010)
	<i>Bacillus subtilis</i> fmbJ	Fengycin	HT29	IC ₅₀ : 20 µg/ml	Cheng et al. (2016)
	<i>Planococcus maritimus</i> SAMP MCC 3013	Glycolipid	HCT	IC ₅₀ : 31.233 ± 5.08 µg/ mL	Waghmode et al. (2020)
	<i>Pseudomonas</i> sp	Biosurfactant	Colon cancer cell line (SW480)	IC ₅₀ : 168.52 µg/ ml	Haque and Hassan (2020)
Leukemia	<i>Bacillus subtilis</i> natto T-2	Crude cyclic lipopeptides (CLPs)	Human K562 leukemia cells	IC ₅₀ :10 µM	Wang et al. (2007)
	<i>Exophiala dermatitidis</i>	Biosurfactant	U937, Monocytic leukemia cells	IC ₅₀ : 49.85 µg/ mL	Chiewpattanakul et al. (2010)

(continued)

Table 2 (continued)

Type of cancer	Strain	Biosurfactant	Studied cancer cell line/s	Activity at concentration	References
Hepatocellular carcinoma	<i>Pseudomonas aeruginosa</i> BN10	Rhamnolipids	Human pre-B leukemia line BV-173	IC ₅₀ : 50 µM	Christova et al. (2013)
			SKW-3 (T-cell chronic lymphocytic leukemia)	IC ₅₀ : 54 µM	
			HL-60 (human promyelocytic leukemia)	IC ₅₀ : 67 µM	
Hepatocellular carcinoma	<i>Wickerhamiella domercqiae</i>	Sphorolipid	H7402 human liver cancer cells	IC ₅₀ : 28.66 µg/ml	Chen et al. (2006b)
			Human Bel-7402 hepatoma cells	IC ₅₀ : 35 µM	Liu et al. (2010)
Cervical cancer	<i>B. subtilis</i> HSO121	Surfactin-like lipopeptides	HeLa	IC ₅₀ : 29.89 µg/mL	Chiewpattanakul et al. (2010)
	<i>Exophiala dermatitidis</i>	Biosurfactant	HeLa	IC ₅₀ : 41.41 ± 4.21 µg/mL	Waghmode et al. (2020)
	<i>Planococcus maritimus</i> SAMP MCC 3013	Glycolipid	HeLa	IC ₅₀ : 77.50 µg/ml	Jiang et al. (2021)
	<i>Bacillus velezensis</i> strain T701	Lipopeptide	HeLa		

involve the regulation of NF- κ B, AP-1, PI3K/Akt, and ERK pathways (Park et al. 2013). Glycoprotein (BioEG) formed by *L. paracasei subsp. paracasei* A20 exhibits potential activity when evaluated against breast cancer cells (Duarte et al. 2014).

5.2 Colon Cancer

Colon cancer serves to be the second most occurring cancer, leading to death in Europe. It is the second most common among women, and among men, it is the third most prevalent cancer (WHO 2012). Few studies are reported on the cytotoxic activity of biosurfactants against colon cancer cells. Surfactin isolated from *Bacillus subtilis* inhibits the proliferation of LoVo colon cancer cells by suppressing the PI3K/Akt cell survival signaling through DNA fragmentation, morphological alteration, and modifications in cell regulatory proteins (Kim et al. 2007). Halobacillin is the biosurfactant produced by marine *Bacillus* sp. and it was found to prevent the development of human colon tumor cells (Trischman et al. 1994). The *Planococcus maritimus* SAMP strain produced glycolipid that exhibited cytotoxic activity against HCT cell lines at 31.23 $\mu\text{g}/\text{mL}$ (Waghmode et al. 2020). Lipopeptide obtained from the marine bacterium, *B. circulans* DMS-2 showed inhibition against HCT-15 and HT-29 colon cancer cells and the cell viability was inhibited by 90% at a higher concentration of 290 μM (Sivapathasekaran et al. 2010).

5.3 Leukemia

Leukemia is a kind of cancer of blood forming cells. The leading types of leukemia are acute myeloid leukemia, chronic myeloid leukemia, acute lymphocytic leukemia, and chronic lymphocytic leukemia (American Cancer Society). Radiation, chemotherapy, and transplantation of bone marrow are the main therapeutic options for treating leukemia. Biosurfactants from microbes were found to be effective against leukemia cells in vitro. The glycolipids such as succinoyl trehalose lipid (STL) and mannosylerythritol lipid (MEL) exhibit cytotoxic activity against human promyelocytic leukemia cell line (HL60) (Isoda et al. 1997). A peptide isolated from a fish induced apoptosis in human U937 lymphoma cells by enhancing caspase-3 and caspase-8 action (Lee et al. 2003). In a study, biosurfactant from yeast-like fungus *Exophiala dermatitidis* obtained from palm-oil contaminated soil showed cytotoxicity toward monocytic leukemia (U937) cells (Chiewpattanakul et al. 2010). The lipopeptide from *B. subtilis* can destroy K562 cells at a concentration of 100 μM , with an IC_{50} value of 65.76 μM . Additionally, lipopeptides can upsurge the reactive oxygen species production in K562 cells, suppressing the Bcl-2 expression, promote cytochrome-C (Cyto-C) production followed by cell death induction (Zhou et al. 2018).

5.4 Liver Cancer

Liver cancer is known to be a worldwide health-related problem and it is estimated that more than one million people will be affected by liver cancer in 2025 (Llovet et al. 2021). Hepatocellular carcinoma (HCC) is the widely prevalent liver cancer which is found in more than ~90% of cases (Ozakyol 2017). Liver cancer is the second most prevalent cancer leading to death in men (Bray et al. 2018). Effective therapeutics are needed for the treatment of liver cancer. A study reported that sophorolipid produced by *Wickerhamiella domercqiae* exhibits cytotoxic activity against human liver cancer cells H7402 by inhibiting the cell cycle at the G1 phase, activating caspase-3, and accumulating Ca²⁺ concentration in the cytoplasm, thereby inducing apoptosis (Chen et al. 2006b) (Table 1). A study reported that surfactin CS30–2 isolated from *Bacillus* sp. CS30 showed cytotoxic activity against liver cancer cells (Huh7.5) in a dose-dependent manner. Surfactin CS30-2 induced the production of reactive oxygen species and disturbed cell membrane, and caused cell death (Zhou et al. 2020).

5.5 Other Cancer Types

Surfactin-like lipopeptides were also studied for cytotoxic activity against human oral epidermoid carcinoma (KB-3-1), pancreatic (SW-1990), and rat melanoma (B16) cancers with the IC₅₀ value of 57 ± 2.6, 58 ± 1.6, and 20 ± 1.6 μM, respectively (Liu et al. 2010). Surfactin isolated from *Bacillus atrophaeus* exhibits cytotoxic activity against A549 lung cancer cells by inhibiting G0/G1 cell cycle progression and apoptosis (Routhu et al. 2019). A yeast-like fungus *Exophiala dermatitidis* was reported for its cytotoxic activity against cervical cancer cells (HeLa) (Chiewpattanakul et al. 2010). Furthermore, various biosurfactants such as succinoyl trehalose lipids act against basophilic leukemia by constraining the development of the cells (KU812) (Isoda et al. 1996); sophorolipids act against promyelocytic leukemia (HL60) by networking with the plasma membrane (Isoda et al. 1997) and also against esophageal cancer cells (KYSE109/KYSE450) by growth inhibition (Shao et al. 2012); e-poly-L-lysine acts against cervix adenocarcinoma cells (HeLaS3) by inhibiting its growth (El-Sersy et al. 2012) and viscosin acts against metastatic prostate cancer cells (PC3M) by migration inhibition process (Saini et al. 2008). A lipopeptide extracted from *B. amyloliquefaciens* caused apoptosis in human oral squamous cell carcinoma cell line (Kuo et al. 2015).

6 Conclusion

This chapter discusses the biodegradation and cytotoxic effects of biosurfactants produced by microorganisms. The biosurfactants, glycolipids, phospholipids, and lipopeptides had the potential to degrade poorly soluble polycyclic aromatic hydrocarbons and increase their bioavailability. Recently, many microbial biosurfactants have been identified, but studies on their cytotoxic effects are limited. Furthermore, the interest in screening cytotoxic effects of biosurfactants and identifying their mechanisms is now increasing. Biosurfactants also exhibit promising cytotoxic activity against breast, colon, liver, and leukemia cancer cells. Studies on cytotoxicity of biosurfactants on esophageal cancer, cervix adenocarcinoma, and metastatic prostate cancer are very limited. Biosurfactants could be safe and readily producible in large quantities than synthetic surfactants and had the potential to be the alternative to synthetic anticancer drugs. However, more *in vivo* studies and clinical trials are highly warranted for promoting biosurfactants as anticancer drugs.

7 Challenges and Future Prospects of Biosurfactants

Biosurfactants are recognized as potential substances for biodegradation, emulsification, ingredients in cosmetics and pharmaceutical formulations and also for their many other biological properties. Many commercial companies are interested in developing biosurfactant-based products for bioremediation/biodegradation of pollutants. Most importantly, biosurfactants are attracting researchers and industrialists for their anticancer potentiality. They show promising anticancer activities against various important cancer cells with less toxicity to host cells. Due to the toxicity and other side effects of available anticancer drugs, biologically produced compounds like biosurfactants are highly warranted for clinical management and treatment of cancer patients. The availability of such reports on anticancer activities of biosurfactants is gaining more support and importance for the selection and development of anticancer drugs using microbially produced biosurfactants. However, more research studies should be conducted to further explore biosurfactants as potential anticancer drugs.

Important Websites (Companies, Organizations, and Research groups)

1. Jenei Biotech: <https://www.jeneibiotech.com/biosurfactants>
2. Evonik Biosurfactants—<https://corporate.evonik.com/misc/micro/biosurfactants/index.en.html>
3. Logos Technologies: <https://www.logostech.net/>
4. Holiferm: <https://holiferm.com/>
5. Allied Carbon Solutions USA/English site: <https://www.allied-c-s.co.jp/english-site>
6. Dispersa: <https://www.dispersa.ca/>
7. TeeGene Biotech: <https://www.teegene.co.uk/>

8. Unilever: <https://www.unilever.com/news/news-search/2022/building-a-clean-green-foamproduction-machine/>
9. BioCollection Inc.: <https://gust.com/companies/biocollection-inc>
10. Kaneka: https://www.kaneka.co.jp/en/business/qualityoflife/nbd_002.html
11. The Ecover Company, <http://www.ecover.com/>

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Comparison of Biodegradability, and Toxicity Effect of Biosurfactants with Synthetic Surfactants



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1 Introduction

The size of the surfactant market in 2019 was \$39,901 million worldwide and is expected to grow to \$52,417 million by 2025. Most surfactants used today are synthetic and are applied daily in large amounts in homes and industries (Johnson et al. 2021). However, despite the universality of these components, their environmental impacts are generally overlooked since most of the products that add them are sold as disposable and end up being released into the environment. Surfactants reaching aquatic environments have already been reported as organic pollutants, having been detected in surface waters (Stuart et al. 2012).

Surfactants in industrial, domestic, and medical wastewater present challenges for water treatment plants. Due to the molecular properties of surfactants, they become difficult to remove from water (Siyal et al. 2020), causing residual surfactant content to remain even after treatment. The biodegradability deficiency of synthetic surfactants causes resistance in the environment, even though in some cases the partial degradation products proved to be more toxic than the original surfactant molecule. In addition, surfactants can also increase the spread of different pollutants such as heavy metals, causing extra problems for the ecosystem (Johnson et al. 2021).

In this context, biosurfactants emerged as an alternative to synthetic surfactants (Fig. 1). In contrast to synthetic surfactants, biosurfactants are made up of natural

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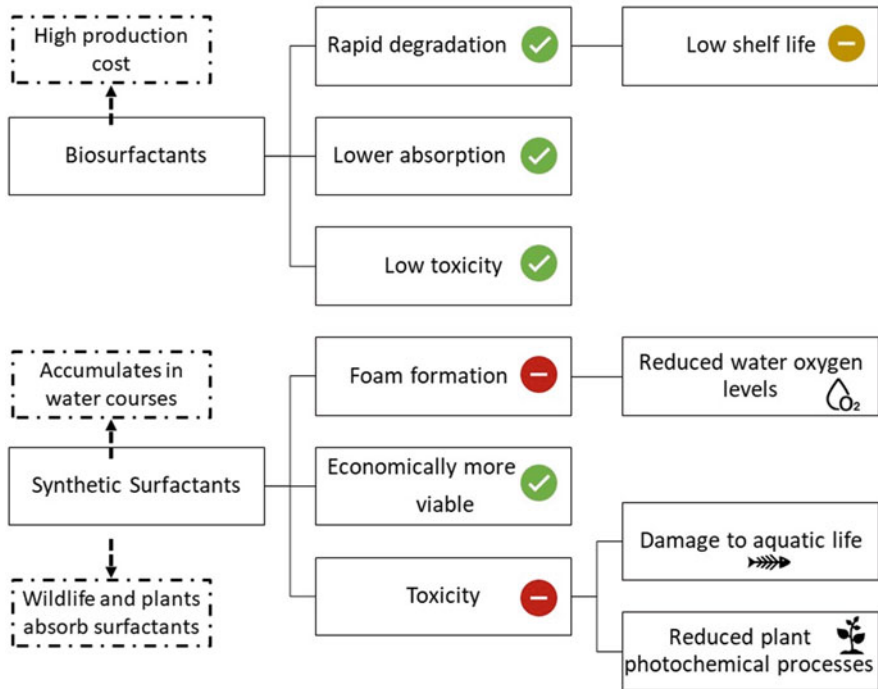


Fig. 1 Surfactants and biosurfactants: comparative advantages and disadvantages. **Source:** The authors

molecules such as lipids, sugars and proteins and are produced by microorganisms. This unique composition gives them preferential properties such as better biodegradability or less toxicity, and yet can maintain their surface properties equivalent to synthetics (Berg 2010; Uchegbu et al. 2013), increasing their acceptance since they generally do not pose an ecological threat.

In this chapter, the environmental results of synthetic surfactants are explored, in addition to uses where biosurfactants have been used as an equivalent, including their comparative advantages and disadvantages involving the low toxicity and high biodegradability of biological biosurfactants in relation to synthetic surfactants. The low toxicity and high biodegradability of biological biosurfactants compared to synthetic surfactants have been reported in recent years and will be exposed in this chapter.

2 The Environmental Impact of Synthetic Surfactants

To reduce the interfacial tensions between two liquids or a liquid and a solid, surfactants are used, thus increasing the cleaning effectiveness of water and other liquids (Siyal et al. 2020). Although there is a great concern related to the environmental impact of synthetic surfactants due to the toxicity of some of these molecules to living organisms and its effect on biological activity (Chen et al. 2018; Fei et al. 2020; Lee et al. 2017; Pradhan and Bhattacharyya 2017).

Surfactants (e.g., cetylpyridinium bromide) caused damage to human lymphocytes, which turns out to be harmful to mammalian cells (Hrabak et al. 1982) and rabbit corneal epithelial cells (Grant et al. 1992). In addition, although few surfactants have less toxicity, their degradation products can have disadvantageous effects, such as impaired estrogen production (Blasco et al. 2003). Surfactants used in agricultural products have also been shown to have a noxious effect on bees through oral toxicity to them (Chen et al. 2018). Since bees are essential for plant pollination, that effect is a huge concern because a loss in the bee population can lead to food deficits (Hristov et al. 2020).

Studies showed that not only do animals suffer harmful effects linked to surfactants, but also showed that these molecules are harmful to photosynthesis, one of the crucial processes for plant life (Masakorala et al. 2011). The occurrence of surfactants in the water can direct to stable foam formation on the surface, thereby reducing the sunlight that reaches the river and sea bed cause of the turbid nature of foams with extreme net fraction, which causes the impact on photosynthesis processes in plants (Lee et al. 2017). Another study revealed that surfactants used in gray water caused toxicity to lettuce; it was observed that these consequences can be relieved with the use of microorganisms that are capable of breaking down the particular surfactant experimented (Bubenheim et al. 1997), although some surfactants in gray water can be feasibly biodegraded and not cause harmful effects for crops.

Because they demonstrate a biodegradability lack in several parts of their molecular structure (Pradhan and Bhattacharyya 2017; Tmáková et al. 2016) when released into the environment, synthetic surfactants remain in systems for a long time and it can be accumulated, especially in places with little movement of material, for example, in sediments, or rivers and lakes with slow-movement currents. Studies have shown that there was a rapid decrease in surfactant concentration remote from the sewer outlet during winter months (high water flow), but the decline was more gradual during summer because the water flow was notably slower (Scott and Jones 2000).

Other effects of synthetic surfactants can be to diffuse other contaminants, including heavy metals, to a broader area. Due to the surface-active properties of surfactants in the environment, they can also improve the solubility of toxic organic compounds in the soil, which can increase the mobility of toxic compounds and additionally contaminate the aquatic environments (Johnson et al. 2021).

As described in the introduction, most surfactants lead to the aquatic environments, through product use and disposal, industrial and domestic processes, or

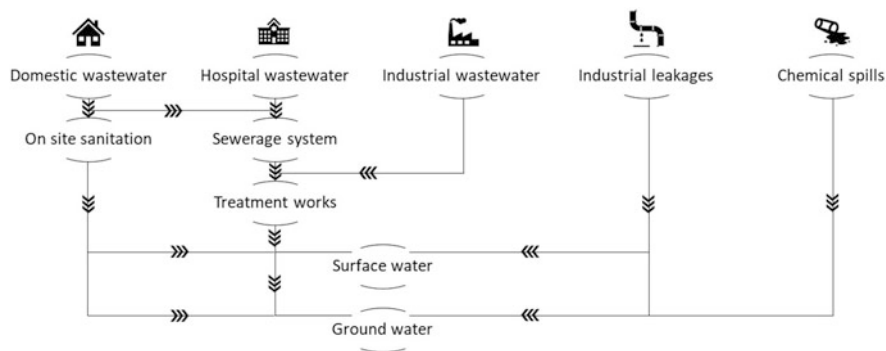


Fig. 2 Pathways of water contamination by surfactants. **Source:** The authors

unintentional events (Fig. 2). Therefore, the biodegradability of biosurfactants is a huge advantage, due to their decomposition while discharged into the environment, instead of continuing and possibly accumulating to significant levels of toxicity, likely the case of synthetic surfactants. Consequently, biosurfactants are suitable for applications in cosmetics and detergents (Moya Ramírez et al. 2016), for instance, where significant amounts of product finish up in the drain. Due to their reduced toxicity to flora and fauna, biosurfactants loose into the environment pose less harmful effects than synthetic surfactants (Hogan et al. 2019; Jahan et al. 2020; Santos et al. 2017).

One of the foremost barriers to the development of the surfactant market for remediation is the shortness of knowledge and studies about their impact on the environment and the toxicity of these molecules (Franzetti et al. 2006). The occurrence of synthetic surfactants in the aquatic environments in the past three decades has developed in large toxicity. Therefore, an extended database of toxicity tests in laboratories of assorted commercial surfactants has been built up over the years. Contrastingly, the biosurfactants' toxicity in the environments has been poorly studied (Santos et al. 2017).

Most synthetic surfactants have a toxic impact on human cells, so the regulation restraining the use of these compounds in products designed for human utilization. The substitutes biosurfactant with low toxicity bring down these restrictions making them *generally recognized as safe* (GRAS) for food (Nitschke and Costa 2007). Therefore, products planned for direct human utilization such as food, skin products, pharmaceuticals, and nutraceuticals, the peculiarity of being digestible and presenting low toxicity has great influence, giving biosurfactants an important advantage over synthetics.

Due to concerns related to the impact in the environments of synthetic surfactants and the consumer preference trend towards more natural products, the prospective replacement of synthetic surfactants with biosurfactants is being evaluated (Mulligan et al. 2014). The biosurfactants offer better biodegradability and low toxicity avoiding the negative effects of synthetic surfactants. A study showed a contrast in the toxicity of three synthetic surfactants and three biosurfactants concluding that

biosurfactants were less toxic than synthetic surfactants against some species of invertebrates (De Cássia et al. 2014).

3 Cytotoxicity of Surfactants and Biosurfactants

Currently, environmental, human, and animal exposure to synthetic surfactants is increasing. Thereupon, cases of people, animals, and environments experiencing toxic effects have also increased. In this regard, some countries have already declared that it is required to assess the irritant potential and cellular damage caused by new pharmaceutical products or ingredients (Basit et al. 2018). Absence or low cytotoxicity is desirable when biosurfactant application is being proposed for humans or as a food additive. Toxicity characterization tests study short-term, acute, and long-term, chronic, substances using a variety of species and measures, e.g., mortality, growth effects, behavioral effects, duration of effect, recovery potential, bioaccumulation, and others (US-EPA 2019).

Synthetic surfactants have been shown to have antagonist effects or low compatibility, particularly in cosmetic or pharmaceutical products, where the association between the use of some surfactants and the increase of dermatitis, skin, and eye irritation has been reported (Mehling et al. 2007; Shiratori et al. 2017). In this sense, biosurfactants emerged as a substitute for synthetic surfactants, since in contrast to them, biosurfactants are products produced by microorganisms formed of natural molecules (Fig. 3). This unique composition gives them the advantage of lower toxicity, while maintaining surface properties similar to synthetics (Rodríguez-López et al. 2018; Rodríguez-López et al. 2019a).

Studies have evaluated the toxicity of a biosurfactant produced by *Sphingobacterium detergens* and it was observed that the compounds tested were

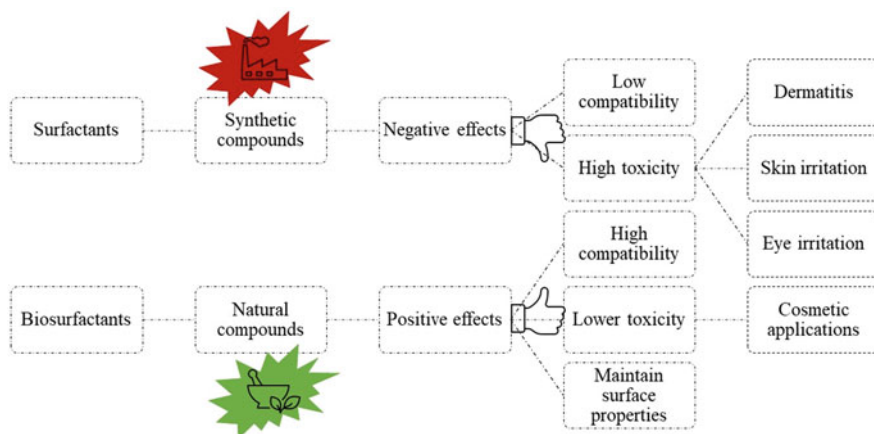


Fig. 3 Surfactants and biosurfactant effects. **Source:** The authors

capable of reducing cell proliferation and inducing apoptosis (Burgos-Díaz et al. 2013). Furthermore, the results achieved with this biosurfactant were better when compared to a synthetic surfactant frequently used in cosmetic products, due to low toxicity effects. In another work, the antibiotic activity against pathogenic bacteria and fungi of a biosurfactant produced by *Pseudomonas aeruginosa* PG1 was demonstrated, along with its non-cytotoxic outcome on mouse fibroblasts (NCTC clone 929), showing a lower cytotoxicity effect of biosurfactants, compared to synthetic surfactants (Makkar et al. 2017).

The results of a biosurfactant produced by *Lactobacillus pentosus* BS5 showed a confident outcome on fibroblasts, reaching 113% cell viability at 1 g/L, whereas the results showed no significant differences between the amount observed in the different concentrations (Rodríguez-Lópezlópez et al. 2020). Another study showed a similar behavior for other biosurfactants, such as a lipopeptide produced by *Streptosporangium amethystogenes* sub sp. *Fukuiense* A1-23,456. The biosurfactant evaluated showed a proliferative effect on bone marrow cells of BALB/c female mice (Cameotra and Makkar 2004). Other lipopeptides, a surfactin, produced by *Bacillus subtilis*, have been shown to have a proliferative and differentiating outcome on mammalian cells (Rodrigues et al. 2006). These studies agree with previous results, whereupon the irritative response of the biosurfactant produced by *L. pentosus* was irrelevant (Rodríguez-López et al. 2019b).

A recent study evaluated the cytotoxicity of the cell-associated biosurfactant produced by *L. pentosus* NCIM 2912 on mouse fibroblasts (ATCC L929), human embryonic kidney 293 cells (HEK-293), and human epithelial cell lines type 2 (HEP-2). The cytotoxicity assay performed did not demonstrate any substantial effect in reducing cell viability compared to PBS (pH 7.0). Cell-associated biosurfactant showed between 90–99% viability when cultured with HEK 293, mouse fibroblasts, and HEP-2 cell lines (Sharma et al. 2021).

Aquatic toxicity was demonstrated in a study in which they evaluated using Microtox and Zebrafish, in addition to human cell cytotoxicity assays - xCELLigence and MTS - to examine monorhamnolipid toxicity. Results showed that new rhamnolipids with altered stereochemistry or congener composition may have different properties. This research showed that these differences caused changes in the biodegradation measurement for zebrafish toxicity and xCELLigence human lung cell toxicity. For acute aquatic prokaryotic toxicity using the Microtox evaluation or for the human MTS cell cytotoxicity investigation, there were no significant differences detected (Hogan et al. 2019).

4 Biodegradation of Biosurfactants

Chemical products that have passed specified screening tests for ultimate biodegradability are allocated as “readily biodegradable” by the Environmental Protection Agency. Screening tests are demanding, so the readily biodegradable status requires that compounds will promptly and biodegrade in aquatic and aerobic environments

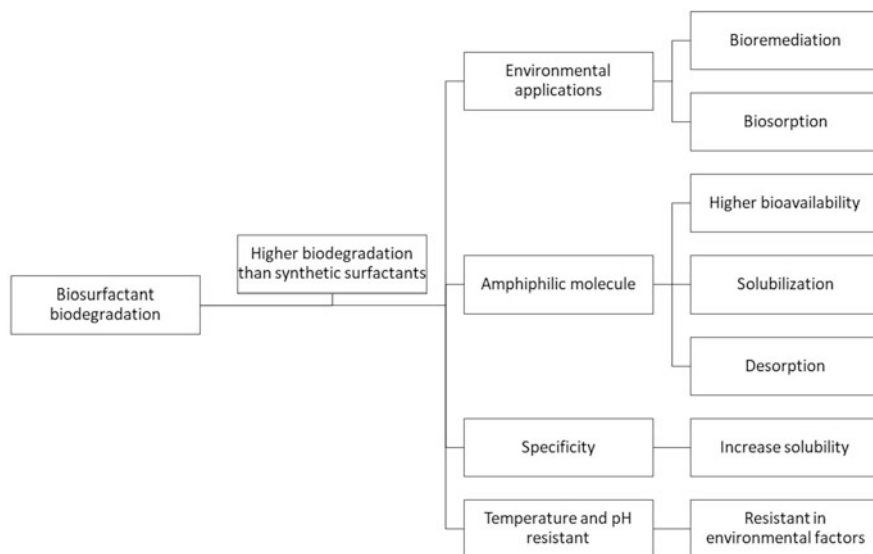


Fig. 4 Advantages of biosurfactant biodegradability. **Source:** The authors

(USEPA 1998). During the process, various tests of biodegradability are imposed, including CO₂ measurement to assess mineralization. To be treated as readily biodegradable, the CO₂ measurement prerequisite values greater than 60% within 10 days after the measured CO₂ exceeds 10% of theoretical CO₂, this 10-day interval needs to be within a 28-day evaluation (Böhmer 2009).

Synthetic surfactants have been shown to cause chronic toxicity to animals in aquatic environments at concentrations above 0.01 g/L, even though some surfactants are more toxic than others (Singh et al. 2002), such as quaternary ammonium-based surfactants, most of them cannot be biodegraded by microorganisms (Garcia et al. 2016). To avert these complications, the use of biosurfactants rather than synthetic surfactants can be an attractive substitute in several industrial sectors (Fig. 4).

Studies have shown that the biodegradation of biosurfactants begins instantly after its culture, being manifested in values of BOD/TOD (Biochemical Oxygen Demand to Total Oxygen Demand). Hirata et al. (2009) tested the biodegradability of sophorolipid biosurfactants produced by the non-pathogenic yeast *Candida bombicola*, which reached a level of 61% after 8 days of cultivation. In the same study, the biodegradation of two other biosurfactants, surfactin and arctogactin, was evaluated, with results comparable to the first, however, the synthetic surfactants did not show biodegradability after 8 days.

Another study revealed that the biodegradation of a biosurfactant rhamnolipid happens under aerobic and anaerobic conditions, exhibiting a soluble COD removal efficiency of 74% and 47.2% after 10 and 6 days, respectively. While the Triton X-100, a synthetic surfactant, was not biodegradable under anaerobic circumstances

and it was moderately biodegradable under aerobic circumstances, with soluble COD results of 47.1% at concentrations under 900 mg/L after 10 days (Mohan et al. 2006). Another lipid biosurfactant, mannosylerythritol (MEL), produced by *Candida Antarctica* also showed results of easy biodegradation. This biosurfactant was degraded by activated sludge microorganisms, exhibiting almost complete degradation in 5 days, while synthetic surfactants were barely degraded after 7 days of the experiment (Kim et al. 2002).

Studies have shown the biodegradation of a rhamnolipid in two different soil, clay and sand. In this study, the biodegradation of this biosurfactant was comparatively low in the first two days of the experiment, increasing strongly on the third day, also after seven days of evaluation, the biosurfactant was practically completely degraded, showing 92% of degradation in the two types of soil evaluated (Pei et al. 2009). A separate study evaluated a rhamnolipid and showed complete degradation after 4 days of incubation by a mixed bacterial population isolated from the soil (Fiebig et al. 1997).

A recent study showed the mineralization of monorhamnolipids for all treatments evaluated with percentages ranging from 34% to 92%. Bio-mRL, Rha-C10-C10(R, R), and Rha-C10-C10(S, R) were treated as readily biodegradable showing 60% or more mineralization. Although the mixture of diastereomer was readily biodegradable, showing 69% mineralization, it was noted that Rha-C10-C10 (S, R) (51% mineralization) and Rha-C10-C10 (S, S) (34% mineralization) did not follow the readily biodegradable norm for this test (Hogan et al. 2019).

Biosurfactants have also been shown to biodegrade in marine environments. One study showed that an exopolysaccharide biosurfactant was readily biodegradable in the marine environment by bacterial strains and its mineralization exceeded 90% using *Pseudoalteromonas* sp., while when using *Vibrio proteolyticus* the mineralization showed a smaller efficacious, with 60% (Cappello et al. 2011).

Tests of biodegradability were performed in liquid and soil using five biosurfactants, produced by *Bacillus* sp., *Flavobacterium* sp., *Dietzia maris*, and *Arthrobacter oxydans*, and a synthetic surfactant. These studies showed that the capability of degradation is dependent on the bacteria used. The culture mix of the five biosurfactants in soil showed a biodegradability of 42.5% to 73.4%, meantime, the biodegradability of the synthetic surfactant on a 7 days incubation study was reduced, showing only 24.8% degradation (Lima et al. 2011).

Another recent study submitted a biosurfactant, spontaneously fermented, composed of crude extract collected in the corn wet milling industry in a biodegradation study, showing biodegradation percentages between 3 and 80%. This work was carried out without the addition of external microbial biomass, under different conditions of temperature, biodegradation time and pH (Rodríguez-López et al. 2019a). In conclusion, it was determined that the biodegradation of the biosurfactant, with the absence of an inoculum addition, is associated with conditions of the environment. Prompt biodegradation of the biosurfactant would not be advantageous, due to the risk that it could be biodegraded before performing its function. This is very important for the use of biosurfactants in various applications. Thus, it is

possible to establish harmony among the stability and biodegradability of a biosurfactant in different environments and industrial formulations.

5 Synthetic Surfactants and Biosurfactants in the Cosmetic Industry

Today, a considerable portion of surfactants compose the formulations of cosmetic and personal care products, reaching values of up to 50%. These large amounts of synthetic surfactants in these products can increase the risk of side effects, so it is necessary to shorten the amount of surfactants in the products while maintaining the same capability (Moldes et al. 2021). However, biosurfactants are a good alternative to replace synthetic surfactants in commercialized products, as they avert side effects, are less toxic, and have greater biodegradability (Jahan et al. 2020).

Unlike synthetic surfactants, biosurfactants can be promptly biodegraded by microorganisms (Otzen 2017; Rodríguez-López et al. 2020) and, because they are formed by lipids, sugars, and proteins, they have less cytotoxicity, which makes them suitable ingredients for the food industries, cosmetics, and pharmaceuticals (Marcia Nitschke et al. 2017; Vecino et al. 2017). The application of biosurfactants in various industrial sectors may reduce the number of cases, like allergies and side effects caused by synthetic surfactants incorporated in cosmetic products (Boozalis and Patel 2018; Kosumi et al. 2017; Martínez-González et al. 2017).

Biosurfactants have higher production costs when compared to synthetic surfactants and, for this reason, most biosurfactants are applied in the cosmetics and pharmaceutical industries, due to the small amount used in each product. Biosurfactants obtained directly from fermented agro-industrial streams, as such, are extracted from maize steeping water, has its costs are reduced (Vecino et al. 2014, 2015). This type of biosurfactant has costs that are competitive with the production costs of synthetic surfactants, as they are obtained directly from corn wet milling waste streams. This type of extraction has an international patent, as it is a new source of biosurfactants (Moldes et al. 2014). The biosurfactant extracted in this way has already been tested in the cosmetics industry, as part of the formulation of hair care (Rincón et al. 2017; Rincón et al. 2020) and skin care (Rincón-Fontán et al. 2020; Rodríguez-López et al. 2022) products.

Microbial biosurfactants have been applied in cosmetic, personal care, and pharmaceutical formulations, obtaining optimistic results with fewer side effects than formulations with the application of synthetic surfactants (Fig. 5). These biosurfactants have a broad possibility of applications, acting as anti-aging agents (Piljac and Piljac 2007), cleansers in shampoos (Allef et al. 2016), body wash (Kulkarni and Choudhary 2011) and others. In addition, biosurfactants are also being used in the pharmaceutical area, where they can be incorporated in several products due to their antimicrobial (P. Das et al. 2008), anti-adhesive (Nickzad and

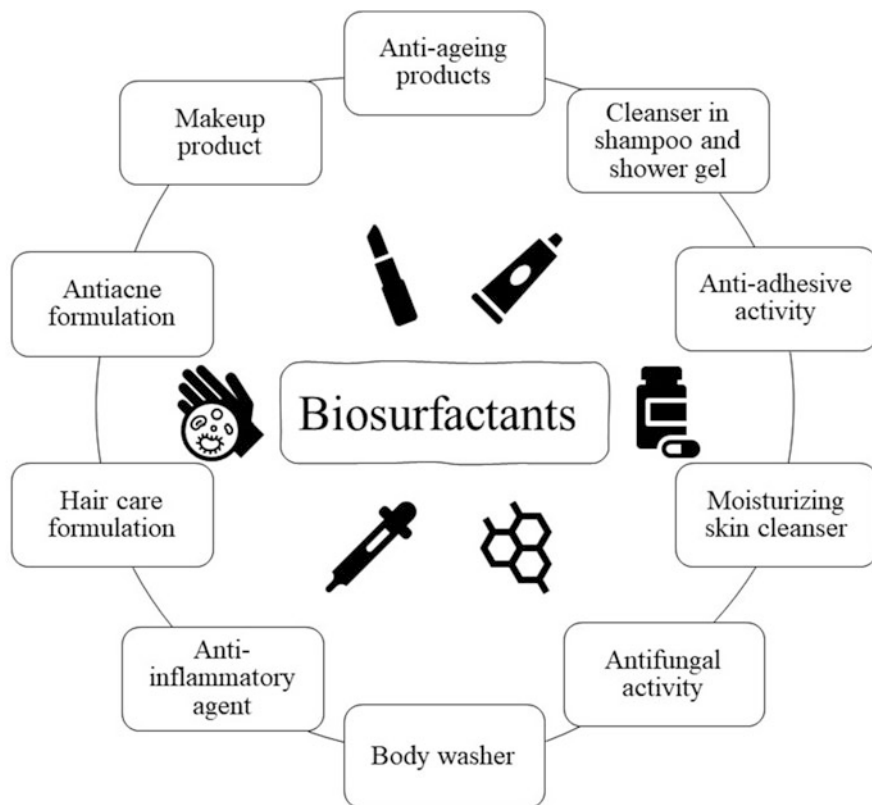


Fig. 5 Biosurfactant application in cosmetic and pharmaceutical formulations. **Source:** The authors

Eziel 2013), anti-inflammatory (Hagler et al. 2007) and anti-viral (Wu et al. 2015) capacities, which is why they are considered substances with enormous potential.

A major advance for the cosmetic industry is the development of new products with sun protection effects, a study evaluated the production of a “green” sunscreen with a mineral base and a biosurfactant. In this work, the synergistic relationship between the mineral and the biosurfactant and the protection against the negative effects of the sun provided by the product (Rincón-Fontán et al. 2018) was observed. In addition, in another work, the same compounds were evaluated as stabilizers in a cosmetic containing vitamin E, and the presence of the biosurfactant increased the emulsion volume by 70% after 22 days (Rincón-Fontán et al. 2019).

In one study, a biosurfactant produced by *Lactobacillus paracasei* was evaluated as a stabilizing agent in oil–water emulsions, with the presence of essential oils and a natural antioxidant extract of grape seeds (Ferreira et al. 2017). The assays were performed using a 3 T3 mouse fibroblast line, being compared with a synthetic surfactant. As a result, emulsion values around 100% were obtained in 7 days when

the biosurfactant was used together with the antioxidant extract and values of 97% of emulsion value only with the biosurfactant, while the synthetic surfactant showed a great inhibitory effect of the fibroblast cells.

Biosurfactants are also being applied in other personal care products, such as toothpaste and mouthwash. A study evaluated the replacement of a chemical surfactant, normally used in commercial toothpaste, for a biosurfactant produced by *Nocardiopsis* VITSISB (I. Das et al. 2013). It was observed that the biosurfactant was more effective in the formulation and less toxic than the synthetic surfactant. In another study involving personal care, different types of mouthwashes were formulated from biosurfactants produced by *Pseudomonas aeruginosa* UCP 0992, *Bacillus cereus* UCP 1615, and *Candida bombicola* URM 3718 with the addition of peppermint essential oil (Farias et al. 2019). From these formulations, the antimicrobial activity against oral microorganisms, as well as its toxicity in mouse fibroblasts and macrophages were evaluated. The results showed that using biosurfactants in mouthwashes makes them less toxic than commercials that use synthetic surfactants.

None of these studies evaluated the biodegradation of the tested biosurfactants, which would be another advantage of their use. On the other hand, several works have confirmed that the toxicity of surfactants and biosurfactants can reach the ecosystem and cause damage. In addition, chemical surfactants can also produce allergic and other reactions when included in cosmetic formulations, such as skin reactions (Mangodt et al. 2019; Martínez-González et al. 2017), dermatitis (Kosumi et al. 2017; Warsaw et al. 2018), eye irritation (Cotovio et al. 2010), and scarification (Rieger and Rhein 2017).

Undesirable reactions of surfactants can be prevented by using biosurfactants. Nonetheless, the threat of incorporating natural ingredients or biosurfactants into cosmetic formulations is a safety assessment in agreement with the Scientific Committee on Consumer Safety (Mellou et al. 2019). For this purpose, *in vitro* tests need to be applied to meet the safety of cosmetic ingredients, especially their cytotoxicity.

6 Biosurfactants Applied in the Food Industry

A diverse number of studies in the literature report the advantages of biosurfactants over synthetic surfactants, as discussed above. Especially in the food industry, the superiority of biosurfactants is important mainly in food processing and the final quality of the products (Ribeiro et al. 2020a, b, c). The properties of biosurfactants, such as resistance to temperature oscillations, acidity, and salinity, allow the quality of the product to be positively influenced. Recently, studies have shown that biosurfactants produced by yeast are stable at temperatures up to 250 °C using thermogravimetry (Ribeiro et al. 2020a, b, c). In addition to these properties, biosurfactants have greater biodegradability and reduced toxicity, which have already been proven by analyzes that show that the cytotoxic potential against

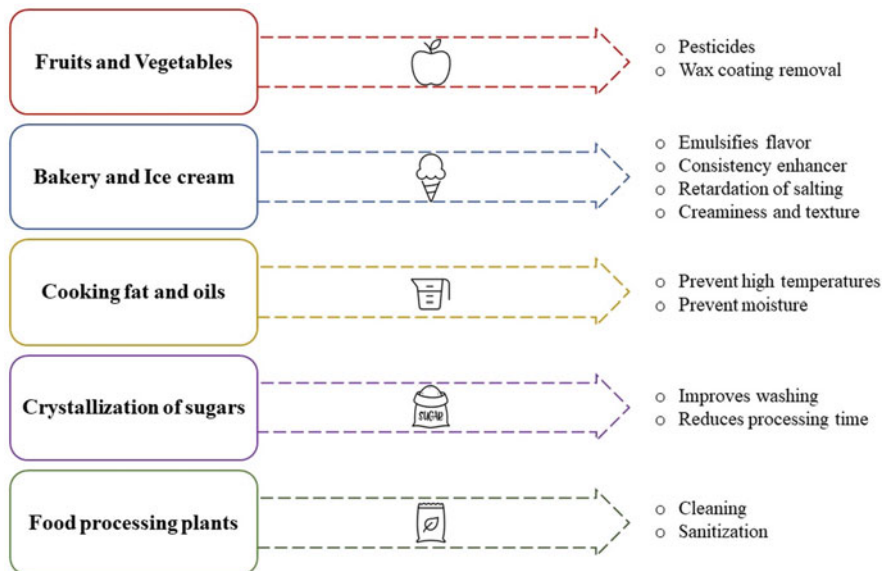


Fig. 6 Biosurfactant applications in the food industry. **Source:** The authors

mammalian cells assess the demands of consumers who prefer to consume natural and safer foods. Biosurfactants have also been shown to prevent associated human health risks that synthetic surfactants generally used in foods can cause when consumed in high amounts.

The great diversification of biosurfactant molecules increases the power of biomolecules selected with suitable properties for each application. As well as the possibility of connecting more than one property, such as antioxidant, anti-adhesive, and antimicrobial activity, which are also advantages that exceed the normal properties of synthetic surfactants and that can increase even more the value of the food (Zouari et al. 2016).

To generate formulations that guarantee the safety of consumers and do not cause damage to health, the food industry determines strict rules for the control of microorganisms. In addition to food safety, it is extremely important for the consumer that the final product has a high level of quality and acceptance, as interest in foods with fewer artificial and chemically synthesized compounds and rich in natural ingredients is growing (Garcia et al. 2020; Nwoba et al. 2020).

Due to increasing demand in the past years, new products are being developed to substitute synthetic additives with natural compounds, but with microwave cooking and irradiation, the natural molecules can lose some of their unique properties. Along these lines, new additives with thickening, stabilizing, and emulsifying properties, as well as antioxidant, anti-adhesive, and antimicrobial properties, are being produced, isolated, and identified, (Faustino et al. 2019; Nitschke and Costa 2007); some the biosurfactants applications are shown in Fig. 6.

The literature reports biosurfactants with great emulsifying properties, in some cases, they are called bioemulsifiers. It is important to consider that emulsifying and dispersing additives do not necessarily need to be able to decrease the surface tension of water or hydrocarbons, but rather confer the reduction of surface energy between the phases and the formation of static and electrostatic barriers, preventing the adhesion of the particles (Campos et al. 2013; Campos et al. 2019).

With the same objective of emulsifiers, the use of biosurfactants in bakeries was raised to reduce the use of synthetic additives currently commercialized and to improve the viscosity of the products. Although little explored, there are some reports of biosurfactants used in bakery formulations that showed improvements in the texture profile of the dough, in addition to reducing the calories in muffins and cookies, which demonstrates great industrial potential (Bandyopadhyay et al. 2014).

In addition to emulsifying and stabilizing functions in food formulations, biosurfactants also have antimicrobial and anti-adhesive effects and hence can be used to increase the shelf life of products. Thus, by controlling the adherence of microorganisms to the contact surfaces of food and equipment, biosurfactants can contribute considerably to the development of procedures to prevent microbial proliferation, ensuring safe and quality products (Kieliszek et al. 2017). Therefore, potential applications of biosurfactants have been suggested as anti-adhesive agents in the processing of dairy products, which is common for biofilm formation and accumulation of microorganisms on the pasteurizer (equipment responsible for heat exchange) (Campos et al. 2013; Marcelino et al. 2020).

The dense need of consumers for “completely natural” foods makes studies aimed at the food industry want to identify natural alternatives to the many synthetic ingredients used in food formulations today (Ribeiro et al. 2020a, b, c). Thus, biosurfactants are the perfect alternative to their synthetic equivalents, as they have low toxicity, remarkable physicochemical characteristics, bioavailability, biodegradability, and synthesis from renewable sources.

7 Future Directions

To make it possible to use only biosurfactants in industries, it is necessary to plan strategies that can use a combination of non-pathogenic strains with a high level of production, in addition to the use of renewable substrates so that it is possible to achieve total cost reduction and also the reduction of synthetic molecules. The strains used should preferably be those that can produce greater amounts of biosurfactant using one or more renewable substrates, such as residues from milling corn, coffee, and sugar cane, among others. It is also evident that the use of non-pathogenic microorganisms has a better promising effect compared to their individual effects, as they can be applied in the pharmaceutical, medical, and cosmetic industries. Thus, future research should focus on combining these strategies for the complete removal of synthetic surfactants.

8 Conclusions

The growing need for surfactants by industries, mainly cosmetic, pharmaceutical, and food, has caused a large consumption of synthetic surfactants, which are frequently toxic, irritating, and non-biodegradable. Biosurfactants emerged as a substitute for synthetic surfactants, being biologically based compounds that can be obtained from renewable sources, thus reducing the use of synthetic sources, mainly petrochemicals. However, the biosurfactants produced, which use biological reactions, as secondary metabolites, may show an auspicious alternative, whereas they have lipids, carbohydrates, or proteins in their composition, making them with better biocompatibility and biodegradability than the synthetic surfactants. In addition, biosurfactants have low toxicity, high biodegradability, stability under extreme conditions, and several possibilities for industrial applications.

However, biosurfactants are still not widely used in the industry, despite their great and important advantages. Technological production, mainly related to the substrate used in the culture medium, is still the highest production cost of biosurfactants and the main problem. In addition to this production cost, there are still high costs in the extraction and purification phases of these molecules. For this reason, it is needed to further study the use of renewable, inexpensive sources that can be used as a substrate in the production of residual sources that can be used for the spontaneous production of biosurfactants, in addition to seeking an increase in global productivity, obtaining microorganisms that produce greater quantities. Finally, as a result of the current move towards “green” consumption, it is prospective that studies will increase significantly in the development of cosmetic, personal care, pharmaceutical, and food products in which synthetic surfactants are substituted by renewable and eco-friendly biosurfactants.

Companies, Organizations, and Research Groups Working on the Topic

<https://www.unilever.com/news/news-search/2022/building-a-clean-green-foamproduction-machine/>

<https://www.dispersa.ca/>

<https://www.ulster.ac.uk/staff/im-banat>

<https://scholar.google.com/citations?user=2kQzN7kAAAAJ&hl=pt-BR&oi=sra>
<https://scholar.google.com/citations?user=k1OF-vAAAAJ&hl=pt-BR&oi=sra>

<https://www.allied-c-s-usa.com/>

<https://www.teegene.co.uk/>

<https://corporate.evonik.com/misc/micro/biosurfactants/index.en.html>

https://scholar.google.com/citations?user=0XilX_YAAAAJ&hl=pt-BR&oi=sra

<http://www.debiq.eel.usp.br/~silvio/integrantes.htm>

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Surface Activity and Emulsification Properties of Saponins as Biosurfactants



Sweeta Akbari, Nour Hamid Abdurahman, and Viacheslau Kudrashou

1 Introduction

Saponins are the secondary metabolites widely distributed in many plants and some marine animals (Oleszek 2002; Skountzou et al. 2017). Saponins are classified as steroid or triterpenoid glycosides where this classification is based on the aglycone part of the molecule. The saponin word is obtained from a Latin word called “sapo” meaning soap that makes it highly amphipathic with hydrophilic and hydrophobic properties (Den Brok et al. 2016; Singh et al. 2017). Saponins are commonly found secondary metabolites in plants. Saponins are also found in foodstuff such as onion, spinach, peanuts, garlic, [asparagus](#), and many other plants. Essentially, it protects plants from the attacks of pathogens and herbivores. In addition, food and medicine industries are interested to use them as an active constituent in their products (Augustin et al. 2011). The division of saponins as triterpenoids and steroidal glycosides helps to distinguish the types of saponins by the sugar chains attached at different positions (Pagureva et al. 2016; Poojary et al. 2015). In fact, many plants have indicated a great phytochemical and medicinal properties which are useful in

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treating some diseases such as tumor, cancer, diabetes, heart problems, and infections (Riasat et al. 2018).

Despite of having the above properties, saponin also possesses emulsifying and foaming characteristic which makes it a useful additive for food, pharmaceutical and cosmetic products. Saponins are also known as biosurfactant or surface-active materials with bioactivity. Currently, the production of biosurfactants from natural sources are becoming very attractive for industries due to its low risk and eco-friendly properties (Marchant and Banat 2012). The surfactants obtained from natural sources have similar properties to the commercially available surfactants by containing hydrophobic (water-heating or oil-loving) and hydrophilic (water-loving) compartments (Fracchia et al. 2012). Biosurfactants (saponins) can be acquired from plants and some sea animals (Boruah and Gogoi 2013; Cheeke 2010; Cheok et al. 2014).

Plants contain different types of bioactive compounds such as flavonoids, phenolic acids, tannins, alkaloids, lectins and saponins (Akbari et al. 2019; Bernhoft 2010; Kumar et al. 2017; Mohan et al. 2015; Moyo et al. 2013). The benefits of these compounds to human health are widely investigated. Bioactive compounds of plants act as antiviral, anticancer, antiinflammation, antimicrobial, and antioxidant agents (Yang et al. 2013a, b; Mgbeahuruike et al. 2017; Mohan et al. 2015). Among them, saponin has attracted many researchers from health to wastewater treatment studies, since besides their wide biological activity they have also indicated good surface activity which supports their application in food, pharmaceutical, and cosmetic emulsions (Akbari et al. 2018). Saponins are high molecular weight biological compounds with distinct properties. The application of saponins in food sources was neglected for a long time and in some cases their uses have been limited due to their hemolytic activity, toxicity and antinutritive effects in foods (Dias and Sales 2017). However, recent studies have appreciated their potential nutritive value in foods and health benefit such as anticancer activity and cholesterol-lowering activity (Guclu-Ustundag and Mazza 2007; Ma et al. 2017; D. Zhang et al. 2020).

Due to the surfactant properties, saponins are often used in food, cosmetic, and pharmaceutical industries. Studies indicated that saponins are able to stabilize nanoemulsions and nanosuspensions with a very small droplet size of less than 100 nm (Aswathanarayan and Vittal 2019; Ven et al. 2010). Currently, QS-21 and Californian tree *Yucca schidigera* are the most popular source of saponins for commercial uses (Góral and Wojciechowski 2020; Wojciechowski et al. 2014).

The ability of saponins to reduce the Low-density lipoprotein (LDL) cholesterol level in the body and exhibiting a high antiviral and adjuvant activity made this compound a promising candidate for future development of medical drug production (Marrelli et al. 2016; Shi et al. 2004). This chapter reviews the structure, surface activity and emulsification properties of plant-based saponin as biosurfactants, CMC, and stabilizing the nanoemulsions using saponins.

2 Structure Diversity and Properties

Saponins are naturally occurring phytochemicals with a vast functional and structural diversity, out of four plants at least it occurs in three plants. These bioactive compounds are composed of a hydrophilic sugar chain (glycone) and a hydrophobic sapogenin (aglycone), which make them highly amphipathic (Navarro del Hierro et al. 2018; Sidana et al. 2016). Based on the aglycone structure, they are classified as steroid and triterpenoid saponins. The subcategories of steroid and triterpenoid saponins also exist. For example, steroid saponins can be further classified as spirostanol and furostanol saponins and triterpenoid saponins can be found as dammarane, lupanes, tirucallanes, hopanes, taraxasteranes, oleananes, ursanes, lanostanes, cycloartanes, and cucurbitanes types (Böttcher and Drusch 2017; Vincken et al. 2007). Based on sugar chain structures, saponins with one, two, and three sugar chains are described as monodesmosidic, bidesmosidic, and the uncommon tridesmosidic, respectively (Fig. 1a–c). The sugar chains of monodesmosidic (with one sugar chain) and bidesmosidic (with two sugar chains) saponins are normally attached at C-3 and C-28 of the aglycone, respectively (Guclu-Ustundag and Mazza 2007). In bidesmosidic saponins, usually the sugar chain attached at C-3 through an ether linkage and at C-28 through an ester linkage (triterpene saponins) or at C-26 an ether linkage for furostanol saponin type. The most common sugars (monosaccharides) are D-galactose (Gal), D-glucose (Glc), D-galacturonic acid (GalA), D-fucose (Fuc), D-glucuronic acid (GlcA), D-xylose (Xyl), L-arabinose (Ara), and L-rhamnose (Rha) (Guclu-Ustundag and Mazza 2007; Lorent et al. 2014).

3 Surface Properties of Saponins

In saponins, the hydrophilic part (head) is a sugar chain, and the hydrophobic part (tail) can be a steroid or triterpenoid which is also called sapogenin (Fig. 2a, b). Saponins are dissolvable in water with higher molecular weights in the ranges of 600 to 2000 Daltons (Goel 2012; Peter et al. 2004). One of the important characteristics of saponins is the foaming properties which makes them able to act as surface-active agents. Surface-active agents are widely used chemicals in different industries

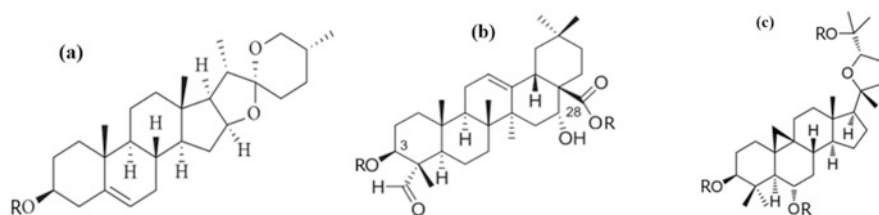


Fig. 1 Examples of monodesmosidic (a), bidesmosidic (b) and tridesmosidic (c) saponins. Figure adapted with the permission from ref. Scognamiglio et al. (2015), copyright 2015, Elsevier

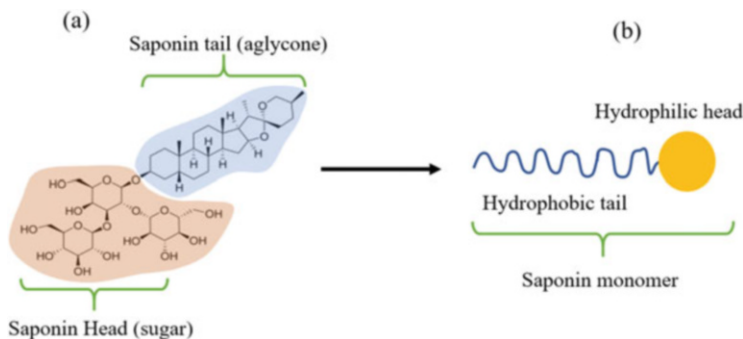


Fig. 2 Chemical structure of saponin (a) and saponin monomer (b)

which include paper products, detergent, paint, petroleum, pharmaceuticals, textile, cosmetics, food, water and soil treatment (Elazzazy et al. 2015; Guclu-Ustundag and Mazza 2007; Gupta et al. 2013).

A wide range of saponins with various structure and functional properties can be found in the nature, this is due to availability of different sapogenins and sugar chain attached at different positions (Lorent et al. 2014). The terms hydrophilic and hydrophobic are referred to water loving and water hating in an amphipathic compound, respectively. In fact, in a saponin molecule, the hydrophilic and hydrophobic parts represent the interfacial properties (Liu et al. 2017; Vincken et al. 2007). Plant-based surfactants (saponins) are biocompatible, biodegradable, and less toxic to humans and the environment. Many studies found that saponins can stabilize the emulsions of oil-in-water (O/W) and the multiple emulsions of water-in-oil-in-water (W/O/W). However, there is no data available to report the use of saponin in water-in-oil (W/O) type emulsions. The reason might be the high hydrophile–lipophile balance (HLB) values of saponins. Surfactants with higher HLB values ($HLB > 11$) are water soluble (hydrophilic) and preferred for O/W emulsions. However, low HLB value ($HLB < 9$) surfactants are oil soluble (lipophilic) and are suitable for W/O emulsions (Schramm et al. 2003; Williams 2007). Generally, the HLB numbers can be between 1 to 30 or more (Williams 2007). Table 1 shows the HLB values of some plant-based saponins.

The amphiphilic nature of saponins is due to the existence of water-soluble sugar chain and lipid-soluble aglycone in the saponin molecule (Fig. 2a, b). Therefore, saponins can be used as wetting, foaming and emulsifying agents and even immunological adjuvants (Akbari et al. 2018; Rekiel et al. 2020; H. X. Sun et al. 2009). The amphiphilic properties of saponins have also indicated a significant role in biological activity of saponins which is also related to the defense of plants against pathogens (Chen et al. 2014; Góral and Wojciechowski 2020). Saponins also display a good rheological properties and high viscoelasticity. The *Quillaja* Saponin (QS) saponins have indicated a potent dilutional rheology and shear compared to some proteins and other biosurfactants. Pagureva et al. (2016) studied the rheology of pure aescins containing more than 95% saponin and horse chestnut extract

Table 1 HLB values of some plant-based saponins

Saponin	HLB	Reference
QS-21	36.3	Oda et al. (2003)
Saponaside A	20.3	Sun et al. (2006)
Lablaboside F	25.5	Sun et al. (2006)
Onjisaponins	24.1	Nagai et al. (2001)
Soyasaponin A1	26.9	Oda et al. (2003)
Soyasaponin A2	21.4	Oda et al. (2003)
Soyasaponin I	13.6	Oda et al. (2003)
Soyasaponin II	12.2	Oda et al. (2003)
Soyasaponin III	10.0	Oda et al. (2003)
Dehydrosoyasaponin I	13	Oda et al. (2003)
Tea saponin	16.26	Yu and He (2018)
Q-Naturale (<i>Quillaja</i> saponin)	> 9	Piorkowski and McClements (2013)

containing 20% saponins. Their findings indicated that pure aescines have a high surface viscoelasticity in both shear and dilatational deformations with a surface tension of 45 mN/m above $c = 10^{-4}$ M. However, the extract of horse chestnut did not indicate a remarkable viscoelastic behavior.

4 Critical Micelle Concentration

Naturally, most of the saponins are soluble in water. In an aqueous system depending on solubility of saponins they accumulate at the air–water interface with orientated hydrophobic tail towards the air and hydrophilic head orientated in waterside (Fig. 3a). In an aqueous solution, when the liquid surface is fully saturated with surfactant molecules this point is called critical micelle concentration (CMC) at this point the surface tension reaches its minimum value (Fig. 3b). Further addition and dissolving of surfactant molecules begin to form micelles. Micelles are defined as colloidal dispersions in nano sizes created from aggregation of amphiphilic molecules in a three-dimensional shape (Lorent et al. 2014; Mukherjee et al. 2016). Generally, a pure fraction of saponins forms micelles in an aqueous system with spherical shape. However, micelles may also appear in rod-like (cylindrical), worm-like, or bilayers shapes as can be seen in Fig. 3c. The size and shape of micelles depends on the nature and molecular geometry of the surfactants (Böttger et al. 2012; Samal et al. 2017). The CMC can be obtained from a plot of saponin concentration versus surface tension or interfacial tension and it varies between saponins and their extracts. The micellar properties can be affected by temperature, pH, and salinity (Jiang et al. 2014; Shah et al. 2016). CMC indicates the efficiency of a surfactant; lower CMC means less surfactant molecules in a system to saturate interface and form micelles. Micelles solubilize the oil and dirt by lifting them into the solution from the surface (Jha et al. 2016; Williams 2007).

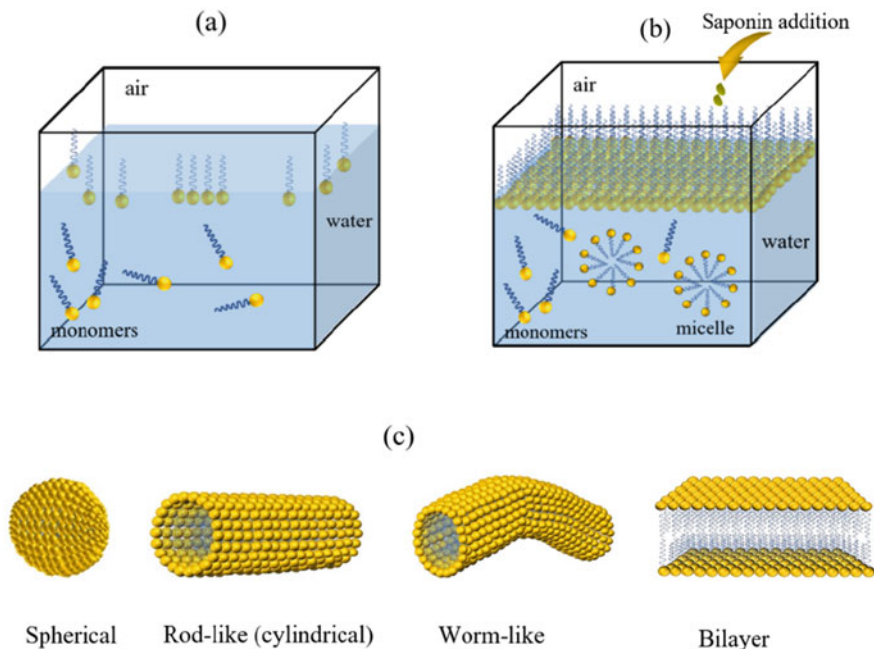


Fig. 3 Saponin behavior in aqueous solution (a), micelle and CMC formation (b); different shapes of micelle (c)

5 Saponins as Biosurfactants

There are many natural food-grade emulsifiers, such as polysaccharides, proteins, saponins, and phospholipids. The emulsification properties of these compounds are due to their amphiphilic properties. Among them, saponins are more effective to produce smaller droplet size even at low concentrations. This may be due to low molecular weight 1.67 kDa and high tendency to adsorb to the droplets surface and create thin interfacial layers.

Biosurfactants have several advantages over the synthetic surfactants and they can be obtained from different sources. The advantages of biosurfactants over the chemical derived surfactants are the bioavailability, biodegradability, environment-friendly, high foaming, and low-cost. Hence, their applications in food, cosmetics, and pharmaceutical products are considered safe and less harmful (Bhadoriya and Madoriya 2013; Fracchia et al. 2015; Nitschke and Costa 2007). The wide applications of biosurfactants have made them multifunctional agents from food to petroleum industries. The bulk application of chemically derived surface-active agents in industrial processes may result in contamination of soils, rivers, and even oceans by the industrial discharges. The characteristic and usefulness of biosurfactants as presented in Fig. 4 include solubility enhancement, reduction of surface tension and low CMC (Jha et al. 2016; Mulligan 2009). When a biobased surfactant is able

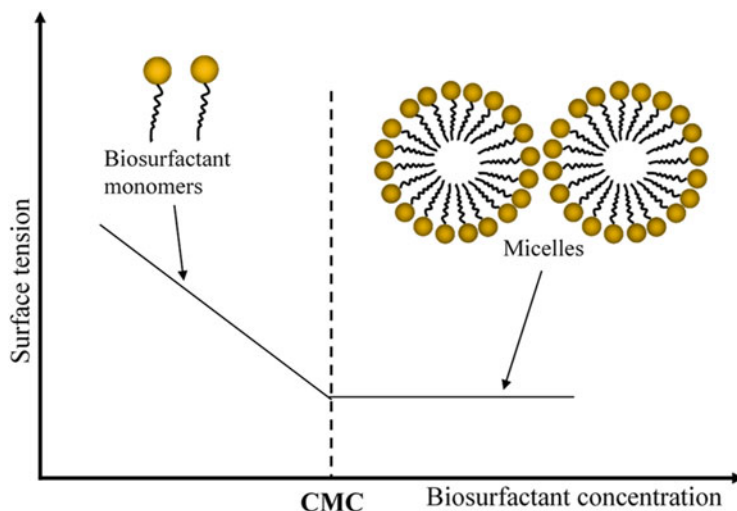


Fig. 4 Biosurfactant monomer and micelle formation

to reduce water surface tension from 72 to around 40 mN/m and interfacial tension between a polar and non-polar liquid and for water against n-hexadecane from 40 to 1 mN/m (Mulligan 2005) is considered effective. In surface and colloid chemistry, CMC is an important characteristic of surfactants defined as the maximum concentration of surfactant monomers in water which can be influenced by temperature, pH, and ionic strength of the solution (Mondal et al. 2015; Pacheco et al. 2010; Shah et al. 2016).

Saponins can be obtained from different plants and vegetal sources. The most common sources of industrial saponins are the saponins obtained from *Quillaja saponaria* tree. Recently, a food-grade emulsifier extracted from the bark of *Quillaja saponaria* tree is introduced in the market with a trade name of Q-Naturale® (Dammak et al. 2020; Yang and McClements 2013). It is claimed that saponin-based amphiphilic molecules are the major surface-active components of Q-Naturale® (Yang et al. 2013a, b). Studies revealed that O/W nanoemulsions prepared with *Quillaja* saponin as emulsifier indicated high stability in the pH ranges of 3 to 7 (Ahmadi and Jafarizadeh-Malmiri 2020).

An O/W and W/O emulsions are consisting of three main components namely, oil/lipid phase, an aqueous phase based on water, and one or more emulsifier. However, these three compounds are not the only phenomenon affecting the formation of an emulsion. Emulsion formation also requires a mechanical energy to disperse one phase into another, and the function of emulsifier is to wrap the dispersed phase in the form of small droplets in micro or nano sizes (Fig. 5) (Akbari et al. 2018). The process of emulsion formation is called emulsification. It is a dynamic process and to form a stable emulsion a mechanical energy is required to break the dispersed phase into very small droplets and disperse it in the continuous phase. Commonly, in emulsification process, two different phases with different

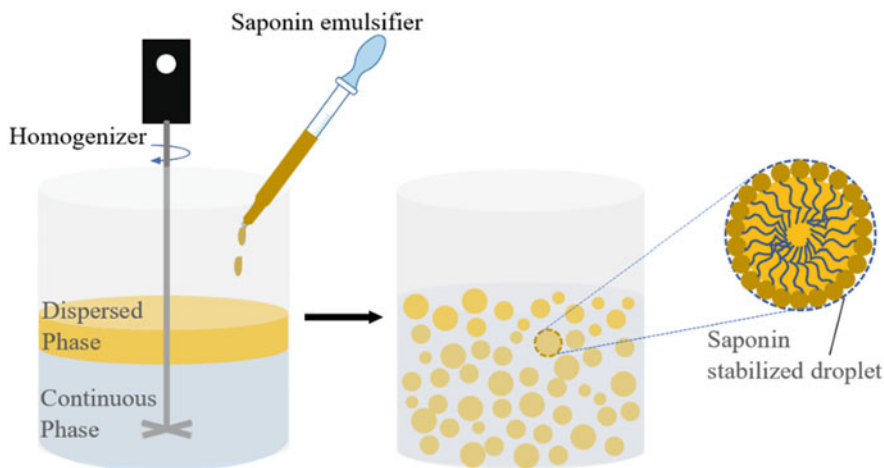


Fig. 5 Fabrication steps of an O/W emulsion stabilized by saponin as emulsifier

densities are required. Emulsifications are carried out using many methods such as mixing with rotor-stator systems, simple shaking, liquid injection through porous membranes, ultrasound generators and high-pressure homogenizers. To produce a stable emulsion, only homogenization is not sufficient. In this case, surfactants or stabilizers are required to make stable emulsion system. Emulsification plays an important role in many industries such as food, cosmetic, pharmaceuticals, and crude oil industries (Mnif and Ghribi 2016).

In addition, type and concentration of emulsifier have key role to form small sized droplets (Ahmadi and Jafarizadeh-Malmiri 2020).

Saponins are surface-active agents containing both hydrophobic and hydrophilic moieties. The important parameters of surfactants are the foaming, wetting and emulsification properties which reduce the surface tension of the water or an emulsion (Varjani and Upasani 2017). Surface tension is the measurement of a liquid-gas or air.

The wettability of a sample is related to the contact angle measurement which indicate the degree of wetting when a liquid and solid interact. (Duan et al. 2018) reported that the smaller the contact angle the better the wettability of the surfactant. The purpose of surfactant application is to enhance the wettability of aqueous solutions on a hydrophobic surface. The wettability of a surfactant is usually evaluated by the degree of the contact angle (θ) created on a solid substrate. When the θ is above 90° , the surfactant is considered to have poor or non-wetting. In the case if the θ is less than 90° , then the surface can get easily wetted and provides a good wetting property as shown in Fig. 6. The foaming property of the saponins is due to the combination of aglycone backbone and sugar chain in their structure. However, in rare cases, some saponins without foaming property have also been observed (Kregiel et al. 2017). Basically, the foam testing is usually performed according to Ross–Mile method (Ross and Miles 1941) in which after the

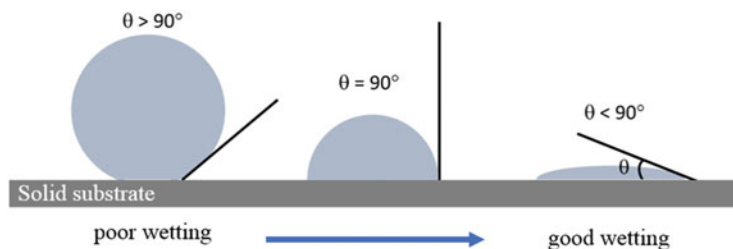


Fig. 6 Wetting property of a surfactant solution. Source: (Akbari et al. 2019b)

mechanical agitation of the surfactant solution the height of the produced foam is measured. Initially, the first foam observed after the agitation is called the foamability while the foam by the time is the measure of foam stability.

6 Saponin-Stabilized Nanoemulsions

Nanoemulsions are system of two immiscible liquid phases where one phase disperses into another. They can be prepared in the forms W/O or O/W emulsions stabilized by an amphiphilic surfactant and the droplet diameter size is usually less than 500 nm. Nanoemulsions have applications in food (Ahmadi and Jafarizadeh-Malmiri 2020; Aswathanarayan and Vittal 2019), cosmetic (Sonneville-aubrun et al. 2018), pharmaceutical, and drug delivery systems (Pathak et al. 2018) and vaccine development process (Ledet et al. 2013). There is not much different between the sizes of a nanoemulsion and a microemulsion. However, nanoemulsions are kinetically stable systems and can be stable for several years, while microemulsions and thermodynamically stable (Ledet et al. 2013). Chen et al. (2017) studied a high internal phase emulsion stabilized by QS-coated nanodroplets for color performance. They first prepared a monodispersed QS-coated nanodroplets (154 nm) via an ultrasonic homogenization and then the prepared nanodroplets were used as stabilizer to form O/W High internal phase emulsion ($\phi = 0.75$). They found that the emulsion droplet size and polydispersity index (PDI) were dependent on concentration of saponin (154 nm: PDI = 0.20) and a very fine emulsion was obtained when the concentration of QS was around 15 mg g^{-1} and beyond that stayed constant (Fig. 7a). The SEM result (Fig. 7b) also confirmed that the average droplet size is about 160 nm indicating a monodispersed spherical morphology showing a good agreement with dynamic light scattering (DLS) results (Fig. 7c). In addition, 15 mg g^{-1} of QS coating indicated a highest surface charge (ζ -potential) – 53.1 mV (Fig. 7d). They also found that the prepared nanoemulsion indicated colloidal stability without any creaming during 90 days of storage. Kaur et al. (2016) claim that the minimum value of zeta potential in a stable dispersed system should be around $\pm 30 \text{ mV}$. Table 2 shows nanoemulsions stabilized by saponin.

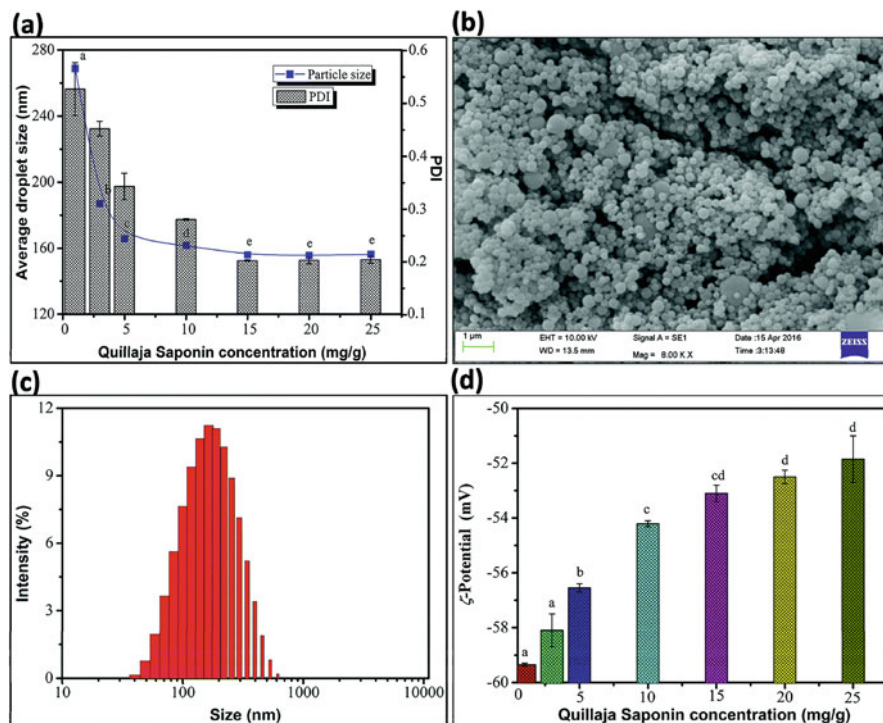


Fig. 7 (a) influence of QS saponin on average droplet size and PDI, (b) SEM analysis of nanoemulsions, (c) particle size distribution formed at concentration of QS at 15 mg g^{-1} with 10% sunflower oil and (d) variation in the ζ -potential of the QS-coated nanoemulsion versus concentration of *Quillaja* saponin (Chen et al. 2017)

7 Conclusion

Current climate change and increase in environmental pollution is a calling for an urgent need of eco-friendly materials to be used in chemical industries. Biosurfactants are a better alternative and a sustainable replacement for chemical surfactants. Plant-based biosurfactants are cost-effective, less toxic, efficient, biodegradable and environmentally friendly surfactants. Therefore, they are in huge demand in terms of application and investigation. This chapter summarized the surface activity and emulsification properties of plant-based biosurfactants. Studies indicated that QS-21 saponins possess high surface activity and emulsification properties. In addition, these saponins are potent in creating micro- and nanoparticles/nanoemulsions around 20 nm which can be supported its application in food, cosmetic and pharmaceutical industries. It is also suggested that among most of the plant biosurfactants, QS-21 saponin can be used for the preparation of oil-in-water emulsions at different concentrations due to its high HLB value.

Table 2 Nanoemulsions prepared with saponin as natural emulsifier

Emulsion phases description	Phase ratio	Saponin concentration (wt%)	Saponin origin/type	Mean droplet size (nm)	Process parameters	Homogenization technique	Emulsion condition	value of zeta potential (mV)	References
O/W, lemon oil (2% w/w), corn oil (2% w/w)	0.04:1	1 + 5 saponin-Tween 80	Food-grade saponin	52 ± 10	70 °C, 15 min, 2 min sonication, 40 W	Ultrasonication	Stable for up to 30 days	-41 ± 8	Kaur et al. (2016)
O/W	1:9	1.5	<i>Quillaja</i> bark saponin	20	3 min, 11,000 rpm, 100 MPa	Ultra-Turrax homogenizer	> 30 days	< -40	Schreiner et al. (2020)
O/W, sun-flower oil 50 wt%	1:1	Saponin to oil ratio (2:10)	Pharmaceutical grade saponin	< 200	Pre-homogenized at 20,000 rpm for 1. Then, sonicated at 600 W, 33 min. pH 7	Homogenizer+ sonication	Stable for up to 30 days	< -59.8	Nejatian and Abbasi (2019)
Orange peel oil/citrate buffer	N/A	6	Q-Naturale®	69	22,000 psi, multiple passes, Ph 3.6	Microfluidizer	2 weeks	> -20 m	Zhang et al. (2016)
O/W	1:9	2	Q-Naturale®	120-190	100 MPa for 4 passes	High-pressure homogenizer	N/A	N/A	Ozturk et al. (2015)
Corn oil/water	1:9	1%	Q-Naturale®	140-160	103 MPa for single	Dual-channel microfluidizer	14 days	> -60	Luo et al. (2016)
Thymol/water	N/A	Thymol:saponin ratio 6:1 (w/v)	<i>Quillaja</i> saponin	274 ± 2	50 min, pH 5.5	Sonication	3 months	-31	Kumari et al. (2018)
O/W	1:9	1	Tea saponin	200	50 MPa for three cycles	High-pressure homogenizer	30 days	-50	Zhu et al. (2018)
O/W	0.5:10	0.5	<i>Quillaja</i> saponin	146	112 MPa 10 passes	Microfluidizer	20 days	N/A	Doost (2018)
	1:99	1.5		89	5 passes at 20,000 psi	Microfluidizer	N/A	-41.6	(continued)

Table 2 (continued)

Emulsion phases description	Phase ratio	Saponin concentration (wt%)	Saponin origin/type	Mean droplet size (nm)	Process parameters	Homogenization technique	Emulsion condition	value of zeta potential (mV)	References
Fish oil/water			<i>Quillaja</i> saponin						Uluata et al. (2015)
Miglyol oil/water	1:9	1	<i>Quillaja</i> saponin	107	1500 bar for 4 times	Microfluidizer	21 day	-56	Salminen et al. (2020)
Thyme oil/water	N/A	0.28 mL thyme oil and 0.94 g saponin	Saponin	184.51	70 °C heat and stirrer, 15 min, 30 rpm	Heat and stirrer	N/A	-22.51	Ahmadi and Jafarizadeh-Malmiri (2020)
Sunflower oil/water	N/A	15 mg/g	<i>Quillaja</i> saponin	154	70% power for 300 s under	Ultrasonication	3 months	-53.1	Chen et al. (2017)

Acknowledgments Sweeta Akbari is grateful from the Institute of International Education's Scholar Rescue Fund (IE-SRF) and Finnish National Agency for Education (EDUFI) for supporting her postdoctoral research in Tampere University.

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Part II
Biosurfactants: Current Industrial
Applications

Biosurfactants as Emulsifying Agents in Food Formulation



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1 Introduction

Chemical compounds observed within phases with diverse hydrogen bonds and levels of polarity are called surfactants. It can either be natural or synthetic. The natural surfactants are majorly biosurfactants; these comprise amphipathic molecules with hydrophobic and hydrophilic portions. In biosurfactants, the non-polar portion contains hydrocarbon chains, while the polar portion can either be amphoteric, ionic (anionic or cationic), or nonionic. These features position biosurfactants to form emulsions and minimise interfacial and surface tensions (Santos et al. 2017). These features aid the usage of biosurfactants in diverse industries that engage with detergency, foam formation, lubrication, the solubilisation or dispersion of different phases, and emulsification (Shakeri et al. 2021). In another definition, biosurfactants refer to the surface-active biomolecules generated from microorganisms possessing

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diverse usages (Ribeiro et al. 2020). Currently, a lot of studies are being carried out on biosurfactants because of their distinct characteristics such as relative ease of preparations, low toxicity, and specificity. The unique functionalities of biosurfactants have propelled their usage in different industries that include petroleum, organic chemicals, petrochemicals, metallurgy, mining, agrochemicals, cosmetics, fertilisers, pharmaceuticals, beverages, foods, and others. Biosurfactants are used as demulsifiers, spreading agents, wetting agents, foaming agents, detergents, functional food materials, and emulsifiers.

Moreover, biosurfactants can enhance the transportation of nutrients through membranes and influence different host–microbe interactions. Compared to the natural and synthetic surfactants, biosurfactants are advantageous due to their digestibility, biocompatibility, biodegradation, effectiveness when considering different environmental occurrences (temperature, salinity, and pH), reduced toxicity, higher surface activity, complex and larger structure, and the capability to form liquid crystal and molecular assembly (Ribeiro et al. 2020).

Recently, biosurfactants are being considerably used in the food industry. The sector in which these products have been expanding more intensely in recent years is the food industry (Dikit et al. 2019; Gudiña and Rodrigues 2019). Moreover, they are being employed in food formulations to achieve different goals that include inhibiting the growth of pathogenic microorganisms, improving the viscosity, stabilising the salad dressings, reducing energy values through the replacement of fats, and improving the dough textures of cookies, cakes, and bread. Importantly, the use of biosurfactants in food formulations exhibits no side effects on animal or human health because they originated from nature (Campos et al. 2015). They are also being utilised in food formulations due to their antioxidant, anti-adhesive, and antimicrobial properties. Biosurfactants are as well capable of improving the alterations to destruction emanating from oxidation and enhancing the shelf life of the product (Ribeiro et al. 2020).

Biosurfactants have been used in diverse ways in food formulations, dressings, and preparations as food additives. Biosurfactants including sophorolipids, surfactin, and rhamnolipids are being utilised in different food preparations. Currently, biosurfactant-based products are available in the market. Based on different reports on the usages of biosurfactants in food formulations, this chapter focuses on the roles of additives, the importance of biosurfactants in the food sector, and biosurfactants as emulsifiers in food formulations. These point to the useful information that can help the food sector in introducing biosurfactants into their food formulations as emulsifiers.

2 Roles of Additives and Importance of Biosurfactants in the Food Sector

2.1 Roles of Additives in the Food Sector

Right from ancient times, using preservatives and flavouring materials in foods has been a common practice to maintain the outstanding qualities of foods. Nutritionally

enriched, appealing appearances, good flavour, and safe for consumption are minimum requirements for acceptable food products. Furthermore, affordability and cost are important factors considered by the consumers of any food product. Several ingredients/additives are being used in the food industry; these have caused higher demands by the consumers. Nowadays, consciousness is being exercised regarding originality and safety in the production of foods. Additives including hydrocolloids, pentosanases, and enzymes (hemicellulases, lipase, amylases, and so on) are being utilised mostly to enhance the consistency and texture of foods. Additionally, other benefits of additives include improved shelf life and enhanced freshness (Munif et al. 2012). During the preparation of any formulation in the food industry, some important parameters include freshness maintenance, safety maintenance, improving the appearance and texture, and maintenance and improvement of the nutritional value of foods. These parameters are explained as follows:

2.1.1 Freshness Maintenance

Foodborne diseases such as botulism caused by hazardous microbes associated with foods are life-threatening. Using an antioxidant as preservatives is common in averting the oxidation of fats and oils that are present in foods; this minimises or delays the occurrences of unpleasant flavours.

2.1.2 Safety Maintenance

It is known that foods can spoil due to the occurrences of diverse microbes including yeast, bacteria, actinomycetes, and moulds. The growth of microbes in foods is aided by air. Thus, achieving an excellent quality is the main challenge facing the food products that are being consumed by animals and human beings.

2.1.3 Improving the Appearance and Texture

Naturally, the introduction of sweeteners and flavouring spices is mostly done to increase the taste of food products, whereas the use of colourings is mainly to enhance the appearances of food products and make them appealing to the consumers. Other than the regular components, the thickeners, stabilisers, and emulsifiers are utilised to attain the desired rheological behaviour, homogeneity, texture, appearance, alkalinity, and acidity of food (Kourkoutas and Banat 2004).

2.1.4 Maintenance and Improvement of Nutritional Value

Several food products comprise different vitamins, minerals, fibres, proteins, fats, and sugars that can influence the nutritional values of the foods. Therefore,

additional nutritional substances may be included in the food formulation to further improve the nutritional values. Nevertheless, caution should be observed not to alter the taste and quality of the food.

2.2 Importance of Emulsifiers and Surfactants in the Food Sector

Diverse findings had established the benefits of biosurfactants relative to chemical surfactants. These benefits are essential in the food industry; they are important in achieving final food quality. Resistance to different salinity, acidity, and temperature can enhance the maintenance of original features of biomolecules that can significantly impact the final food quality. Some studies had outlined the thermal stability up to 250 °C temperature of yeast biosurfactants when using thermogravimetry (Ribeiro et al. 2020a, b). Moreover, the properties such as reduced toxicity and biodegradability obtained from analysis using some cell lines had been reported to attain consumer requirements that select natural and safer foods; this also assists in avoiding problems associated with human well-being imposed by some of the synthetic additives that are utilised in food formulations. Structural variations of biosurfactants can as well prompt the biomolecule selections alongside the properties suitable for a particular application. Characteristics that include antimicrobial, anti-adhesive, and antioxidant are benefits associated with the use of biosurfactants in foods as compared to the synthetic surfactants (Zouaria et al. 2016).

Over the years, the use of surfactants and emulsifiers has been regularly employed in the formulations of diverse food products. Breweries, bakeries, fermented products, and dairy commonly utilise surfactants and natural and synthetic emulsifiers. Mostly in the dairy products including curd, milk, creams, and cheese, the use of food-grade emulsifiers/surfactants is permissible. Furthermore, products such as dressings, salad, deserts, and mayonnaise can be supplemented with emulsifiers to enhance their storage, appearance, and aroma, other than their nutritional values. Other benefits of using biosurfactants as emulsifiers are stabilisation of flavour oils and improved properties in dairy formulations and bakeries (Ribeiro et al. 2020).

Getting to know more about the different characteristics of emulsifiers is imperative in their exploration industrially. Lower molecular weight compounds including fatty alcohols, glycolipids, lecithins, and monoglycerides can significantly minimise the interfacial and surface tensions. However, higher molecular weight compounds such as polysaccharide molecules and proteins tend to stabilise the emulsions (Satpute et al. 2010). Under this situation, the electrostatic interactions activate an efficient penetrating power. Diverse foods show a colloidal system with different kinds of aggregations made up of drops and particles that propel the gel-like appearance. Surface tension reduction helps emulsion formation within immiscible phases and increases their texture. A similar mechanism is observed in the foam formulation (Campos et al. 2013). The food formulations establish different phases

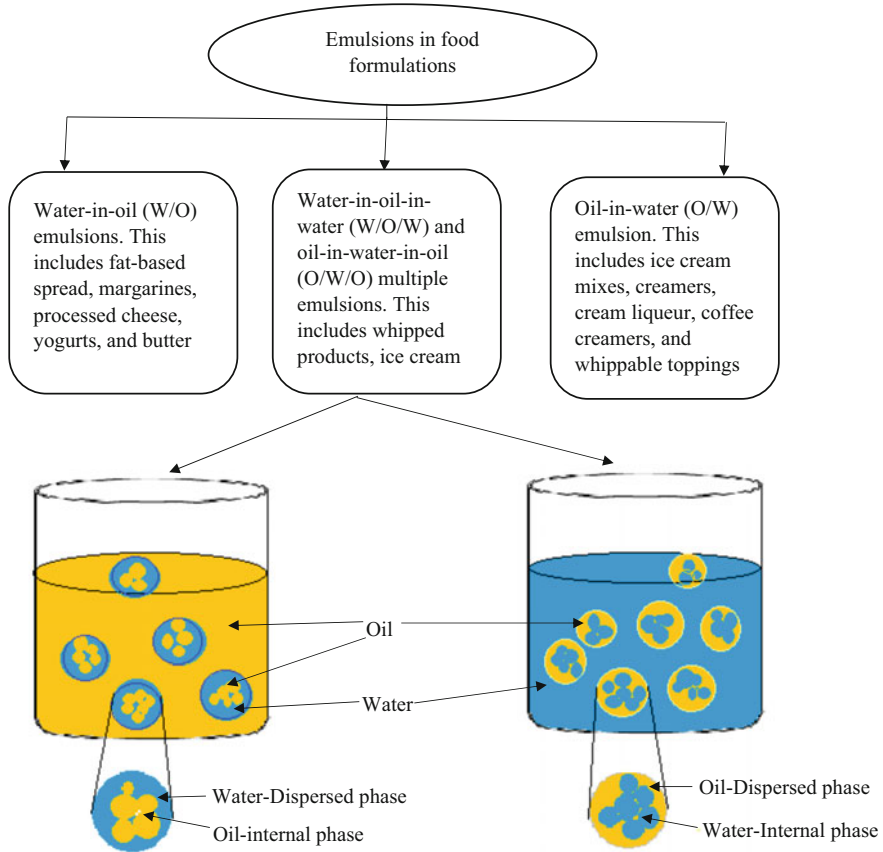


Fig. 1 Types of emulsions in food formulations

amidst particles (Kralova and Sjöblom 2009). There are three main kinds of emulsions used in food formulations (Fig. 1).

The aim of adding emulsifiers in food formulations is to retain or alter certain physical (appearance and constancy), biological (safe for consumption), and chemical (taste, temperature, and pH) distinctiveness to food products that undergo diverse processes such as dressings, preparation, manufacturing, packaging, storage, transportation, and handling. Biosurfactants are mostly utilised as gelling, stabilising, and thickening agents; nevertheless, the emulsification characteristic is of great importance in food formulations. This is due to the properties such as consistency and texture the biosurfactants impose on the food products. Additionally, the factors that include aroma solubilisation and phase dispersion are as well impacted by the characteristics of emulsifiers and emulsification phenomena. The purpose of emulsion stabilisation is attained through the assemblage of fat globules; this is done using emulsifiers and stabilising aerated approach (Berton-Carabin et al. 2014).

Food products including margarine, cream, mayonnaise, chocolate, salad dressing, and butter need extensive use of emulsifiers (Partridge et al. 2019). An emulsifier from *Candida utilis* is being employed in the formulation of salad dressings; this emulsifier provides innovative texture modification to the salad dressings. Liposan as a good example of an emulsifier with adequate emulsification property is being used in the formulation of edible oils that are commercially available in the market (Santamaria-Echart et al. 2021). Several emulsifiers from biosurfactants with higher molecular weight possess significant stabilising behaviour compared to arabic acid and carboxymethyl cellulose. The outstanding changes are established due to the introduction of emulsifiers in food products (Marchant and Banat 2012). The emulsifiers from *Enterobacter cloacae* function as a viscosity enhancer; this encourages the usage of lower pH (acid-containing) products such as ascorbic acid and citric acid (Ask et al. 2006).

3 Biosurfactants in Food Formulations as the Emulsifiers

In the food industry, emulsifiers are used to minimise the surface tensions between two immiscible phases to allow for adequate mixing. There are two major kinds of emulsifiers commercially available for use in the drink and food industries; they are emulsifiers generated from synthetic means and lecithin from egg and soy (Santamaria-Echart et al. 2021). As the functional food market is fast-growing, the need for organic or natural ingredients in their formulations can propel the emanation of new emulsifiers. Because the biosurfactants possess emulsifiers and surface-active actions, the market values for biosurfactants have greatly increased over time in the food industry (Ekambaram et al. 2022). Besides, the potential market value is not only encouraged based on the surface activity but also on the environment-friendly features, distinct properties and structures, low toxicity, and increase in demands for organic or natural-based ingredients (Rodrigues et al. 2006; Saravanan and Vijayakuma 2015).

Surfactants are mostly employed as emulsifiers in the food industry. Lower molecular weight surfactants including lecithins, monoglycerides, fatty acids, and fatty alcohols are amphiphilic molecules that can effectively minimise the interfacial tensions during the emulsification phase. However, the higher molecular weight surfactants include polysaccharides (gum arabic pectin, alginate, cellulose derivatives, dextran, starch, and xanthan) and proteins (whey, casein, and gelatin proteins) are involved in the stabilisation and formation of emulsions (Tan and McClements 2021).

The system of emulsion is essential in the dispersion and solubilisation of food formulations; this is as well helps the texture, appearance, and steadiness of foods (McClements 2016). The primary goal of the emulsifier is to stabilise the emulsion by managing the agglomeration of phases. Biosurfactants possess a significant benefit in food production when compared to synthetic surfactants. Patents had been published on the utilisation of rhamnolipids in food production to enhance

the shelf life of dairy and bakery products, and consistency in the stability and volume of dough (Mnif et al. 2012). A study investigated the influence of adding lipopeptide biosurfactant from *Bacillus subtilis* in the production of bread to improve the quality. The result showed that the introduction of the emulsifier at the concentration of 0.075% increased the crumb structure and specific volume when compared with a similar formulation by utilising soya lecithin (Mnif et al. 2012).

Moreover, biosurfactants from *B. subtilis* strain had been reported to emulsify diverse edible oils; this has shown a great prospect of its usage in the food system. In another study, adding the 0.10% biosurfactants from the *B. subtilis* as emulsifiers in cookies formulation resulted in the texture properties of the dough; these include adhesiveness, cohesiveness, springiness, and hardness as compared to the dough with glycerol monostearate (commercial synthetic emulsifier) (Zouari et al. 2016). The polymeric biosurfactants integrated with protein moieties, fatty acids and polysaccharides enhanced improved emulsification and stabilisation of emulsions (Uzoigwe et al. 2015). The emulsifiers from yeasts, mostly from the genera *Yarrowia*, *Pseudonysms*, and *Candida*, are generally recognised as safe (GRAS); this property is important for the food system as compared to other biosurfactants from bacteria (Campos et al. 2014). A surface-active compound that can be employed as an emulsifier in food formulation had been reported from *Candida utilis* strain (Campos et al. 2014). This same *C. utilis* had been used in the formulation of sunflower oil-based mayonnaise. The inclusion of guar gum and biosurfactants produced mayonnaise with 30 days of stability at 4 °C. Furthermore, rats were used to investigate the toxicity of these biosurfactants; the results established no toxic impact on the rats. This showed a clear prospect of using biosurfactants from *C. utilis* in food formulations (Campos et al. 2015).

Another potential usage of biosurfactants is the formulation of micro- and nano-emulsions; these can function as conveyors of essential food components including functional molecules, probiotics, and vitamins (Sagalowicz and Leser 2010). In the food system, nano-emulsions can be used to preserve foods or decontaminate equipment. The antimicrobial and fungicidal activities of sunflower oil-based surfactin nano-emulsions were investigated against *Bacillus cereus*, *Salmonella* Typhi, *Listeria monocytogenes*, and *Staphylococcus aureus*; the results reflected a noticeable decline in the microbial population when used in apple juice, milk, raw chicken, and vegetables (Manoharan et al. 2012). Biosurfactants obtained from *P. aeruginosa* were used to formulate a nano-biosurfactant; better emulsification was achieved compared to synthetic surfactant and butter (Farheen et al. 2016).

3.1 Food Formulations as Enhanced by Biosurfactants

The food sector urges uncompromising microorganism control measures to achieve products with the consumer safety at hand. Besides, the product must possess a higher degree of acceptance and quality by the consumers. Because of the high demand in recent times, new food formulations are being established using plant-

based compounds including gum arabic and lecithin to replace the synthetic additives; this is being generally accepted (Hasenhuettl and Hartel 2019). Nevertheless, the use of plant-based ingredients is limited regarding the products that require microwave cooking and irradiation; this is because the chemical compounds in plants can degrade over time. Hence, novel additives possess emulsifying, stabilising, thickening characteristics as well as antimicrobial, antibiofilm and antioxidant properties (Nitschke and Costa 2007).

In the previous studies, different emulsifiers have been used to improve the dough's rheological properties, bread volume, and crumb texture. Most importantly, edible-grade emulsifier provides adequate softness and strength to crumbs; this aids the usage of biosurfactants in bread production. The shelf life and quality of bread can be enhanced using biosurfactants from *B. subtilis* SPB1 (Mnif et al. 2012). This study reported that the bread was improved in terms of the voided fraction of loaves, specific volume, and shapes when compared with soya lecithin (common commercial surfactants). Using an SPB1 emulsifier concentration of 0.075% w/w, the texture profile of the bread was improved. The introduction of SPB1 emulsifier also declines chewiness, adhesion values, and firmness. Moreover, the emulsifier provided the bread with a stronger protein networks and gas retention potential for the doughs during the fermentation process, leading to an increased bread volume.

In recent times, biosurfactants are being utilised as capping agents and to synthesise green nanoparticles (Ganesh et al. 2010). This has encouraged more studies in this area. Ganesh et al. (2010) reported that *P. aeruginosa* produced RHL-mediated silver nanoparticles. In another study, NaBH₄ from *P. aeruginosa* strain showed the synthesis of RHL reverse micelles. Micro-emulsion system in heptane was used to synthesise NiO nanoparticles (Palanisamy and Raichur 2009). The ZnS nanoparticle rods are formulated by utilising a capping agent (Narayanan et al. 2010). A microbial system such as *Brevibacterium casei* had been employed to formulate glycolipids in conjunction with Ag nanoparticles.

3.2 Evacuating Heavy Metals from Foods Using Biosurfactants

There is a health-related problem associated with the presence of heavy metals in foods. Diverse plants and the growth phases, surrounding environment, soil condition, and presence of heavy metals are the factors that determine the uptake of heavy metals. Thus, adequate observation of the presence and accumulations of heavy metals in food products is essential to avert the negative outcomes of these heavy metals. Several efforts are being put in place to treat wastewater in the food industries to minimise the level of heavy metals. Novel technologies are being developed to solve the issues of heavy metals in food products. Nonetheless, organised solutions are yet to be established to solve the issue (Hidayati et al. 2014).

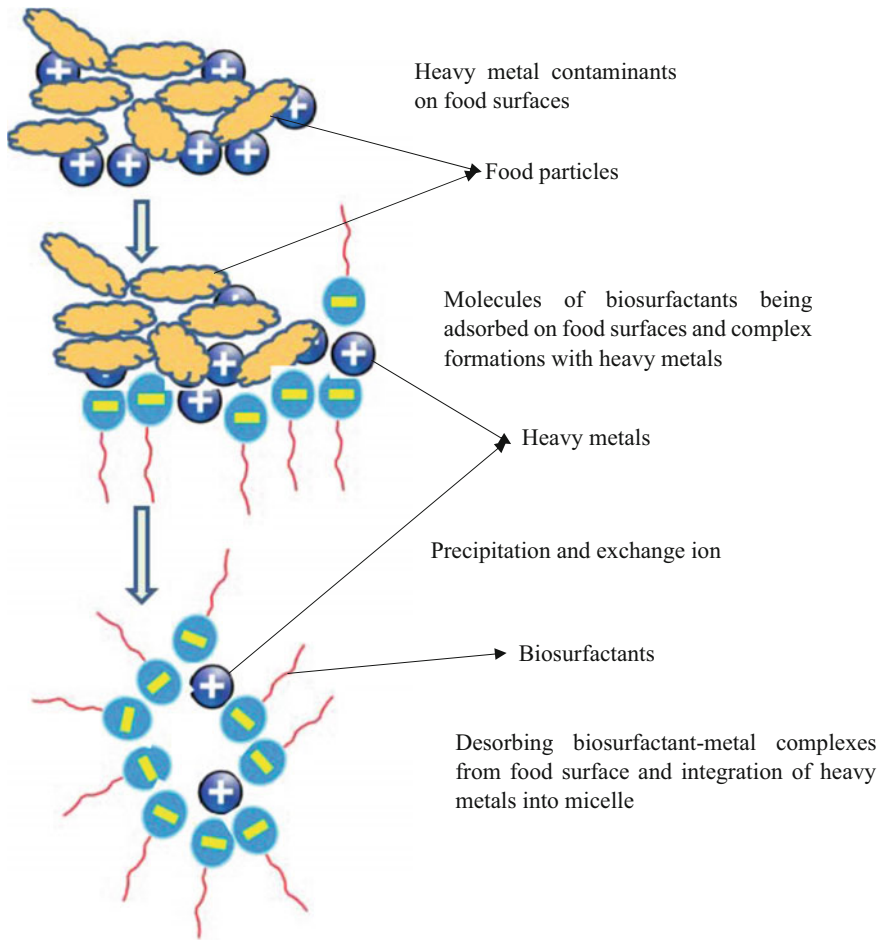


Fig. 2 Process of eliminating heavy metals from food surfaces using biosurfactants (Wang and Mulligan 2004)

The ionic surfactants join the heavy metal and precipitate through the ion exchange phenomenon. Hence, the heavy metals can be evacuated in the form of aggregates (Fig. 2). Moreover, heavy metals can efficiently be removed from foods using RHL in the form of microemulsion (Wang and Mulligan 2004). Just like soil surface, heavy metals are proposed to form complexes with biosurfactants; these metals can then be evacuated from foods to the surrounding solutions. Predominantly, biosurfactants of anionic nature (RHL) can effectively evacuate charged metals due to surface activity between heavy metals and biosurfactants (Xu et al. 2011). About 70% of cadmium was reported removed from radish, potato, onion, and garlic by utilising surfactin from *Bacillus sp.* MTCC 5877 (Anjum et al. 2016). This study showed that biosurfactants can successfully remove heavy metals from

food products. Besides, biosurfactants can minimise biofilm formation; thus, they avert the occurrence of microbes from different food surfaces. Giri et al. (2017) reported that biosurfactants isolated from *B. licheniformis* VS16 can remove cadmium from potato, radish, ginger, and carrot. Besides, this can eliminate films of different pathogenic organisms. Therefore, surface-active molecules can enhance the cleanliness of environmental pollution.

3.3 Sanitations of Food Processing Using Biosurfactants

Microorganisms are responsible for food spoilage; due to this, several techniques are being considered to resolve this problem in the food sector. Nonetheless, food products, vegetables, and fruits should comprise similar safety and nutritional values until consumption. The most common chemicals used to preserve food from spoiling are organic acid, chlorine compounds, tri-sodium phosphate, ammonia compounds, and iodine solution (Hricova et al. 2008). The use of these chemicals has been reported to sustain some setbacks including failure to maintain the integrity of the food products such as appearance, colour, and taste. This has propelled the effort of sourcing alternatives for food formulation to overcome the challenges of food spoilage (Dilarri et al. 2016).

Several microbial systems can withstand the surrounding environment; hence, the production of biosurfactants from microbial cells has proven to importantly improve food products against spoilage (Mellor et al. 2011). Mellor et al. (2011) further reported that the presence of biosurfactants in food formulation increases the bacteria count in the stored foods. It was evident in the study that the total bacteria count of *P. fluorescens* increased on chicken stored aerobically for 72 h. Biosurfactants influence the nutrient bioavailability for bacteria growth; thus, it makes them hostile to sustaining and increasing food spoilage (Jirku et al. 2015). Moreover, a study outlined that pathogen such as *S. enteritidis* which is foodborne has a legitimate propensity to stick to the surface of lettuce leaves. A study suggested that biosurfactants from *Salmonella enteritidis* SE86 c influence lettuce leaves and confer resistance to sanitiser (Rossi et al. 2016). This study used scanning electron microscopy (SEM) to investigate lettuce leaves; lumps were formed by the considered organisms as the produced biosurfactants favoured the stomata invasion. Furthermore, it was reported that the biosurfactants influence the adherence capacity and enhance the resistance ability of organisms over sanitiser. Another form of biosurfactant which is RHL was investigated for sanitation purposes and fruit washing. The results established that RHL is effective in preventing microorganism growth and increasing the shelf life of fruits. Thus, RHL propelled fruit sanitation (Dilarri et al. 2016).

3.4 *Biosurfactants as Food Additives*

Based on the report by European Food Safety Authority (EFSA), substances that are purposely added to food formulation for precise technological tasks are called food additives. These food additives can be grouped into nutritional additives, preservatives, colouring agents, flavouring agents, miscellaneous additives, and texturing agents (Brannen and Haggerty 2002). Examples of food preservatives are antioxidants and antimicrobials; nutritional additives comprise vitamins, amino acids, and fibres. The flavouring agents are flavour enhancers, flavours, and sweeteners. The texturing agents are stabilisers and emulsifiers that are used to modify the mouth-feel or texture of food products. Moreover, miscellaneous additives are lubricants, chelating agents, anti-foaming agents, and enzymes (Brannen and Haggerty 2002). The use of food additives continues to increase in recent times; using additives is associated with the occurrence of food industrialisation (Pandey and Upadhyay 2012). In 2021, it was estimated that the market value of food additives will be over US\$ 39.85 billion (Nitsche and Sousa 2018).

4 Conclusion

This chapter has reviewed the roles of additives and the importance of biosurfactants in the food industry and biosurfactants as emulsifiers in food formulations. The increasing demands for biosurfactants in food formulation as emulsifiers cannot be underestimated. The increase in market demand for biosurfactants has encouraged more studies. Several functional and biological properties are associated with the biosurfactants regarding food preparations and formulations. Recent techniques including the combination of biosurfactants with nanoparticles are being used in the current food formulations. Recently, applications of biosurfactants in the food industry can be reached through an innovative modification that cannot be found in conventional emulsifiers. The useful information in this chapter can aid the food industry to introduce more biosurfactants into their food formulations as emulsifiers. Besides, this will encourage more studies to unveil more important biosurfactants that can be useful as emulsifiers in food formulations.

In the future, biosurfactant-based products can be employed in cleaning filtration membranes or ultrafiltration to eradicate biofouling in the food industry. One of the main challenges facing the food industry is biofilm control; this can be tackled by using green preparation to remove biofilm or using biosurfactant-based products as cleaning agents. Moreover, emulsification of fat and oil that is paramount to bakery and related preparations can further be achieved using unconventional emulsification solutions. These solutions can be utilised to prepare thickening agents, stable emulsions, and nano-emulsions that can enhance bakery formulations in respect of the texture.

Acknowledgments The authors acknowledge the financial assistance (industrial grant, UIC190806) of Eastern Unity Technology, Malaysia.

Important Websites Indicating Companies with Industrial Applications of Biosurfactants

<https://research.tees.ac.uk/en/publications/biosurfactants-and-bio-emulsifiers-from-algae-3>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6046428/>

https://www.tateandlyle.com/ingredient/frimulsion-stabiliser-systems?utm_source=google&utm_medium=cpc&utm_campaign=FRIMULSION&gclid=CjwKCAjw7cGUBhA9EiwArBAvos5iAZn2qDzLhjLjaP4UicJexQNqbAewIgo0S14xlWeEcF2TeCHChxoCaPkQAvD_BwE

https://www.foodchem.com/?campaignid=8392115017&DEV=c&PLC&target&keyword=food%20additive%20companies&AD=402581407376&feeditem&gclid=CjwKCAjw7cGUBhA9EiwArBAvogXffn0dG6-fDtJb-ryHqveKb1AOawAV6JcFKBSuq1HBS3iaWpEP_xoCtucQAvD_BwE

Conflicts of Interest The authors declare none.

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Application of Biosurfactants as Anti-Corrosive Agents



Saman Zehra, Mohammad Mobin, and Ruby Aslam

1 Introduction

The main reason why metallic structures fail is corrosion, which is a highly difficult and serious industrial conundrum (Zehra et al. 2021). On the other hand, microbial corrosion or the biocorrosion processes are the results of electrochemical reactions that are influenced or driven by microorganisms, which are often present as a biofilm. Microorganisms are capable of quickly degrading many economically used metals and alloys, including stainless steel, alloys based on nickel and aluminum, and materials like concrete, asphalt, and plastics. Additionally, several protective oils, varnishes, and emulsions are vulnerable to microbial deterioration. Biofouling, biodeterioration, and microbiologically induced corrosion are three categories of microbial processes that degrade both organic and inorganic materials (MIC) (Javaherdashti 1999; Videla and Herrera 2005; Parande et al. 2005). Figure 1 illustrates the general distinction between corrosion and biocorrosion.

To minimize or control the severe attack of corrosion and biocorrosion, several methods are followed in different conditions. The application of anti-corrosive agents, i.e., corrosion inhibitors (Kumar 2008; Mobin et al. 2016a, b) and biocides, has been accepted as the primarily practicable methodology, adapted in various industries to prevent and control corrosion and biocorrosion, respectively.

It has been known that various types of chemicals can be used as corrosion inhibitors. Numerous studies have been conducted on the properties of these chemicals. Some of these include sodium dodecyl sulfate, cetyl trimethyl ammonium bromide (Arjmand et al. 2016), tetradecyltrimethylammonium bromide (Javadian et al. 2017), and Gemini (Heakal and Elkholy 2017; Aslam et al. 2021). Among the commonly used types of chemicals for treating corrosion in oil and gas fields is

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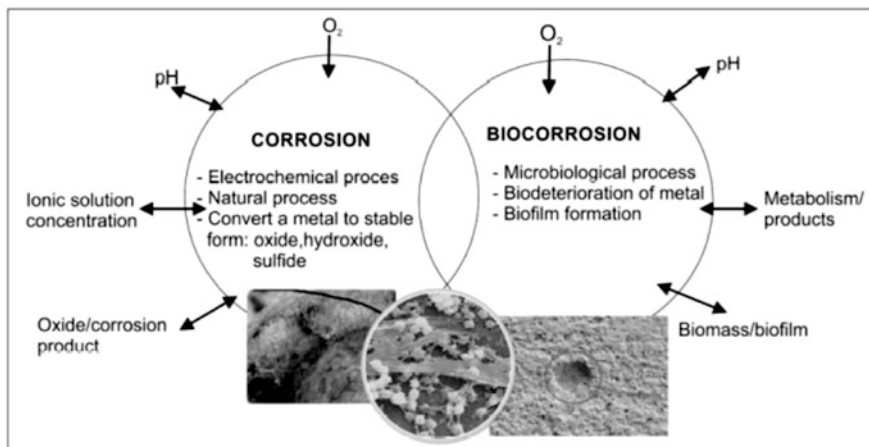


Fig. 1 Corrosion versus biocorrosion

polyoxyethylene alkylate, primary alcohol ethoxylate, and polyethylene glycol ester (Hill and Romijn 2000; Ali et al. 2010; Malik et al. 2011a). However, these surfactants can pose a threat to the environment due to their negative effects on various biological regions.

When selecting an inhibitor to manage corrosion issues in various industries with various conditions, extreme care must be used. It should be biodegradable, easily producible, affordable, stable in harsh environments, and environmentally beneficial. Hence, researchers are very much interested in green inhibitors rather than chemical ones. Over the past few decades, numerous green inhibitors from various sources have been thoroughly researched. Biosurfactants, or biologically active molecules, substitute new compounds for the control of corrosion; few studies have attempted to use biosurfactants to suppress corrosion issues outside of specific environments (Dagbert et al. 2006a, 2008; Araujo et al. 2016). The various factors that affect the development and use of corrosion inhibitors must be considered when it comes to choosing new chemical biocides. These include their antimicrobial properties, low toxicity, and their eco-friendly attributes.

The prospective microbiological strains of bacteria, fungus, and yeast can easily make biosurfactants when given an agricultural waste substrate (Kumar et al. 2015; Peele et al. 2016; Parthipan et al. 2017a). Environmental, petrochemical, agricultural, cosmetic, medicinal, food manufacturing, and pharmaceutical industries all use biosurfactants (Freitas de Oliveira et al. 2013; Dhasayan et al. 2015; Parthipan et al. 2017b). Unique and significant properties of biosurfactants include biodegradability, environmental friendliness, antimicrobial/anti-biofilm properties, and high flexibility (Araujo et al. 2016; Mani et al. 2016; Parthipan et al. 2017c). Biosurfactants also kill microorganisms by directly compromising the integrity of their cell walls or plasma membranes. Any target organism finds it challenging to acquire resistance to the

biosurfactant due to the severity of such damage to the cell barrier (Pereira et al. 2012; Pornsunthorntawee et al. 2008).

So, this chapter aims to cover the applicability of the biosurfactants as anti-corrosive agents for corrosion and biocorrosion. Several examples from the already reported literature where biosurfactants had been utilized as corrosion inhibitors and biocides are also discussed in the chapter.

2 An Introduction to Biosurfactants

Biosurfactants are surface-active molecules that have a variety of uses in medicine, including as adhesives and antibacterial agents (Pérez et al. 2002; Le 2011; Fawzy et al. 2018a, b). One of the examples is biosurfactants, which are typically environmentally safe and economically advantageous (Mulligan et al. 2014a). Such biosurfactants are used in the chemical and pharmaceutical industries, in agriculture, and in solving environmental problems due to a diversity of physical, chemical, and biological characteristics (Müller et al. 2012). Figure 2 shows several applications of biosurfactants. Their effectiveness is based on their capacity to dramatically lower the surface and interfacial tension of water at low concentrations and to produce stable emulsions.

The main properties of biological surfactants are not inferior to those of synthetic ones. A natural mixture of extracellular rhamnolipids and polysaccharides is



Fig. 2 Outline of some applications of biosurfactants

produced by the strain *Pseudomonas sp.* PS-17 is known as the rhamnolipid biosurfactant complex (Karpenko et al. 2009). Indicators of the rhamnolipid biosurfactant complex's high surface activity include the emulsification, solubilization, and lowering of surface and interfacial tension of solutions to 29.0 and 0.17 mN/m, respectively. It is biodegradable, of moderate toxicity, and an efficient surfactant at various temperatures, pH levels, and salt concentrations. It has undergone a fair amount of research.

The molecular weight or chemical charge of biosurfactants can be used to categorize them because the chemical makeup of these substances varies substantially among different kinds of bacteria. Low-molecular-weight surfactants, which reduce the surface tension between two immiscible liquids, and high-molecular-weight emulsifiers, also known as polymeric surfactants (or bioemulsifiers), which promote the formation of oil-in-water or water-in-oil emulsions and are frequently exopolysaccharide-based, are two groups of surface-active substances. Glycolipids, phospholipids, fatty acids, lipopeptides, and lipoproteins are the main chemical constituents of biosurfactants with low molecular weight. These structures can be polymers, single macromolecules, particle structures, or biosurfactant structures (Banat et al. 2010a; Makkar and Rockne 2003; Satpute et al. 2010). Low-molecular-weight surfactants' main job as surface-active agents is to lessen surface and/or interfacial tension between two immiscible liquids, liquid and solid, or liquid and gas phases. This is because of their low molecular weight. While glycolipids are the biosurfactants that have been studied the most and are made up of various sugars linked to β -hydroxy fatty acids, lipopeptides are built of cycloheptapeptides with amino acids linked to fatty acids of varying chain lengths (carbohydrate head and a lipid tail).

Biosurfactants are an essential biotechnology product for many industrial applications, including those in food, cosmetics, cleaning products, medicines and medicine, and oil and gas. The global market for biosurfactants earned more than USD 1.5 billion in sales in 2019, and between 2020 and 2026, it is anticipated to increase at a pace of more than 5.5% CAGR (Ahuja and Singh 2020).

Biosurfactants are surface-active amphiphilic chemicals that are created by specific bacteria, fungi, and yeasts. They are sometimes referred to as biologically derived surfactants. *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, and *Acinetobacter calcoaceticus* are the predominant species (Vijayakumar and Saravanan 2015; Santos et al. 2016). The compounds can be digested inside the cell, discharged into the surrounding environment, or become a component of the cell membrane (Mulligan et al. 2014b). They are non-ribosomally produced chemicals that demonstrate considerable emulsification and surface activity. Molecular weights of the varied category of biomolecules known as biosurfactants range from 500 Da to 1000 kDa.

Biosurfactants have been divided into many classes based on their chemistries and microbiological sources. There are five main categories: neutral lipids, phospholipids, polymeric substances, lipopeptides, and glycolipids (Kosaric and Vardar-Sukan 2015). The distinctive physicochemical properties of diverse biosurfactants are influenced by their varying chemical compositions. The complex components of

biosurfactants come from a wide range of biological-chemical substances. Some of these include fatty acids, dicarboxylic acids, alkyl glycosides, and sugar molecules. The hydrophobic and hydrophilic moieties of the biosurfactant's molecules are typically separated. While the hydrophobic moiety frequently consists of saturated or unsaturated long-chain fatty acids, the hydrophilic moiety is made up of anions, cations, amino acids, or polysaccharides (Płaza 2018; Fenibo et al. 2019). They all exhibit various surface tension, interfacial, and emulsification characteristics. Biosurfactants, which are known to have broad-spectrum antimicrobial activity, are used in a range of industries, including agriculture, oil, food, cosmetics, biotechnology, pharmaceuticals, and environmental remediation techniques (Mulligan et al. 2014b; De Almeida et al. 2016; Kubicki et al. 2019).

The biosurfactants are produced by the bacterium and are either released extracellularly or partially adhered to the cell membrane (Sun et al. 2018). When the bacterium is cultivated in substrates that are insoluble in water, the latter arrangement frequently takes place. It is suggested that intracellular biosurfactants are used for gene and nutrition uptake, to help host cells neutralize hazardous substances by sequestration, to support cell differentiation, and, ultimately, to make it easier to store carbon and energy (Van Hamme et al. 2006). A water-insoluble substrate's phase boundary is made more accessible for nutrient intake and metabolism by the generating organism when surface tension is reduced by biosurfactants. Additionally, biosurfactants make it simpler for microbes to migrate across liquid–liquid, liquid–solid, and liquid–air interfaces. This property results from the decreased surface tension between the various phases, which helps with organism movement in potentially hazardous situations (Sun et al. 2018; Van Hamme et al. 2006). Biosurfactants, or natural surfactants made by microorganisms, have advantages over synthetic equivalents. Extended foaming properties, low toxicity, high selectivity, and specificity of action at high pH and low temperatures are a few of these (Geetha et al. 2018). The special connection between biosurfactants and environmental sustainability was reported in a review article published by Olasanmi and Thring (Olasanmi and Thring 2018).

Due to the vast range of uses for surfactants and biosurfactants, including in the fields of chemistry, biology, medicine, water and soil pollution prevention, and corrosion inhibition, these fields are expanding quickly. An overview of chemical surfactants, including corrosion inhibitors, is given in the review of Malik et al. (Malik et al. 2011b). The adsorption of the biosurfactant functional group onto the metal surface is the most crucial step in corrosion inhibition.

3 Biosurfactants as Anti-Corrosive Agents for Corrosion

An international issue, corrosion has an impact on a wide range of businesses, including shipping, sewage systems, building and construction, drinking water systems, and oil refineries. When oxygen and moisture are present, materials will corrode. This is an electrochemical process that involves the ionization (oxidation)

of the metal during the anodic reaction and the reduction of chemical compounds during the cathodic reaction (Beech and Gaylarde 1999). Finally, it affects the material's functionality and changes its physical characteristics (Fang et al. 2002; Garcia et al. 2012).

Zin et al. (Zin et al. 2018a) investigated the ability of rhamnolipid biosurfactant produced from *Pseudomonas sp.* PS-17 to suppress corrosion of aluminum alloy. It has been proved that the biosurfactant rhamnolipid successfully prevents metal corrosion in artificial acid rains. The rise in biosurfactant concentration can improve the effectiveness of corrosion inhibition. However, once the concentration of micelles reaches a critical level, the improvement in inhibition is not as strong. It is believed that the mechanism by which the biosurfactant molecules are attracted to the surface of an aluminum alloy is the catalytic mechanism for corrosion. Even though it is not completely ruled out that the formation of a salt film between rhamnolipid and aluminum ions on the anodic sites of the alloy could be the cause of the corrosion, it is still believed that the biosurfactant molecules can effectively resist corrosion when exposed to a corrosive environment. The addition of a biosurfactant to the environment can also increase the repassivation kinetics of the alloy. The results of SEM investigation of the uninhibited alloy after 7 days of exposure showed localized corrosion damage close to cathodic intermetallic inclusions on the aluminum alloy surface. However, when the solution was inhibited with 0.5 g L^{-1} of the biosurfactant complex, only minor corrosion damage was seen. Indirect proof of the existence of an organic biofilm is provided by increased specimen charging on the inhibited sample.

The effects of two green biosurfactants, namely, sodium N-dodecyl asparagine and sodium N-dodecyl arginine, on the dissolution of a mild steel alloy were studied by Fawzy et al. (Fawzy et al. 2018c). They found that both the inhibitors inhibited the corrosion of MS-37-2 in aqueous NaCl solutions. Following the Langmuir process, the B-Surf compounds were prepared by using MS-37-2. The resulting compounds were then adsorbed on various substrates. The process was endothermic and spontaneous, which is regarded as the kind of adsorption that occurs naturally. The ability of the inhibitors to block the two biosurfactants was improved by the addition of transition metal ions.

In a US patent application filed in the USA, Gunawan and colleagues (Gunawan et al. 2016) stated that a biosurfactant could prevent or inhibit the corrosion effects of certain well-treatment applications by adding a corrosion inhibitor to the formulation. The biosurfactant was selected from a variety of different types of lipid compounds, including polyol, glutinous, ornithine, and mannosylerythritol.

4 Biosurfactants as Biocides for Biocorrosion

A well-known and extremely destructive phenomenon is biocorrosion. The rapid corrosion of steel, cast iron, copper alloys, stainless steel, aluminum, and nickel alloys has been linked in published cases to bacteria and fungus. Additionally,

materials including plastic, stone, concrete, and wood can be ruined by microorganisms. They are acknowledged to have an impact on society, the environment, and the economy. Although microorganisms have advantages to the biogenesis of minerals and bioleaching, many kinds of microbes are also in charge of deterioration and corrosion.

Microorganisms, which are frequently present as biofilm, impact or drive electrochemical reactions that result in the microbial corrosion (biocorrosion) processes. Microorganisms are capable of quickly degrading many of the metals and alloys that are commonly used in industry, including stainless steel and alloys based on nickel and aluminum as well as substances like concrete, asphalt, and plastics. Additionally, some oils, emulsions, and coatings for protection are susceptible to microbial deterioration. Biofouling, biodeterioration, and microbiologically induced corrosion are three categories of microbial processes that degrade both organic and inorganic materials (MIC). In marine, freshwater, and soil environments, biofouling refers to the attachment of micro- and macroorganisms to material surfaces, resulting in the production of fouled layers of biofilms. Biodeterioration is the word used to describe the degradation of nonmetallic materials such as rubber, wood, plastic, and cement brought on by microbial activity. More than 30% of corrosion damage is caused by microbially influenced corrosion (MIC), which occurs in a variety of settings such as soil, freshwater, and seawater. By eating the hydrogen and secreting enzymes and acidic metabolites, the microorganisms in this process promote corrosion (Kip and van Veen 2015). Sulfate-reducing bacteria, sulfur-sulfide oxidizing bacteria, iron-oxidizing/reducing bacteria, manganese-oxidizing bacteria, and bacteria producing organic acids, exopolymers, or slime are the principal bacterial species linked to corrosion. Biofilm is usually linked to corrosion caused by microbes. These bacteria live in naturally occurring biofilms, frequently creating complex, synergistic consortia. Microorganisms build a biofilm on the surface and start, promote, or worsen corrosion events during biocorrosion through cooperative metabolism (Javaherdashti 1999; Videla and Herrera 2005). Initial adhesion and biofilm formation are influenced by microbe–metal interactions. A biofilm can be compared to a gel that contains extracellular polymeric substance (EPS), a suspension of cells, and inorganic debris and constitutes 95% water (Kip and van Veen 2015). Reversible attachment, irreversible attachment, starting maturation, complete biofilm, and dispersal of planktonic cells are the five processes of biofilm formation that are highlighted. The biofilm's microbial community's variety and expansion are highly reliant on its environment. Both aerobic (in the top zones) and anaerobic (in the lower zones close to the substrates) microorganisms can develop in the biofilm due to the oxygen gradients present throughout. The parameters of the metal-to-bulk solution interface are altered by biofilms, such as the kinds and concentrations of ions, oxygen, and pH, which change the metal's electrochemical behavior (Parande et al. 2005; Videla and Herrera 2007). Redox processes carried out by microorganisms have a considerable impact on the characteristics of minerals in the environment (Mansour and Elshafei 2016). The bacteria biofilm promotes biocorrosion by changing several variables, including pH, pressure, oxygen levels, and nutrition. Recently, it has become clear that bacteria can prevent or inhibit corrosion as well as

cause it. This process is known as microbiologically induced corrosion inhibition (MICI). Then, the biofilm can be employed to change the environment at a metal surface or create antibacterial agents, which can either speed up or slow down the corrosion process because it is a multispecies combination of microbes.

One of the most potent bioactive compounds is an antimicrobial agent produced by microbes, and its discovery was regarded as one of the greatest achievements of the twentieth century. Since their discovery, numerous broad- and narrow-spectrum antimicrobial drugs have been applied globally in human medicine, industry, and agriculture to eradicate or stop the spread of harmful microbes (Malik et al. 2011b; Ullah and Ali 2017). Surfactants, which are made synthetically and biologically, are one of the most widely used antimicrobial substances. As was previously mentioned, biosurfactants (biologically derived surfactants) are secondary metabolites and surface-active amphiphilic compounds that are produced by bacteria, fungi, and yeasts. The dominant species are *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, and *Acinetobacter calcoaceticus* (Vijayakumar and Saravanan 2015; Santos et al. 2016). The capacity of biosurfactants to aggregate into micelles and provide a protective layer on the metal surface is proportional to their capacity to adsorb. This layer slows down or stops the materials' deterioration. Biosurfactants also kill microorganisms by directly compromising the integrity of their cell walls or plasma membranes. Any target organism finds it challenging to acquire resistance to the biosurfactant due to the severity of such damage to the cell barrier (Banat et al. 2014; Satpute et al. 2016).

The postulated mechanisms for how bacteria contribute to the biocorrosion processes are described in the review by Lin and Ballim (Lin and Ballim 2012), and various methods for biocorrosion control are offered. Additionally, Kip and van Veen (Kip and van Veen 2015) listed several techniques as probable MIC-inhibition mechanisms. The first is the development of a protective biofilm. Biological corrosion control techniques, such as biocompetitive exclusion and the utilization of bacteria that produce antimicrobials and create biofilms, are showing great promise as more efficient, sustainable, and long-term corrosion control techniques. The process of chemicals being precipitated by microbes to shield the material from corrosion is the second MICI mechanism. This study describes several examples of naturally occurring mineral precipitation layers. MIC is relevant to practically every significant industry, and several biological, physical, and chemical techniques are employed to regulate them. Although corrosion is a rather straightforward process in and of itself, studying it in situ is challenging and complex. Like this, complex processes involving microorganisms that carry out diverse electrochemical reactions and secrete distinct secondary metabolites (microbiological/biofilm processes) affect biocorrosion. Electrochemical/physical techniques and conventional microbiological culture-dependent methods have up till now offered some understanding of corrosion activities. The identification and function of microbial communities, which are connected to corrosion in many materials and settings, are nonetheless little understood. Using omics-based techniques, it is now possible to obtain insights into microbial communities and their metabolism thanks to the advancements made in modern science.

Finding innovative solutions based on natural sources and with suitable attributes, such as effective antibacterial activity, economically viable, low toxicity, and ecologically friendly features, is critical due to restrictions associated with the use of chemical biocides. As an alternative to chemical biocide, biosurfactants kill microorganisms by directly compromising the integrity of the cell wall or plasma membrane. Any target organism finds it challenging to acquire a resistance to the biosurfactant due to the severity of such damage to the cell barrier (Banat et al. 2010a; Satpute et al. 2010). For instance, lipopeptides cause pores to form in the target organism's cell membrane, which causes an imbalance in the flow of ions into and out of the microbial cell and is fatal to the injured cell (Satpute et al. 2010). Additionally, the lipopeptide biosurfactant substances made by the *Bacillus* species especially exhibit growth inhibitory and lytic effects against a variety of bacteria. These include several viruses, fungi, and bacteria, both Gram-positive and Gram-negative (Van Hamme et al. 2006; De Rienzo et al. 2015; Rienzo et al. 2016; Efremenkova et al. 2019). Rhamnolipids, a glycolipid-based biosurfactant largely made by *Pseudomonas* species, exhibit algicidal, anti-amoebic, and zoosporicidal characteristics. Additionally, numerous bacteria, fungi, and even viruses are successfully eliminated by these lipid molecules (Soberon-Chavez 2011; Banat et al. 2010b). The study, which was conducted by Rienzo and colleagues (Rienzo et al. 2016), focused on the capabilities and characteristics of biosurfactants, such as sophorolipids and rhamnolipids, and sodium dodecyl sulfate (SDS), in combination with specific organic acids, to disrupt the formation of bacterial biofilms. The findings of the study suggest that these two types of biosurfactants have different modes of action against bacteria. For instance, while the antimicrobial properties of sophorolipids are concentrated between the stationary and exponential phases, the growth of these compounds is suppressed in the exponential phase. The scientists noted that sophorolipids could help break the *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* structures that have formed on glass coverslips. They noted that these structures could be useful in disrupting the formation of bacterial and fungal biofilms. The findings suggest that sophorolipids have a significant potential to be utilized in the treatment of bacterial and fungal infections. For instance, degradable sophorolipids can be used to remove the deposits of *Bacillus pumilus*. Surfactin produced by *B. subtilis* has also been shown to prevent the formation of these structures (Dusane et al. 2010; Mireles et al. 2001).

Bacillus species can create biofilms and effectively secrete a variety of antimicrobial substances, including biosurfactants from the family of lipopeptides, polymyxin B, and gramicidin S. They appear to be promising candidates to make sulfate-reducing bacteria-fighting antibiotics (SRB). According to studies by Jayaraman et al. (Jayaraman et al. 1999) and Zuo et al. (Zuo et al. 2004), naturally occurring or genetically created *Bacillus* species can produce antimicrobial chemicals in the biofilm, which prevent the growth of SRB, a cause of corrosion, and slow down the corrosion rate of mild steel. It has been demonstrated that both pure gramicidin S and the supernatants of gramicidin S producers suppress the growth of the SRB. It has been established that these antimicrobial agents alter

cytoplasmic and outer membranes as part of their mode of action. The use of microorganisms within a biofilm complex to prevent the colonization of SRB is an effective and cost-effective way to address this issue. The reduction in the concentration of corrosion inhibitors and biocides can be achieved using microorganisms within a complex.

To prevent the corrosion of stainless steel, a biosurfactant was created by the bacterium known as *Pseudomonas fluorescens* by Dagbert et al. (Dagbert et al. 2006b). This biosurfactant prevented the steel from corroding. In the marine industry, stainless steel is commonly used. The impact of the biocomplex on the surface of a newly cut Al-Cu-Mg aluminum alloy was studied by Zin et al. (Zin et al. 2018b). The alloy in synthetic acid rainwater was successfully suppressed by the rhamnolipid biosurfactant complex, which is made up of monorhamnolipid, dirhamnolipid, and polysaccharide biopolymer. With a rise in biosurfactant concentration, the effectiveness of inhibition grew stronger. Over the threshold micelle concentration, the inhibition was minimal. The creation of a monolayer barrier film because of biosurfactant adsorption on the surface of the aluminum alloy was likely the mechanism of corrosion inhibition. It was discovered in the past that the supernatant culture from *Pseudomonas sp.* PS-17 and the rhamnolipid biocomplex both prevented the corrosion of aluminum D16T alloy in distilled water and 0.1% sodium chloride (Pokhmurs'kyi et al. 2014).

To treat the corrosion of carbon steel (API 5LX), a biosurfactant was developed by Parthipan et al. (Parthipan et al. 2018). The use of this eco-friendly microbial inhibitor can help prevent the development of harmful microorganisms in the gas and oil industry. Due to its excellent corrosion resistance, carbon steel is commonly used in the production of various petroleum and gas products. The presence of certain types of harmful microbes, such as acid makers, sulfate-reduction bacteria, and manganese-oxidizing bacteria, can affect the resistance of carbon steel to corrosion. According to estimates, around 30–40% of the corrosion problems in the oil and gas industry are caused by microbes. The authors hypothesized that the low concentrations of a biosurfactant produced by the bacterium, known as F01, could help prevent bacterial strains from damaging the equipment. To identify potential biosurfactants that could be used as an alternative to prevent the development of biocorrosion in oil and gas facilities, Astuti and colleagues (Astuti et al. 2018) conducted a study. The researchers found eight biosurfactants from the rhamnolipid and glycolipid families, which were produced by natural bacteria, to be effective against biocompartment-associated biocorrosion. The researchers noted that biosurfactants could be useful in improving the oil recovery process and reducing the risk of biocontamination in the industry.

A new anti-corrosion agent could be made from the biosurfactant produced by the local bacteria found in oil reservoirs, *Bacillus sp.*, according to a study by Purwasena et al. (Purwasena et al. 2019). A new antimicrobial agent was developed to combat biocorrosion by inhibiting the growth of bacteria in an oil reservoir. The biosurfactant's inhibitory concentrations, minimum inhibitory concentrations, and minimum eradication concentrations were determined to determine the effectiveness of this new agent against different types of bacteria. They were also analyzed to

determine the impact of the biosurfactant on the structure of the community and the rate of corrosion of carbon steel. Due to their ability to create biosurfactants with antagonistic actions against a variety of fungal diseases, bacteria of the genus *Bacillus* are emerging as an alternative, as described in the literature (Silva et al. 2015, 2016, 2017; Soffritti et al. 2019). As a result, these chosen microbes or their by-products are a prospective contender for use as a secure, organic green biocide to protect cultural heritage artworks. Due to the wide variety of secondary metabolites with significant biological activity that *Bacillus* species create, they are worth the treatment (bioactive compounds). They are known to have antagonistic activity specifically against a variety of fungi (Sarwar et al. 2018). Some strains of *Bacillus subtilis* and *B. amyloliquefaciens* are known to produce antifungal peptides, such as surfactins, iturins, and fengycins, as well as antimicrobial polypeptides like subtilin and antifungal peptides like bacilysin and rhizocticin (Sarwar et al. 2018; Sharma et al. 2018).

Iturin-producing strains of the bacteria *B. subtilis*, *B. amyloliquefaciens*, and *B. pumilus* were isolated by Silva et al. (Silva et al. 2017). These strains had high antifungal properties, allowing the researchers to choose them as potential candidates for safe, natural green biocides for biodegrading cultural heritage artifacts. Biosurfactants can also prevent the development of biofilms in addition to being able to disrupt them (Pontes et al. 2016). The greatest approach to combating biofilms may be to prevent them from forming.

In one of their studies, Rajasekar and Ting (Rajasekar and Ting 2010) described the electrochemical behavior of aluminum alloy (AA 2024) when hydrocarbon-degrading bacteria *Bacillus cereus* ACE4 (a Gram-positive bacteria) and *Serratia marcescens* ACE2 are present (a Gram-negative bacteria). Using the BATH assay, which measures bacterial adhesion to hydrocarbons, the hydrophobicity of the cell surface was evaluated. *B. cereus* ACE4 grown on n-hexadecane-containing media had greater hydrophobicity and emulsification indices (86% and E72 85%) than *S. marcescens* ACE2 (60% and E72 75%). This notable shift might be the result of improved biosurfactant synthesis, which increases the hydrophobicity of the *B. cereus* ACE4 cell surface and improves bacterial adherence to the AA 2024 metal surface. Compared to *S. marcescens* ACE2, *B. cereus* ACE4 causes more severe corrosion damage. They also offered some insights into the MIC of AA 2024 produced by two bacteria that break down hydrocarbons in fuel/water mixes. Major fissures can be seen on the metal surface of AA 2024 in SEM photomicrographs (Fig. 3b,c) taken after exposure to the bacterial systems without the removal of biofilm and corrosion product (Fig. 3a).

With both bacterial infected systems, coupons were covered in deposits of thick, brittle, and lumpy corrosion products. After being exposed to the bacteria for 10 days in MSM media, the samples were subjected to SEM examinations, which revealed that *S. marcescens* ACE2 and *B. cereus* ACE4 biofilm had formed on the specimens (Fig. 4a, b, c, d). The AA 2024-accumulating bacteria displayed expected phenotypic characteristics (e.g., clumps of cells and microcolonies). The biofilm that developed on the metal surface is depicted in Figs. 4b, d. Both bacteria were found to have typical rod-shaped cells that were around 1 μ m in size. On the metal

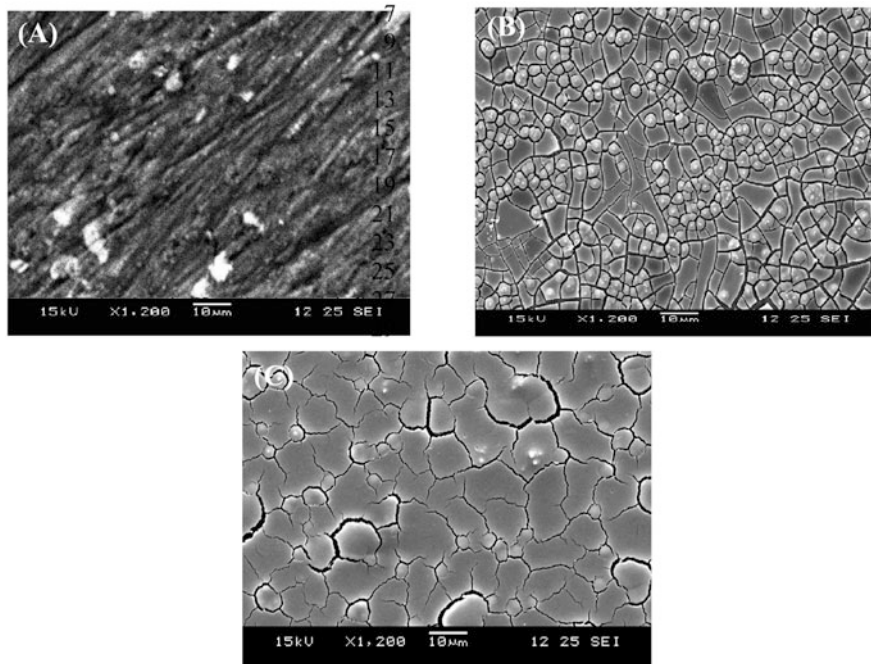


Fig. 3 SEM micrograph of corrosion deposits on the surface of AA 2024 exposed to (a) control, (b) *Serratia marcescens* ACE2, or (c) *Bacillus cereus* ACE4 (Rajasekar and Ting 2010)

surface, there was a layer of biofilm with microbial cell clusters and extracellular polymeric material.

Following the removal of the biofilm, the depth of corrosion brought on by *S. marcescens* ACE2 and *B. cereus* ACE4 was also determined by profiling the pits on the coupon surface using SEM-EDAX analysis (Fig. 5a–d). *S. marcescens* ACE2 and *B. cereus* ACE4 caused pitting corrosion of the metal, in contrast to the control system (Fig. 5a, Fig. 5b–d). Magnesium, aluminum, manganese, and copper oxide deposits of about 9.96 weight percent (*B. cereus* ACE4) and 7.75 wt% were detected by EDAX examination of the metal surface (*S. marcescens* ACE2). The overall percentage of oxides (3.37 wt%) in the two bacterial systems is much higher than in the control. The corrosion damage seen on the coupon surface in MSM medium with both bacteria indicated significant corrosion pits (Fig. 5b, d). This is due to the bacteria's capacity to oxidize aluminum ions to aluminum oxides, which then act as crucial components of alloy pitting corrosion, and to the formation of low-density aluminum hydrated iron oxide and $AlCl_3$ in corrosion tubercles.

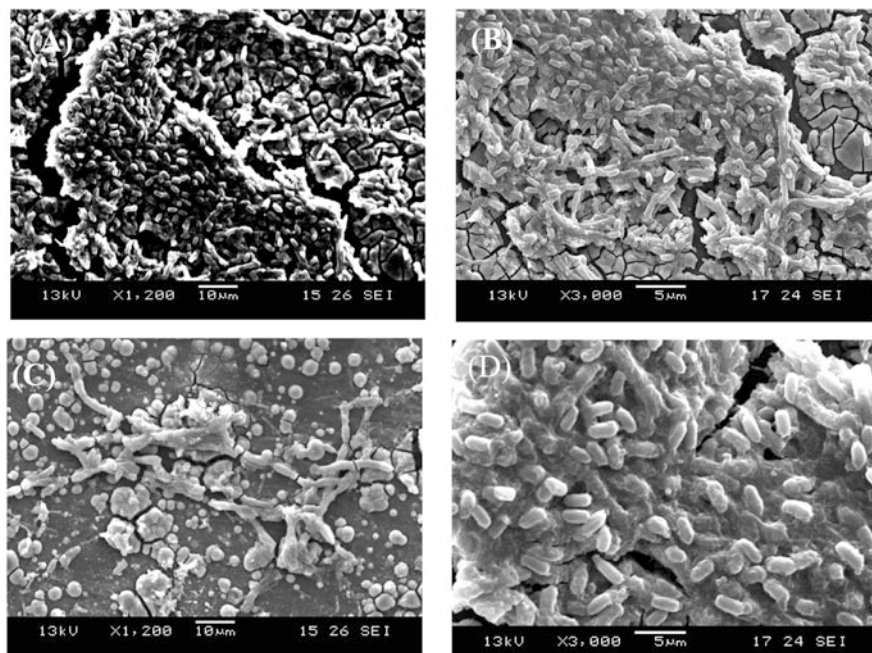


Fig. 4 SEM micrograph of the AA 2024 surface coupon after immersion of MSM medium with (a, b) *Serratia marcescens* ACE2 and (c, d) *Bacillus cereus* ACE4 (Rajasekar and Ting 2010)

5 Conclusion

To replace the chemically derived corrosion inhibitors and biocides currently employed as anti-corrosion agents with green alternatives that are eco-friendly and do not have adverse impacts on the environment or people, innovative research projects are required. Finding superior substitutes and environmentally responsible solutions is becoming a major concern. Extreme caution must be exercised while choosing an inhibitor to control corrosion problems in various industries under various environments. It must be environmentally friendly, easily producible, inexpensive, stable in challenging conditions, and biodegradable. Hence, researchers are very much interested in green inhibitors rather than chemical ones. Similar to this, it is vital to find new solutions based on natural sources and with relevant attributes, including effective antimicrobial activity, economically viable, low toxicity, and environmentally friendly features due to constraints associated with the usage of chemical biocides. Due to this, biosurfactants can be considered as one of the alternatives for harmful chemical anti-corrosive compounds. Although limited research has been carried out in the field, researchers are still far from achieving the primary objectives of developing environmentally friendly biocides like biosurfactants, despite the existence of several studies on the production of new

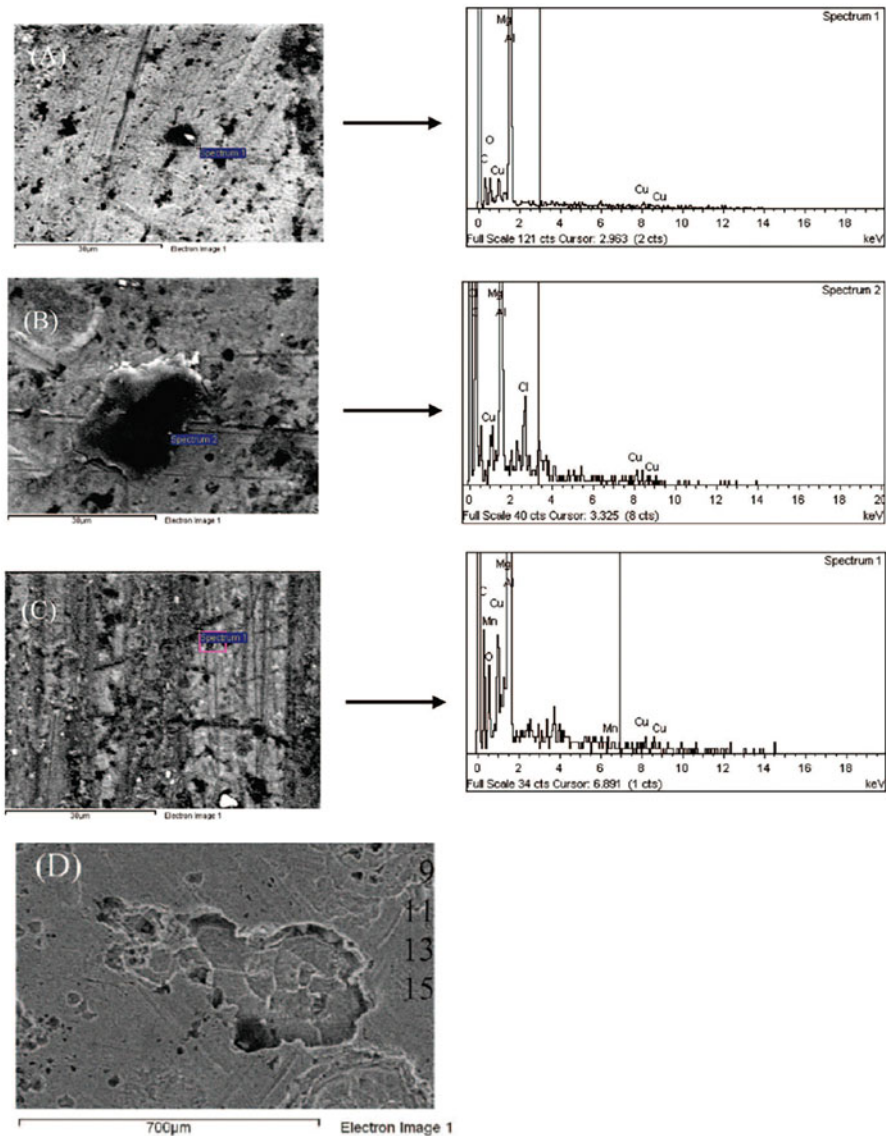


Fig. 5 SEM-EDAX micrograph of typical pits after removing the corrosion products in AA 2024 alloy at 2000 \times ; 10 days of immersion in MSM medium: (a) control, (b) *Serratia marcescens* ACE2, (c) *Bacillus cereus* ACE4, (d) closer view of pit (*Bacillus cereus* ACE4) (Rajasekar and Ting 2010)

forms of green biocides. It is also important to develop adequate on-site technology based on non-intrusive tools.

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Role of Biosurfactants in Nanoparticles Synthesis and their Stabilization



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1 Introduction

Solid particles or particulate dispersions with a size range of 10–1000 nm are called nanomaterials. Nanoparticles are distinguished by their stable shape and submicron size, which alters their chemical and physical properties in contrast to macro-scaled equivalents (Najeeb et al. 2022; Nazar et al. 2021; Najeeb et al. 2021; Mahmood et al. 2020; Abid et al. 2020; Joerger et al. 2000). Vast applications of nanoparticles in environmental cleanup, biosensors, catalysis, optics, targeted drug delivery, and biotechnology require scaled-up processes by modern synthesis methods (Rehman et al. 2019; Chen et al. 2009). Nanoparticles' surface effects and quantum size have potential applications in a variety of sectors due to magnetic, physical, chemical, and structural features not seen in individual or bulk molecules (Duester et al. 2016; Nazar et al. 2011a, 2011b; Myakonkaya et al. 2010; Ozin 1992). Material science still faces difficulties in synthesizing nanoparticles with high monodispersity and a variety of physical shapes.

Nanoparticles are typically created through a variety of chemical and physical complicated processes that involve high pressures, energy, and temperatures, as well as a variety of biologically harmful substances, resulting in pollution (Patel et al. 2005). The metal salts have been reduced to metal atoms by consuming reducing agents like ethylene glycol, hydrides, hydrazine, and citrate, all of which are dangerous to human health and environment. As a result, formation of metal nanoparticles (Me-NPs) has evolved as an alternative ecologically friendly approach throughout the previous decade. The biological methods of nanoparticle synthesis

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are part of a new generation of environmentally friendly processes that are intended to be viable alternatives to chemical and physical methods sometimes referred to as “green synthesis” or “green chemistry” procedures.

Nanoparticles are often synthesized using one of two approaches: top-down or bottom-up (Narayanan and Sakthivel 2010). The bulk materials are gradually broken down to nanosized materials in the top-down technique, whereas molecules or atoms are assembled to molecular structures in the nanometer range in the bottom-up approach. For biological and chemical nanoparticle production, the bottom-up technique is often used. A list of microbes that are known to synthesize various nanoparticles in modified form is presented in Table 1.

1.1 Biosynthesis of Microbial Nanoparticles

Microbial nanoparticle synthesis is a green chemistry strategy that ties microbial biotechnology and nanotechnology together. There has been a report on the production of silver, platinum, tellurium, gold-silver alloy, palladium, gold, selenium, and uraninite nanoparticles. Some bacteria have been reported to produce various forms of nanoparticles. Microorganisms perform this biogenesis of nanoparticles by grabbing target ions from solutions and then enriching the reduced metal in its elemental form via enzymatic activities (as bioreductants) generated by the metabolic activities of microbial cells. The nanoparticles are then classified as extracellular or intracellular synthesis depending on where they originated (Zhang et al. 2011).

Diatoms produce siliceous materials, while magnetotactic bacteria make magnetite nanoparticles (Mandal et al. 2006). Many species produce bio-minerals like calcium carbonate and gypsum layers, which are made up of an organic matrix (polysaccharides, lipids, proteins) and an inorganic component. These creatures have recently been dubbed “eco-friendly nano-factories” that manufacture diverse nanostructures.

2 Biosurfactants

Biosurfactants (BS) are identified as microbial surfactants. They are surface-active chemicals that are fabricated in biotechnological processes as secondary metabolites with the help of microorganisms (e.g., yeast, fungi, and bacteria) (Kubicki et al. 2019). BS production can be noticed at several stages of microbial development, ranging from mid-exponential to stationary evolution. However, Velraeds et al. investigated 15 *Lactobacillus* strains and found that in the stationary phase, they release surface-active chemicals than in the mid-exponential phase.

In contrast, BS synthesis can be augmented or promoted under stressful circumstances, such as by removing one or more nutrients from the standard. BS, like chemical surfactants, are made up of both hydrophobic and hydrophilic particles. As

Table 1 List of microbes well known to produce different nanoparticles in modified form (Thakkar et al. 2010)

Microorganisms	Type of Nanoparticles
<i>Bacteria</i>	
<i>Bacillus subtilis</i>	Ag
<i>Pseudomonas stutzeri</i>	Ag, Au
<i>Pseudomonas aeruginosa</i>	Au
<i>Shewanella algae</i>	Au
<i>Shewanella oneidensis</i>	Uranium (IV)
<i>Lactobacillus</i> strains	Au, Ag, Au-Ag alloy, TiO ₂
<i>Clostridium thermoaceticum</i>	CdS
<i>Klebsiella aerogenes</i>	CdS, Ag
<i>Escherichia coli</i>	CdS, Au
<i>Rhodospseudomonas capsulata</i>	Au
<i>Desulfobacteriaceae</i>	ZnS
<i>Rhodococcus</i> strains	Au
<i>Yeast</i>	
<i>Candida glabrata</i>	CdS
<i>Torulopsis</i> sp.	PbS
<i>Schizosaccharomyces pombe</i>	CdS
<i>S. cerevisiae</i>	Sb ₂ O ₃ , TiO ₂
<i>Fungi</i>	
<i>Verticillium</i> sp.	Ag, Au
<i>Fusarium oxysporum</i>	Ag, Au, Au-Ag alloy, CdS
<i>Colletotrichum</i> sp.	Au
<i>Aspergillus fumigatus</i>	Ag
<i>Trichoderma asperellum</i>	Ag
<i>Phanerochaete chrysosporium</i>	Ag
<i>Schizosaccharomyces cerevisiae</i>	Au
<i>Yarrowia lipolytica</i>	Au
<i>Torulopsis</i> sp.	PbS
<i>Candida glabrata</i>	CdS
<i>Actinomycetes</i>	
<i>Rhodococcus</i> sp.	Au
<i>Thermomonospora</i> sp.	Au
<i>Algae</i>	
<i>Chlorella vulgaris</i>	Au
<i>Phaeodactylum tricorutum</i>	CdS
<i>Sargassum wightii</i>	Au

a result, they are amphiphilic molecules that prefer to bind to fluid phase interfaces such as liquid/solid, liquid/liquid, or liquid/gas (Biniarz et al. 2017). Glucose, phosphate, alcohol, amino acids or peptides, and carboxylic acid make up the hydrophilic moiety, which is traditionally thought to represent the head of these

compounds. The hydrophobic fraction, on the other hand, includes unsaturated, saturated, branched, linear, and hydroxylated fatty acids (Shekhar et al. 2015).

Lipoproteins, glycolipids, glycopeptides, glycolipopeptides, and lipopeptides are only a few of the numerous combinations of these two moieties that result in a variety of categories. Biosurfactant production is also influenced by nutritional requirements (nitrogen and carbon supplies), as well as environmental conditions (agitation speed, oxygen temperature, and pH) (Santos et al. 2016). For example, Winery agro-industrial waste (e.g., grape pruning waste) was employed as a renewable and low-cost carbon source for *Lactobacillus paracasei* biosurfactant fabrication (Vecino et al. 2017). *L. paracasei* formed a glycolipopeptide when the carbon was obtained from glucose, from vineyard pruning debris, according to the researchers. When the sugar supplied was lactose, nevertheless, the biological surfactant was recognized as a glycoprotein.

Several eukaryotic and prokaryotic microorganisms have been documented to create biosurfactants, including several biosurfactants that have been extensively published, and as well as explored their yields, nitrogen and carbon sources used, and extraction methods. *Bacillus* spp. is known for creating a lipopeptide form of biosurfactant, whereas *Rhodococcus* spp., *Pseudomonas* spp., and *Candida* spp. are known for fabricating a glycolipid type of biosurfactant (a few studies also found *Pseudomonas* spp. synthesizing a lipopeptide type of biosurfactant) (Worakitsiri et al. 2011).

The size or chemical structure of BS is used to classify them. Glycolipids (small to medium-size molecules), phospholipids (medium-size molecules), polymeric surfactants (big molecules), lipopeptides (small molecules), lipoproteins (small to medium-size molecules), fatty acids (medium-size molecules), and particulate surfactants are some of the most well-known biosurfactant types (big molecules) (Grasso et al. 2020). Even though biosurfactants have advantages over chemical surfactants in the form of environmental friendliness, toxicity, stability, and specificity, the most significant barrier to widespread adoption is cost.

In numerous parts of the world, research is ongoing to lower production costs and boost output for industrial biosurfactant manufacturing. Use of less expensive substrates, selection and isolation of hyperproducing strains, process parameters, statistical approaches for optimizing nutrient contents, ambient conditions, and the downstream process are some of these strategies (Grasso et al. 2020).

2.1 Sources of Biosurfactants

Biosurfactants are potentially produced by microbes such as bacteria, fungus, and yeast. During their growing phase on water-insoluble substrates, most microorganisms produce a diverse variety of biosurfactants. Bacteria are reported to produce the majority of biosurfactants. Amphiphilic compounds generated extracellularly as

microbial metabolites are known as BS. The majority of biosurfactants are lipopeptide or glycolipid, but we just discovered a glycolipoprotein biosurfactant in the marine fungus *Aspergillus ustus* MSF3 (Kiran et al. 2009). In general, the hydrophobic moiety can be a carbohydrate, an amino acid, a cyclic peptide, a phosphate, a carboxylic acid, or an alcohol, and the hydrophilic moiety can be a carbohydrate, a carboxylic acid, a phosphate, an alcohol, or an amino acid. Microbial surfactants operate as emulsifying agents and/or chelating in nature to help use hydrocarbons more efficiently. Chemical and physical qualities, reduction, surface tension, and emulsion stableness are all key considerations when looking for a possible biosurfactant. *Candida* species have been described to synthesize surface-active agent and have been frequently employed for insoluble substrate fermentation (Sarubbo et al. 1999).

Biosurfactants can play three different roles in applications, according to Rosenberg and Ron (1999): Firstly by raising the surface area of water repellent molecules, secondly by enhancing the bioavailability of hydrophobic water-insoluble particles, and finally after regulating the attachment and detachment of microorganisms to and from surfaces. Different microorganisms produce some of the numerous biosurfactants (Banat et al. 2010).

All other microbes fabricate enzymes, including proteases, glucosidases, and lipases, have been employed to make a range of biosurfactants. Many enzymes were involved in the formation of BS; in particular, lipases are involved in manufacturing many forms of emulsifiers or which that include esters of sugar fatty acids (Capek 2004). Sugar esters are amphipathic synthetic compounds with nonpolar (fatty acid) and polar (sugar) moieties. They engage in a wide range of biological activities (Ozin 1992) and peculiar emulsifying/foaming properties (Bloemer et al. 1990).

2.2 Isolation and Selection of Biosurfactant-Producing Microbes

Because the entire process is dependent on the production capabilities of wild-type isolates, selection and isolation of increased production of microbial strains are two of the most important criteria for any biotechnological industrial fabrication process. Numerous forms of increased production of wild-type and mutant fungus, yeast, and bacteria strains have been described in the biosurfactant production process. Two key requirements for isolating biosurfactant-producing microorganisms are the basis of isolation and the screening method (such as surface and interfacial tension reduction studies, oil spreading method, blood agar plate, and so on) (Walter et al. 2010). As a result, there is an ongoing search for selecting and isolating biosurfactant hyperproducing microorganisms (Walter et al. 2010).

2.3 Use of Cheaper Substrates

Carbon and nitrogen sources, like any other sort of bioproduct, are two of the most important substrates for biosurfactant production. Biosurfactant production has been reported to utilize a variety of substrates, including lipids, carbohydrates, oils, fats, and hydrocarbons. In general, such pure substrates are quite expensive, adding to the production costs, so cheese whey, corn steep liquor, molasses, citrus fruit peels, waste water, waste frying oil, animal fats, potato peels, banana peels, and other agro-industrial waste products have been described to be useful for cost-effective biosurfactant synthesis (P. P. Rane et al. 2017). The usage of waste substrates serves two purposes: waste cleanup and utilization and lowering the cost of producing valuable biological products.

3 Biosurfactants: Types, Structures, and Properties

Surface-active biomolecules synthesized by living cells, primarily microbes, are known as BS (surface-active microbes or biological surface-active compounds) (Banat et al. 2010). They are amphiphilic biological molecules that include both hydrophilic and hydrophobic entities, allowing them to partition or exist at the nonpolar–polar edge (Franzetti et al. 2010). A wide range of microbes, mostly bacteria, fungi, and yeasts, create them. There are several types of biosurfactants and the bacteria that make them. They are formed on the surfaces of microbial cells or excreted extracellularly. So, their water-loving entities can be cyclic peptide, carbohydrate, phosphate, carboxylic acid, alcohol, or amino acid, whereas their hydrophobic entity is usually a hydroxyl fatty acid, alkyl-hydroxy fatty acid, or long-chain fatty acid (Satpute et al. 2010).

3.1 Structure

The structure of biosurfactants, such as the size and position of their functional groups, determines their functional qualities. Rosenberg and Ron (1999) classified BS into two categories: (1) bioemulsifiers are known as high-molecular-weight polymers (HMW) that are more efficacious as emulsion-stabilizing agents and (2) biosurfactants are known as low-molecular-weight surface-active agents (LMW) that are effectively at interfacial tension and at lower surface. The main group contains includes particulate and polymeric biosurfactants such as alasan and emulsan, while the second includes lipopeptides, glycolipids, and phospholipids (Banat et al. 2010). The majority of biosurfactants generated from microbial sources are neutral or anionic. Fatty acid derivatives or long-chain fatty acids make up the hydrophobic portion, whereas amino acid, phosphate, carbohydrate, or cyclic

peptides make up the hydrophilic portion (Kapadia Sanket and Yagnik 2013). The growth conditions and the type of microorganism determine the chemical composition and concentrations produced. Biosurfactant-producing bacteria has been identified from contaminated soils, effluents, and waste water (Marchant and Banat 2012a).

3.2 Types

Microorganism-produced BS is also classified into the following groups: (1) glycolipids—the most known of which are mannosylerythritol lipids, trehalolipids, rhamnolipids, and sophorolipids, which are predominantly carbohydrates that are connected to the long-chain aliphatic acids by an ester group or by hydroxyaliphatic acids; (2) hydroxylated and cross-linked fatty acids (mycolic acids); (3) lipopeptides-lipoproteins—a huge number of cyclic lipopeptides formed primarily by *Bacillus* spp. and divided into four families: iturins, fengycins surfactins, or plipastatins, and kurstakins; (4) lipopolysaccharides—which can have high molecular weights; (5) emulsan, alasan, liposan, lipomannan, and other polysaccharide–protein complexes are polymeric biosurfactants; *Acinetobacter calcoaceticus* RAG-1 produces an extracellular bioemulsifier; *Candida lipolytica* formate liposan, an extracellular water-soluble emulsifier; (6) phospholipids, fatty acids, and neutral lipids are mainly produced by yeast and bacteria that grow on n-alkanes, such as *R. erythropolis* and *Acinetobacter* sp. strain HO1-N. Basic chemical forms of biosurfactants synthesized by microbes are listed in Table 2.

3.3 Properties

Hydrophilic-lipophilic balance (HLB), charge, critical micelle concentration (CMC), and chemical structure are all features of BS (Marchant and Banat 2012b). Some physicochemical properties change at critical micelle concentrations, and the rate at this concentration is typically utilized to quantify the effectiveness of any biosurfactant. Because effective biosurfactants have a less critical micelle amount, they require a less biosurfactant amount to reduce surface tension. Biosurfactants can reduce interfacial and surface tensions, as well as raise the bioavailability and solubility of hydrophobic organic molecules, by forming micelles (Perfumo et al. 2010b). Biosurfactants' interfacial tension (IT) and surface tension (ST) are hence significant features. Water molecules are bound together by strong intermolecular forces, resulting in an increased surface tension of 72 mN/m.

The surface tension of the water is lowered when the biosurfactant is introduced. Surfactin fabricated by rhamnolipids, *Bacillus* spp. fabricated by sophorolipids, and *Starmerella bombicola* synthesized by *Pseudomonas aeruginosa* decrease surface tension to about 30 mN/m at the CMC. Similarly, the interfacial tension between

Table 2 Different biosurfactants fabricated by microbes (Płaza et al. 2014)

Biosurfactant type	Microbial specie
<i>Glycolipids</i>	
Trehalose mycolates	<i>Rhodococcus erythropolis</i> , <i>Arthrobacter paraffineu</i> , <i>Mycobacterium phlei</i> , <i>Nocardia erythropolis</i>
Trehalose esters	<i>Mycobacterium fortuitum</i> , <i>Micromonospora</i> sp., <i>M. smegmatis</i> , <i>M. paraffnicum</i> , <i>Rhodococcus erythropolis</i> , <i>Arthrobacter</i> sp., <i>Nocardia</i> sp.
Rhamnolipids	<i>Pseudomonas</i> spp., <i>Pseudomonas chlororaphis</i> , <i>Burkholderia</i> spp.
Sophorolipids	<i>Candida bombicola/apicola</i> , <i>Torulopsis petrophilum</i> , <i>Candida</i> sp., <i>Candida antarctica</i> , <i>Candida batistae</i> , <i>Candida riococensis</i> , <i>Candida stellata</i> , <i>Candida bogoriensis</i>
Flocculosin	<i>Pseudozyma flocculosa</i>
<i>Phospholipids and lipoproteins</i>	
Phospholipids, fatty acids	<i>Candida</i> sp., <i>Corynebacterium</i> sp., <i>Micrococcus</i> sp., <i>Acinetobacter</i> sp., <i>Thiobacillus thiooxidans</i> , <i>Aspergillus</i> sp., <i>Pseudomonas</i> sp., <i>Mycococcus</i> sp., <i>Penicillium</i> sp., <i>Clavibacter michiganensis</i> subsp. <i>Insidiosus</i>
<i>Lipopeptides and lipoproteins</i>	
Gramicidins	<i>Bacillus brevis</i>
Peptide lipids	<i>Bacillus licheniformis</i>
Serrawettin	<i>Serratia marcescens</i>
Surfactin, subtilisin, subsporin	<i>Bacillus subtilis</i>
Lichensyn G	<i>Bacillus licheniformis</i> IM1307
Amphomycin	<i>Streptomyces canus</i>
Globomycin	<i>Streptomyces globocaciene</i>
Bacillomycin L	<i>Bacillus subtilis</i>
Iturin A	<i>Bacillus subtilis</i>
Putisolvin I and II	<i>Pseudomonas putida</i>
Arthrofactin	<i>Arthrobacter</i> sp.
Fengycin	<i>Bacillus thuringiensis</i> CMB26
Mycobacillin	<i>Bacillus subtilis</i>

hexadecane and water is lowered from 40 to 1 mN/m. Another essential metric of biosurfactants is the hydrophilic-lipophilic balance (HLB), which characterizes features like conductivity, viscosity, density, osmotic pressure, and turbidity. Non-ionic surfactants were the first to be labeled with this title (Van Hamme et al. 2006). The HLB value is a scale from 0 to 20 that shows because a biosurfactant is more likely to form a oil-in-water or water-in-oil emulsion. Lower values suggest goods that form water-in-oil emulsions, while higher values indicate products that form oil-in-water emulsions. This factor can be used to decide which biosurfactant is best for which application. Emulsifiers with a reduced HLB are lipophilic in nature and

stabilize w/o emulsions, whereas those with an increased HLB have the reverse influence and help to increase water dissolving power. The HLB scale measures a biosurfactant's ability to generate oil-in-water or water-in-oil emulsions.

4 Advantages of Biosurfactants

BS have a number of advantages over their chemical counterparts, including:

1. Biodegradability—the chemicals do not survive in the environment, are readily destroyed, and hence do not gather in the environment due to their simple chemical structure and low toxicity (Fracchia et al. 2012).
2. Digestibility and biocompatibility—allowing them to be used freely in cosmetics, medicines, and as functional food additives (Campos et al. 2013).
3. Raw material availability—biosurfactants can be made from relatively inexpensive raw materials that are readily available in large numbers (Makkar et al. 2011).
4. Acceptable production of economics—most biosurfactants can be made from sustainable substrates, including by-products and industrial wastes, which signify a specific place for bulk manufacturing of BS, which is crucial for petroleum-related field technologies like microbial enhanced oil recovery (MEOR), and as detergents and cleaning products (Perfumo et al. 2010a).
5. Detoxification and biodegradation of industrial effluents—bioremediation of polluted soils, oil spill control, and industrial emulsion-stabilizing procedures are all examples of applications in environmental biotechnology (Franzetti et al. 2010).
6. Specificity—some biosurfactant molecules include specific functional groups that can be employed to de-emulsify industrial emulsions, detoxify certain contaminants, in food applications, and develop specific pharmaceuticals (Campos et al. 2013).
7. Effectiveness at extremes of salinity, pH, and temperature (Płaza et al. 2006). With such benefits and compatibility for a wide range of industrial applications, several writers believe that biosurfactants will become more appealing as multifunctional constituents in the future (Marchant and Banat 2012a).

5 Biological Synthesis of Nanoparticles

All efforts for the production and nanomaterial stability are new; the interactions between microbes and metals are long recognized (Beveridge et al. 1996). Metals were extracted and accumulated using two commercial biotechnological processes: bioleaching and bioremediation. Microorganisms are employed to remove pure inorganic metals from their minerals in bioremediation, but microbial degradation is used to eliminate hazardous heavy metals and organic chemicals from the environment. Nanoparticles have been synthesized using living cells, such as silver

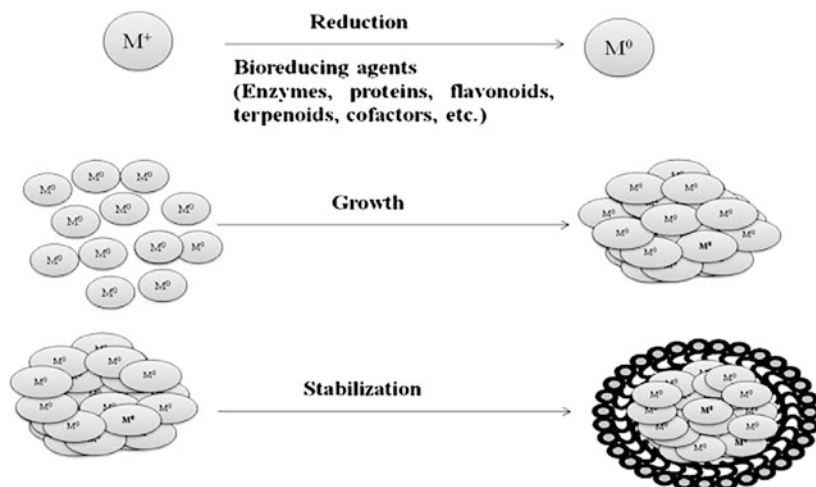


Fig. 1 Synthesis of metal nanoparticles (Me-NPs) (Plaza et al. 2014)

nanoparticles created by fungus *Aspergillus fumigatus* extracellularly (Bhainsa and D'souza 2006). Fungi and bacterial species create gold and silver nanoparticles. Microorganisms produce nanoparticles either extracellularly or intracellularly (Mukherjee et al. 2002) (Fig. 1).

Silver nanoparticle production was accomplished in *Pseudomonas* (Klaus et al. 1999), *Fusarium oxysporum* (Ahmad et al. 2003), the white rot fungus, *Phanerochaete chrysosporium* (Vigneshwaran et al. 2007), filamentous cyanobacterium, *Escherichia coli* (*E. coli*), *Klebsiella pneumonia*, *Plectonema boryanum* UTEX 485, and *Enterobacter cloacae* (Enterobacteriaceae) (Shahverdi et al. 2007). When compared to whole organisms, plant extracts and monosaccharides have been found to be substantial enhancers for nanoparticle formation (Shankar et al. 2004). Silver nanoplates were made using an extract from the unicellular green algae *Chlorella vulgaris* (J. Xie et al. 2007). Proteins present in the extract are thought to have a dual function in the nanosilver formation, reducing and controlling shape. The carboxyl groups in aspartic acid and hydroxyl groups in tyrosine amino acids are proposed to be important for reducing silver ions (J. Xie et al. 2007). In the creation of silver nanoparticles from $AgNO_3$, aqueous extract of geranium plant, plant extracts from live alfalfa, *Capsicum annum* L., *Pelargonium graveolens*, lemongrass, and protein concentration of waste mushrooms are employed as green reactants (Gardea-Torresdey et al. 2003).

5.1 Biosurfactant-Mediated Nanoparticle Synthesis

Biological nanoparticle production is more efficient; it produces nanoparticles at reduced ratio that use reducing mediators, with reaction duration ranging from 24 to 120 hours. A key disadvantage of biological nanoparticle synthesis is the long

reduction time and complex downstream process of nanoparticles that are formed from bacterial extracts. In this context, biosurfactants are emerging as a greener alternative to nanoparticle synthesis. Microemulsion techniques, which include oil, water, and a surfactant, have been proposed to be a viable way for nanoparticle creation (J. Xie et al. 2007). Biosurfactant-mediated synthesis, for example, was used to make silver nanoparticles. To prepare Ag-NPs, 1 mL of biosurfactant solution was mixed with 1 mM AgNO₃ solution, and then 1 mM was added to 20 L of NaBH₄ solution and stirred rapidly for 5 minutes. The color of the AgNO₃-biosurfactant solution was changed to a brown-yellow color. The formation of Ag-NPs is indicated by the brownish-yellow color. In the w/o microemulsion phase, generation of Ag-NPs was also performed in situ. 2 ml neat biosurfactant, 0.5 ml 50 mM aqueous AgNO₃ solution, 0.5 g n-heptane, and 1.5 g n-butanol were added to this procedure and stirred vigorously at room temperature until homogenized distinct micelles were formed. A comparable bulk solution of 100 mM aqueous NaBH₄ solution was used to replace aqueous AgNO₃ and also to construct inverse micelles. Two samples were homogenized for 60 minutes in a stirred environment. After 60 minutes, ethanol was added to the reaction mixture to cleave the reverse micelles (0.5 ml ethanol for 1 ml reverse micelles). Finally, centrifugation was used to separate the silver nanoparticles from the fluid. Nanomaterial production and stabilization using BS is a relatively new development in nanotechnology (Kiran et al. 2010) (Table 3).

Biosurfactants such as surfactin can be produced inexpensively in large quantities, leading to easier fabrication of metal nanoparticles. Biosurfactants minimize mass development due to electrolytic attractive forces and allow for the uniform shape of nanoparticles, making biosurfactant-mediated synthesis more efficient than bacterial or fungal-mediated synthesis. Various surfactants used for the synthesis of nanoparticles are summarized in Table 4.

5.2 Mechanism of Biosurfactant-Mediated Nanoparticle Synthesis

Chemical reduction is widely acknowledged as the most prominent and successful approach for producing huge numbers of nanoparticles. Chemical reduction has the advantage of producing varied forms of nanoparticles by changing the reaction conditions. The newly decreased nanoparticles, on the other hand, are exceedingly unstable and prone to combining into larger formations. Nanoparticles lose their original features and activities when they accumulate. Furthermore, the chemical

Table 3 Biosurfactant-mediated Ag-NPs. (Kulkarni et al. 2019)

S. no.	MDR strains	Zone of inhibition (in nm)			
		20 µl	40 µl	60 µl	80 µl
1	<i>S. aureus</i>	30	36	37	38
2	<i>Candida</i> sp.	28	32	34	36

Table 4 List of different surfactants that are used for the synthesis of nanoparticles (Kiran et al. 2011)

Surfactant	Type of nanoparticle
Alkylthiol molecules	Gold
AOT (sodium bis(2-ethylhexyl) sulphosuccinate)	ZnS
	Copper
	CdS
	Copper nanowires
Double-chained polymerizable surfactants (Surfmers)	BaSO ₄
AOT and phosphatidylcholine	CdS nanorods
C ₁₈ EO ₁₀ nonionic surfactant	CdS
Trioctylphosphine (TOPO); 11-aminoundecanoic acid (AMDAC); Didodecylamine (DDA); Tridodecylamine (TDDA)	Cobalt
Rhamnolipid	Silver
DEHPA	CeF ₃
Lauryl amine hydrochloride (LAHC)	TiO ₂
Gemini surfactant	Silver
Phosphatidylcholine (sodium dodecyl sulfate and CTAB)	Gold
Lytropic mixed surfactant [Nonaethyleneglycol dodecylether (C12EO9) and polyoxyethylene sorbitan monostearate (Tween60)]	Silica
Sodium dodecyl sulfate	TiO ₂ ; V2O5/TiO ₂
Cetyl trimethyl ammonium bromide (CTAB)	Cobalt-ferrite cu nanoparticles
	Gold palladium tin dioxide

reduction method's reactants are harmful chemical agents that may pose environmental and health dangers (Zeng et al. 2007). The contrary microemulsion (reverse micelle) approach has been proposed to control the limitations of chemical reduction and avoid nanoparticle self-aggregation (Xie et al. 2007; Saleem et al. 2018; Saleem et al. 2019; Saleem et al. 2020). The micelles work as "nano-reactors" for the synthesis of nanoparticles with precise size control (Chen et al. 2007). To inhibit crystal formation, Pal and Chauhan (2009) dubbed micelles "microreactors." The most common surfactant-mediated method is globular nanoparticle production in water-in-oil microemulsions.

BS are amphiphathic molecules with hydrophilic and hydrophobic halves that act as dividers at the interface between liquid phases with different degrees of hydrogen bonding and polarity, such as air and water and oil and water (Rodrigues et al. 2006). The water-soluble molecules in the droplet are loaded into the core of microemulsions, and the droplet acts as a "microreactor." Increase in surfactant concentration reduces the proportions of the droplet (microreactor) as the particle size decreases. The amount of water dissolved in the microemulsion has a big impact on the shape and size of the nanoparticles that arise. The impact of the water-surfactant molar ratio (R) on particle size monodispersity and supply has been proved (Han et al. 2008). When the particle size reaches the size of a water pool,

surfactant molecules are adsorbed on the outside of the particle, preventing molecules from sticking together.

As a result, microreactors based on microemulsion droplets, which are critical for fabricating smaller particles in controlled synthesis, might regulate the magnitude and form of nanoparticles produced in such a medium. The attractive forces (van der Waals, repulsive, osmotic, and elastic forces) between reverse micelles lead to micelle collisions and reactant exchange in a microemulsion-mediated borohydride reduction process. The resulting monomeric silver nuclei begin to develop in the micelles and grow to a size determined by the water core of the microemulsion.

6 Role of Biosurfactants in Biosynthesis of Metallic Nanoparticles (Me-NPs)

Identifying the need of developing environmentally acceptable ways for manufacturing biologically dynamic nanoparticles, scientists have begun investigating the synthesis of metallic nanoparticles using biosurfactants as capping agents (Reddy et al. 2009). It was detected that biosurfactants produced by microorganisms could play a very important role in the process of aggregation and stabilization. The size was also reduced with the increase in pH from 5 to 9 at 4 °C as shown in Fig. 2.

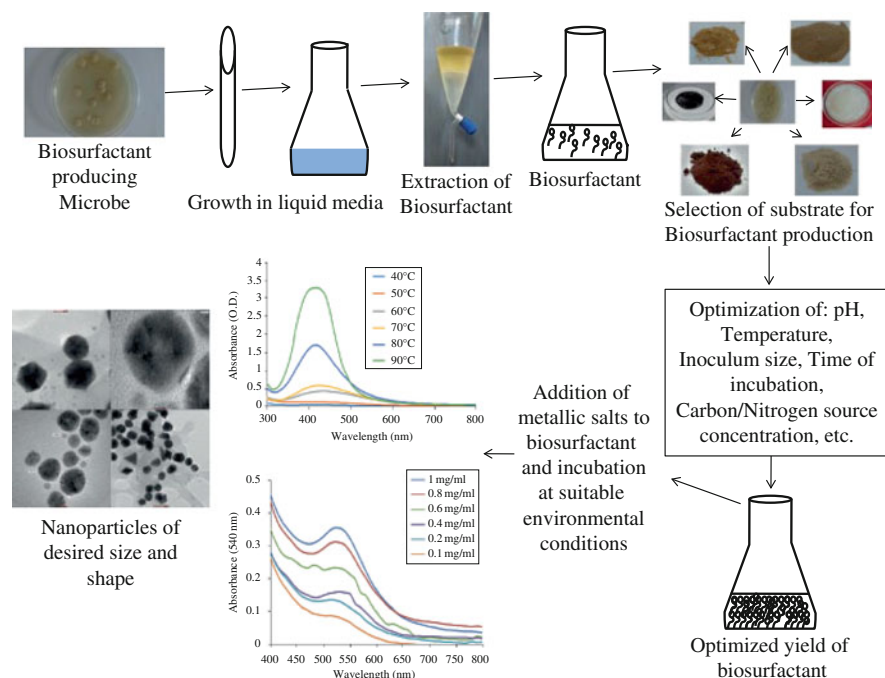


Fig. 2 Biosurfactant production, optimization, and nanoparticle synthesis using a biosurfactant. (A. N. Rane et al. 2021)

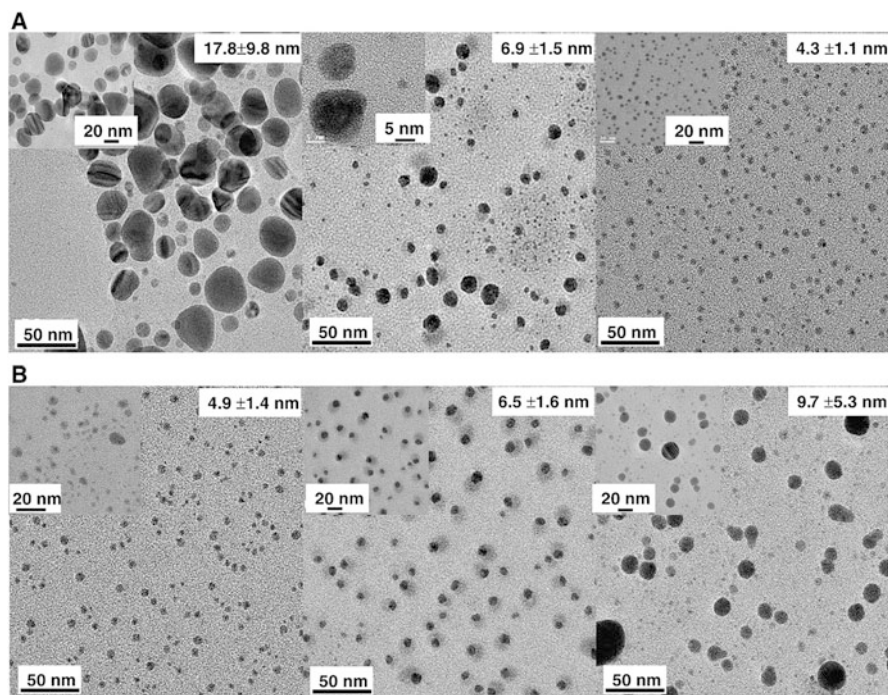


Fig. 3 TEM (transmission electron microscopy) micrographs of silver nanoparticles synthesized at 4 °C and room temperature (RT) at pH values 5, 7, and 9. Reprinted with permission from Elsevier, copyright 2009 {Reddy et al. 2009 #498 }

BS has now developed as a green substitute for both the synthesis and stabilization of nanoparticles (Fig. 3). Adsorbing on Me-NPs, surface stabilization of nanoparticles, and avoidance of subsequent aggregation are the routes of action. The thickness of the adsorbed layer and the kind of surfactant (polymeric, nonionic, ionic, etc.) determine the mechanism of surfactant adsorption (Kvítek et al. 2008). So far, no relative research has been published on the impact of the nature and composition of biosurfactants on the characteristics and abilities to govern the biosynthesis of metal NPs. In the manufacture of metallic nanoparticles, many laboratory-produced samples as well as commercial biosurfactants have been investigated as modifiers and stabilizers.

7 Glycolipids Biosurfactants Produced Nanoparticles

Y. Xie (Y. Xie et al. 2006) reported that AgNO_3 in w/o microemulsion are stabilized by commercial rhamnolipid. The nanoparticles were first produced in rhamnolipid reverse micelles using NaBH_4 as a reducing agent and then removed from the micellar solution and dissolved in heptane. The absorption spectrum of UV-VIS of the generated silver nanoparticles was analyzed, and they were stable for at least 2 months. The AgNO_3 formed in the system were uniform and spherical, according to scanning electron microscopy (SEM) and transmission electron microscopy (TEM). NiO nanorods were stabilized using commercial rhamnolipid (Palanisamy 2008). Two alternative microemulsion techniques were used to make the nanoparticles. The microemulsion was made by closing commercial rhamnolipid in heptane solvent and then introducing $\text{NiCl}_2 \cdot \text{H}_2\text{O}$ solution to mixture while stirring continuously in the first step. In the second method, for nickel chloride solution, the microemulsion was made by substituting NH_4OH . The first and second microemulsions were mixed altogether. Centrifugation was used to separate the precipitated nickel hydroxide, which was then cleaned with ethanol to eliminate heptane and biosurfactant. Nickel hydroxide precipitate was dried at $600\text{ }^\circ\text{C}$ for 3 h for the conversion of $\text{Ni}(\text{OH})_2$ to NiO. The nanorods produced were determined to be 150–250 nm in length and 22 nm in diameter. The pH has an effect on the shape of the produced nanoparticles.

7.1 Lipopeptides Biosurfactants Produced Nanoparticles

Nanoparticles have been made using lipopeptide biosurfactants (Reddy et al. 2009). In the creation of gold and silver nanoparticles, surfactin was used as a template and stabilizing agent. The reduction of aqueous AuCl_4 with sodium borohydrate in the manifestation of surfactin produced by *Bacillus subtilis* was used to make stable gold nanoparticles (Reddy et al. 2009).

Surfactin was extracted by foam fractionation from the culture supernatant and combined with a solution of pale yellow chloroaurate, which became red purple as the oxidation state of metal was changed and nanoparticles of gold were fabricated. The nanoparticles were made at various pH values of 5, 7, and 9, as well as at room temperature and $4\text{ }^\circ\text{C}$. They found that nanoparticles generated at pH 7 and 9 remained stable for two months, whereas nanoparticles synthesized at pH 5 aggregated within 24 h. They also noticed that as pH increased, the average particle size shrank. When compared to those made at $4\text{ }^\circ\text{C}$, the nanoparticles made at ambient temperature were more uniform and monodispersed.

8 Chemical Surfactants and Nanoparticles

Kvítek et al. (2008) studied the effect of a variety of chemically formed surfactants, including anionic surfactants such as SDS, nonionic surfactants such as Tween 80 and Brij detergents, and polymers like polyethylene glycols (PEG) and polyvinylpyrrolidone (PVP), on antibacterial activity and aggregation stability of silver nanoparticles prepared by a modified Tollens process using D-maltose as the reducing agent. Tween 80, SDS, and PVP proved to be good stabilizers among a variety of surfactants and polymers, with the added benefit of purifying the antibacterial activity of enhanced Ag-NPs. They also tried to characterize the mechanism of surfactant and polymer surface interacting with Ag-NPs as well as to specify antibacterial activity. The surfactant adsorption mechanism has not been fully characterized in the case of the Ag-NPs interaction of ionic surfactants. However, Chen and Yeh (2002) proposed that the hydrophilic groups of surfactant particles are adsorbed on the surface of the Ag-NPs, while the hydrophobic tails are absorbed externally to generate the first layer, accordingly, a proposed mechanism for the attachment of SDS molecules to the NP surface.

In the end, an opposite layer is formed, which forms the surfactant water repellent tail interpenetrating between two layers in which water-loving groups are pointing outside. The capability of SDS to upset the cell wall in Gram-positive strains and to raise the absorptivity of the cell wall was linked to the highest surface stabilization and high antibacterial activity in the case of Ag-NPs modified with SDS. Ag-NPs, Ag ions, and Ag have antibacterial and antiviral properties that are well known and widely used (Pérez-García et al. 2011).

9 The Antimicrobial and Cellular Activity of Nanoparticles

Glycolipid biosurfactants and other surfactin molecules have been shown to exhibit antibacterial, anticancer, and antiproliferative properties in a variety of cancer cell lines (Sen 2010). Numerous studies have been published on the usage and manufacturing of surfactin and *Bacillus* bacteria for bacterial, mold, and fungal disease management (Joshi et al. 2008). Recent research has found that carefully formed metal nanoparticles have strong antibacterial activity and that antimicrobial formulations based on nanoparticles could be efficient bactericidal and fungicidal materials (Guzman et al. 2012). The effective stability of silver NP dispersions is linked to the main application challenge. As a result, the aggregation process is prevented, and their antibacterial action is reduced.

The biological approaches for making nanoparticles are still in the early stages of development. Many struggles should be completed to optimize the fabrication process and to better acknowledge the relationships between metallic nanoparticles' physicochemical and antibacterial characteristics. The chemical, physical, and biological properties of Ag nanoparticles, as well as their applicability in environmental,

biotechnological, and medicinal domains, are determined by their size, shape, and controlled dispersion. The rapid and environmentally friendly manufacture of Ag-NPs employing various microorganisms and biosurfactants has revealed a huge potential. The literature contains a summary of nanoparticle synthesis employing microorganisms such as actinomycetes, bacteria, fungus, and yeast. The majority of biosynthetic mechanisms are unknown. Better monodispersity of nanoparticles and control of particle size produced by microorganisms are continuously being required. The effect of procedure factors (pH, temperature, concentration) on Ag-NPs parameters (shape, size, and spread of size) should be investigated, as well as the probability of controlling biogenesis by altering the above-stated parameters. Microbes, growth medium, and synthesis conditions all have an impact on the biological properties of metallic nanoparticles and also physicochemical properties which are still unknown.

10 Use of Lipoproteins and Lipopeptides in the Synthesis of Nanoparticles

The second group of biosurfactants most commonly produced is lipopeptides and lipoproteins. Their structure consists of a fatty acid chain and a peptidic head that gives them unique features that make them more biocompatible and thus suitable for drug delivery (Zhanataev et al. 2020). Nanoparticles have been extensively explored in the medical field, with nanoparticles being used to diagnose and treat a variety of ailments (Zhanataev et al. 2020). For example, in Zhao et al. (Zhao et al. 2017), the inclusion of lipopeptides in pathogen membranes enhanced the vaccine effect, defining the usage of lipid-based assemblies as co-adjuvants in vaccines.

As a result, using it in vaccine formulations boosts immune response by activating toll-like receptors. Moreover, the amphiphilic nature of lipopeptides provides a vast variety of possibilities such as that the three-dimensional structure of the vaccine would vary depending on the length of the lipidic chain, altering the vaccine's immunological capabilities. Furthermore, the lipopeptide-formed micelles can protect the vaccination or medication. As an example, consider the following scenario. Huang et al. (2018) established lipopeptidic nanoparticles, which rely on commercial surfactin, as a chemotherapeutic agent that is doxorubicin nanocarrier. After mixing doxorubicin with triethylamine in chloroform, surfactin was added under ultrasonic emulsification. Organic solvent was extracted from the solution at 40 °C. The usage of surfactin as a nanocarrier improved the drug's chemotherapeutic effectiveness, even in cancer cells that had formerly showed doxorubicin resistance.

Doxorubicin nanocarrier is used as a chemotherapeutic agent in which surfactin was added during ultrasonic emulsification after doxorubicin and triethylamine were mixed in chloroform. At 40 °C, the organic solvent was removed from the solution. According to this study, using surfactin as a nanocarrier increased the drug's

chemotherapeutic efficacy, even in cancer cells that had formerly shown doxorubicin resistance (Mehling et al. 2007).

In this context, using lipopeptides as metal nanoparticle stabilizers has proven to be an appealing option, merging the assembly of surfactants with the biocompatibility of lipids. There have been some reports of lipopeptides being employed in metal nanoparticle compositions. Surfactin, which was first created by *B. subtilis*, is the most illustrative biosurfactant in this category, as previously stated. Different metallic nanoparticles have been synthesized using this lipopeptide. For instance, in Reddy et al. (2009), surfactin was used to stabilize AuCl₄ after it was reduced with sodium borohydride. The production technique was tested at various pH levels, including pH 5, 7, and 9, as well as two temperatures, room temperature and 4 °C. The spherical nanoparticles with an average size of 4.8 nm that were synthesized at higher pH (pH 14, 7, and 9) and at room temperature were the most stable. In this regard, it is worth noting that biosurfactants have a higher stability against temperature, pH, and variations than synthetic surfactants, which opens up a world of possibilities in nanoparticles.

11 Use of Glycolipopeptides, Glycopeptides, and Glycoproteins in the Formation of Nanoparticles

There are a few studies on additional glycopeptide-based biosurfactants in the literature (a form of glycoconjugate particles that are composed of one or more saccharide moieties that are related to proteic fraction) (Faivre and Rosilio 2010) and also on glycolipopeptides (Ekpenyong et al. 2017). Although their application in nanoparticle manufacturing has not been completely eliminated. The research on these biosurfactants is still concentrated on their manufacturing methods and cost reduction. In reality, there are several examples of *P. aeruginosa* in fermentation being used to create glycolipopeptides utilizing various growth media (Ekpenyong et al. 2017). In this scenario, leftover frying oil was used as the carbon source, while urea was used as the nitrogen source. In addition, Vecino et al. (2017) using *L. paracasei* investigated the use of vineyard pruning waste as a carbon source to produce a cell-bound glycolipopeptide biosurfactant. Kiran et al. (2009) used various culture media under varied operational settings such as fermentation duration, temperature, pH, and salinity to optimize the manufacturing of a glycolipoprotein generated by *Aspergillus ustus*.

12 Conclusions and Future Perspective

In the fabrication of metallic nanoparticles by the enhancement of reliable and eco-friendly procedures, biological systems, bacteria, fungi, actinomycetes, and algae, are used in nanotechnology. For the nanoparticles production, the rich microbial diversity indicates their essential potential to behave as potential biofactories. To improve the synthesis rate and monodispersity of the product, the biochemical and molecular mechanisms involved in the synthesis of metallic nanoparticles need to be better understood. Characterization of BS plays part in the production of nanoparticles and has an impact on the size, nature, and spreading of nanoparticles. The structures of metallic nanoparticles are essential to explain the mechanisms that facilitate production of microbes and allow us to regulate nature, crystallinity, and the size of the synthesized nanoparticles. Upcoming study on the functions of biosurfactants is of great importance for the formation of nanoparticles that have exclusive properties and that show their definite applications in agricultural chemistry, medicine, and industries of electronics.

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New Trends in the Textile Industry: Utilization and Application of Biosurfactants



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1 Textile Industry

The textile industry is one of the oldest industries in the world and presents a high-income generation for developing countries (Ranasinghe and Jayasooriya 2021).

The increase of hazardous dye wastewater generated by various industries continues to be a serious public health issue and a major environmental concern (Moyo et al. 2022). The process of removing the dye from these effluents poses more challenges compared to the elimination of the soluble colorless organic substances (Palacios-Mateo et al. 2021). Fig. 1 shows other adverse contributory environmental impacts of the textile industry such as chemicals, energy use, and greenhouse gas (GHG) emissions.

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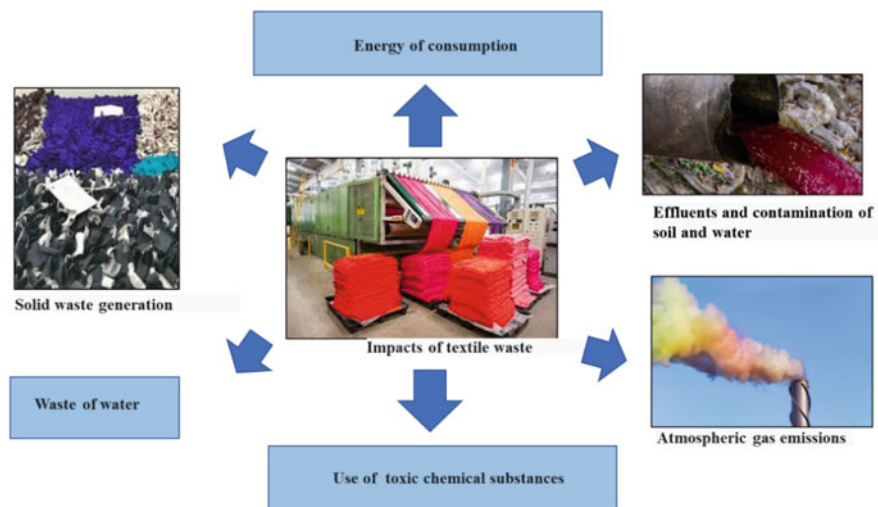


Fig. 1 Main environmental impacts of the textile industry

2 Textile Effluent

The improper releases of effluents from dyeing processes are the source of major environmental concerns among the textile industry. On average, 230–270 tons of water is used to produce 1 ton of textile material (Tavangar et al. 2019).

The wastewater from the finishing process contains mainly metals, organic matter, toxic substances, and surfactants (Partal et al. 2022).

2.1 Dye Toxicity

Every day, around 1.20 tons of this class of compounds are released into the environment. The main source of this loss corresponds to the incomplete fixation of dyes (10–20%) during the dyeing stage of textile fibers or in the stages of processing of jeans (ABIT 2005). The effluents from the dye industry and textile dyeing must be treated before being released into the receiving water bodies and reaching reservoirs and water treatment stations (Bouassida et al. 2018).

The effluents from industrial laundries and dry cleaners in City Toritama (State of Pernambuco), which contain a high organic load, accentuated color, and chemical compounds that are toxic to humans and the environment. They reach a large volume of highly polluting wastewater (55,985.25 m³/month) in addition to 147 m³/month of sanitary effluents, considering that the region does not benefit from basic sanitation (BRAZIL 2005; CPRH 2020).

This easily visible pollution, which is visually detectable even at concentrations below 1 ppm, causes changes in the biological cycle, especially in photosynthesis processes, in addition to being carcinogenic and/or mutagenic (Arslan-Alaton and Alaton 2007). All wastewater generated during the textile processing stages must be sent to equalization tanks. The intense industrial activity and the new products launched in the market make it difficult to enumerate and quantify all the organic products that may be present in the textile effluents (Costa et al. 2018).

The uses of several compounds in industries at different concentrations can be harmful to the human being, being able to cause from small irritations in the eyes and nose to the development of diseases such as cancer, alterations of the chromosomes, damage to organs (kidneys, liver, and lungs), depression, teratogenesis, and mutagenesis (Das and Mishra 2017).

The process of the emergence of textiles was a fundamental factor in the commercial success of the garments sector. Dyes of natural origin extracted from plants, animals, and minerals generally showed little resistance to washing and exposure to light. Thus, the search for resistant and high-fixation dyes gave rise to synthetic dyes at the end of the nineteenth century (Galdino Jr et al. 2020).

3 Conventional Treatment of Textile Industrial Effluents

Textile effluents present, in addition to dyes, high temperature, highly fluctuating pH, a large amount of suspended solids, high content of oxidizable materials, considerable amount of heavy metals, chlorinated organic compounds, and surfactants, making it necessary for efficient application of treatment techniques to minimize environmental impacts (Nigam et al. 2016).

Therefore, textile industries must carry out treatments of their effluents in loco before their discharge into water bodies. In order to meet this requirement, research has been carried out in the search for technologies aimed at reducing the organic load and discoloration of dyes in the effluents (Nor et al. 2021).

Textile effluent treatments may include physical, chemical, physical-chemical, and/or biological processes. There is no universal method of treatment. The methods for discoloration and degradation of dyes involve several technologies due to the complexity, variety, and chemical nature of the dye stuffs present in textile effluents (Sudarjanto et al. 2006).

Several treatments can be efficient in discoloration, but it is necessary to evaluate the possible formation of toxic products during the process. Techniques using bioindicators are useful in evaluating this degradation (Guaratini and Zanoni 2000).

The treatment of textile effluents has been considered one of the most important categories of water pollution control due to the great intensity of color and the high concentration of organic contaminants (Costa et al. 2018). Water, regardless of its quality, can be transformed into potable water, but the costs involved and the reliability of treatment and maintenance can make it impossible to use a certain source of water for supply. Operations are used to treat these effluents, when the

Table 1 Advantages and disadvantages of different methods used in the treatment of textile effluents

Methods	Advantages	Disadvantages
Hydrogen peroxide	It is an environmentally friendly oxidizer.	It is not effective for all classes, as its oxidation potential is not very high.
Electrochemical treatment	Generated compounds are not hazardous. Economic process.	High energy cost. Generates secondary products.
Activated charcoal	Removal of a wide variety of dyes.	High cost.
Membrane filtration	Removal of all types of dyes. Simple process. Environmental friendly: No chemical is used.	Concentrated logo production. Membrane fouling. High energy and maintenance costs. Poor dye mineralization.
NaOCl	Initiates and accelerates breaking of Azo bonds, fast discoloration, cheaper than other oxidants, and easily applicable.	Risks of formation of chlorinated hydrocarbons and increased toxicity.
Ozonation	Applied in the gas phase: No change in volume. Very efficient for color removal.	Short half-life (20 min).
Feton reagent	Effective bleaching of dyes.	Sludge generation.
Ion exchange	Regeneration: Does not lose adsorbent.	Not effective for all dyes.
Coagulation	Highly effective in eliminating metals.	Produces undesirable by-products. Requires precise control of pH. Deo UV.
Adsorption	Efficiency dye removal. Short reaction time.	Challenge in regeneration of adsorbents.
Biological degradation	An economical and efficient alternative in practical application, it treats high-concentration effluents.	Many dyes are stable and resistant to microbiological attacks.

Source: Adapted from Ceretta et al. (2021)

main objective is the elimination of a toxic substance, or its degradation to an innocuous form, biological treatments are used, using a consortium of microorganisms, combined ozonation techniques, advanced oxidative processes, electrokinetic, process, phytoremediation; nanoremediation, and the biodegradation processes (Markandeya et al. 2022).

Table 1 illustrates some advantages and disadvantages of some methods that can be used in the treatment of textile effluents.

4 Role of Biosurfactants in Promoting Environmental Sustainability

Sustainability reconciles economic growth with emerging social and environmental problems. The concept is represented by three pillars that stand for economic, social, and environmental factors. Sustainable technologies are expected to fall within the region that intersects all three concepts (Gayathiri et al. 2022).

In the fields of bioremediation and waste treatment, biosurfactants are ecologically safe. Biobased surfactants are considered as a replacement for their synthetically produced counterparts, as such their reduced toxicity and are biodegradable (Naughton et al. 2019).

4.1 Application of Biosurfactant in the Textile Industry

Processes of textiles using biosurfactants are of interest because of their increasing industrial use such as renewable resources and “green” products (Manga et al. 2021).

Therefore, the use of biosurfactants may represent the best alternative to overcome the toxicity of textile dye and other contaminant compounds.

Montoneri et al. (2009) used biosurfactants from urban wastes compost in textile dyeing and soil remediation and the results were quite promising. Verma et al. (2020) tested biosurfactants as alternative processes can promote enzyme activities that demonstrate the enhancement of decolourization and biodegradation of textile wastewater.

The applications of different types of biosurfactants used in the textile industry is given in Table 2.

Manufacturing industries are staking money on biosurfactants since there is increasing responsiveness among consumers for environmentally friendly compounds’ characteristics and properties (Farias et al. 2021). Table 3 summarizes commercial manufactures of different types of biosurfactants and biotechnological applications.

5 Conclusion and Future Perspectives

The biosurfactants are great candidates to replace synthetic surfactants, and investment in strategies to improve the bioprocessing of these biomolecules is the way to produce biosurfactants on a large scale using abundantly available cheap raw materials and waste products from different industries.

Table 2 Application of different types of biosurfactants used in textile industry

Class/type of biosurfactant	Microorganism	Application	References
Rhamnolipid	–	Removal the oil, chocolate, and stains from cotton clothes	Bafghi and Fazaelpoor (2012)
Sophorolipids	<i>Candida bombicola</i> ATCC22214	Removal the edible oil	Joshi-Navare and Prabhune (2013)
Glycolipids	<i>Candida lipolytica</i> UCP 0988	Removal of motor oil from contaminated cotton cloth	Santos et al. (2017)
Lipopeptide	<i>Bacillus subtilis</i> SPB1	Textile detergents improved their oil and tea stain removal	Bouassida et al. (2018)
–	<i>Cunninghamella echinulata</i>	Textile detergent to clean cotton fabric	Andrade et al. (2018)
Rhamnolipid	<i>Aspergillus versicolor</i>	Removal of a textile dye	Güla (2020)
Rhamnolipid	–	Removal of crystal violet (CV) from the aqueous solution	Verma et al. (2020)
Rhamnolipid	<i>Pseudomonas aeruginosa</i>	Treatment of textile effluents	Silva et al. (2021)
Lipopeptide	<i>Kurthia gibsonii</i> KH2	Biodecolourization and biodegradation of textile wastewater	Nor et al. (2021)

Source: Authors

The advantages and disadvantages of different methods of treating wastewater with dyes were presented in this work, as well as the potential of using biosurfactants in the processes of new eco-friendly technologies.

These bioproducts studies emerge as an alternative in the application of discoloration of dyes, showing potential in the treatment of dyeing wastewater, being a good choice for reducing environmental pollution.

Table 3 List of companies that have developed commercial-scale biosurfactant products for various environmental and biotechnological applications

Company	Website	Biosurfactants
Fraunhofer IGB—Alemanha	https://www.igb.fraunhofer.de/	Cellobiose glycolipids and lipids
AGAE Technologies—EUA	https://www.agaetech.com/	Rhamnolipids
TeeGene Biotech—UK	http://www.teegene.co.uk/	Rhamnolipids and Lipopeptides
Jeneil Biosurfactant—EUA	http://www.jeneilbiotech.com/	Rhamnolipids
Allied Carbon Solutions (ACS) Ltd—Japão	https://www.allied-c-s.co.jp/english-site	Sophorolipids
Rhamnolipid Companies—USA	http://rhamnolipid.com/	Rhamnolipids
Saraya co. ltd.—Japão	http://worldwide.saraya.com/	Sophorolipids
BioFuture—Irlanda	https://biofuture.ie/	Rhamnolipids
TensioGreen—EUA	http://www.tensiogreen.com/index.php	Rhamnolipids
EcoChem Organics Company—Canadá	http://www.biochemica.co.uk/	Rhamnolipids
Logos Technologies—EUA	https://www.natsurfact.com/	Rhamnolipids
Synthezyme—EUA	http://www.synthezyme.com/index.html	Sophorolipids
EnzymeTechnologies—EUA		Bacterial biosurfactant (unknown)
Ecover Eco-Surfactant—Bélgica	https://www.ecover.com/	Sophorolipids
Paradigm Biomedical Inc—USA	http://www.akama.com/company/Paradigm_Bio_medical_Inc_a7bcb2680775.html	Rhamnolipids

Source: Adapted from Farias et al. (2021)

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Biosurfactants as an Eco-Friendly Technology in Heavy Metal Remediation



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1 Environmental Contamination by Heavy Metals

Industrial, agricultural, and domestic wastes are sources of pollutants that contribute to environmental contamination. These pollutants can be from inorganic or organic sources. The inorganic pollutants are substances of mineral origin, such as metals, salts, and minerals. These substances enter the environment through natural processes, and also due to various human activities such as metallurgical and chemical processes, mine drainage, and metal smelting. Organic contamination is caused by biodegradable pollutants, these sources of pollution are naturally found in the environment, however, human activities also contribute to the generation of these pollutants. Some organic pollutants of great concern are polychromatic biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), and petroleum and organochlorine pesticides. Among all pollutants, heavy metals such as Pb, Ni, As, Cd, Hg, Co, Cr, Se, and Zn are highly toxic, even in small amounts (Masindi and Muedi 2018; Silva et al. 2022).

With global industrialization, a large amount of heavy metals have been released into the oceans. Heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), and

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chromium (Cr) are more problematic due to their flexibility, accumulation, non-biodegradability, being resistant, and causing greater potential risk to humans and ecosystems due to its high toxicity. These metals react with bioparticles in the body to form toxic compounds. Toxic characteristics depend on biomagnification and concentration, binding and oxidation states perform a vital role in heavy metal bioavailability (Chen et al. 2021; Rahman and Singh 2020; Carolin et al. 2017).

Second to Ishchenko (2018), industries are one of the main responsible to throw in the environment waste with heavy metals. Among the most active industries causing contamination are the chemical industry production of ceramic products, electronics manufacturing, metallurgy, production of batteries and accumulators, printing industry, production of catalysts, pigments and paints, and stabilizers for polymers. These industries cause the release of metals such as Pb, Cu, As, Ni, Cr, Zn, Co, Cd, and Hg in the environment.

Improper disposal and recycling of electronic waste is a serious threat to human health and the environment. E-waste has toxic chemicals in its composition, including a great diversity of heavy metals, hazardous chemicals, and carcinogens products (Sonone et al. 2020).

Electroplating is a polluting industry that discharges toxic materials and heavy metals through water, atmospheric emissions, and solid waste. It is a great generator of hazardous waste (sludge and rinse water for electroplated parts), and these wastes present a high-risk quotient (HQ) and high carcinogenic risk (CR). Rinse water contains many heavy metals and chemicals, electroplating sludge is the main solid waste generated through electroplating activity and has high concentrations of various heavy metals such as Zn, Ni, Fe, Cu, and Cr. Irrigation activity with electroplating wastewater is capable of causing soil contamination by heavy metals, causing the alteration of its properties and thus, generating a threat to the ecosystem and human health (Xiao et al. 2019; Pinto et al. 2021).

2 Role of Biosurfactants in Metal Remediation

Microbial biosurfactants are secondary metabolites that can present adhered to the cell surface or internally, being secreted out of it, and for their production, a variety of low-cost substrates can be used as a source of carbon and nitrogen, producing biosurfactants with a wide variety of molecular structures and different surface activities (Bezerra et al. 2018; Mishra et al. 2021).

In function to its compatibility with the environment and low toxicity, in addition to numerous other advantages such as biodegradability and stability under varied environmental conditions, the replacement of chemical surfactants by these natural compounds has been studied by many authors over the years. Then, biosurfactants are considered the next generation of industrial surfactants, as well as the “biomolecules of the twenty-first century”, because attend most requirements for industrial projects in various sectors causing low environmental impact (Santos et al. 2016; Jimoh and Lin 2019; Farias et al. 2021).

One of the biosurfactants utilization consists of the bioremediation of contaminated environments, including contamination by heavy metals. Heavy metals are available in nature, but they can also be released inside the environment like a consequence of various humans activities, releasing considerable amounts of these pollutants into nature (Rocha Junior et al. 2019; Vereda et al. 2019; Briffa et al. 2020).

There are currently a lot of conventional methods that can be used to treat environments contaminated with heavy metals, including electrokinetic extraction, chemical precipitation, phytoremediation, soil cleaning, flotation, ion exchange, and surface leveling, as well as several methods used *ex situ*, known as soil washing and solidification. However, some conventional methods have several disadvantages, such as the complexity of the processes, the production of tailings containing toxic compounds that need to undergo new treatments later, incomplete removal of metals, high operating costs, high cost of the chemicals used, and among others (Zamora-Ledezma et al. 2021). Therefore, as it is a green technology, biosurfactants have a high potential for application in the remediation of these environments, given their low capacity to cause damage to nature and their best qualities considered suitable for the removal of heavy metals from soils and waters (Jimoh and Lin 2019).

3 Mechanism of the Process of Heavy Metals Removal by Biosurfactants

Natural surfactants are biologically active compounds that have been successfully used to remove heavy metals. These microbial surfactants have several advantages in the remediation of heavy metals due to their high capacity for biodegradation and activity in environments with extreme levels of temperature, salinity and pH, low toxicity, and small size. As they present a diversity of structures, a wide spectrum of selectivity of metals and binding capacity, they confer greater removal capacity. Biosurfactants have an intense attraction for heavy metals occurring in the formation of a stable biosurfactant–metal complex (Jia et al. 2022; Ravindran et al. 2020; Mulligan 2021).

In the metal removal mechanism, biosurfactant acts as a link between fluid interface due to its amphiphilic nature, causing a reduction of surface tension. The decrease in water surface tension causes an increase in the mobilization of metal from the unsaturated soil, allowing its removal (Fig. 1). In the mechanism of metal extraction through the microbial surfactant, ion exchange, counter-bonding, and precipitation occur. In ion exchange, the negatively charged anionic biosurfactant bonds with positively charged metal cations to form a stronger bond than the bond generated between the metal ion and the soil. During counter-binding, the polar portions of the surfactant micelles bind to the metal ions leaving them soluble in water, thus, the recovery of the metal is carried out by washing. In the end, the

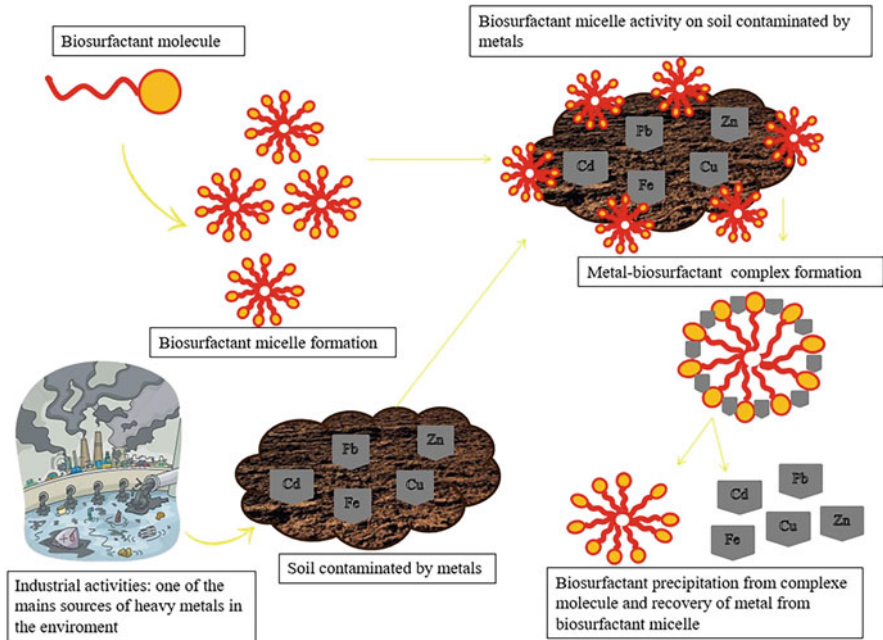


Fig. 1 Representation of heavy metal removal by biosurfactants. Source: Adapted from Srivastava et al. (2021)

metal–biosurfactant complex forms a strong bond where washing with water removes the complex from the soil matrix (Ayangbenro and Babalola 2018).

4 Applications of the Process

Natural surfactants have been extensively investigated with regard to manage and for restoration of environments polluted with oil and consequently suffer contamination by hydrocarbons, despite this, the application of biosurfactants in the ecological remediation of heavy metals in these places remains less explored than it should (Zhao et al. 2016; Karlapudi et al. 2020).

Due to their excellent properties such as high emulsification capacity and surface tension activity, in recent times the application of microbial surfactants has received some attention in the use of heavy metal bioremediation. In the process, biosurfactants increase soil metal desorption through surface accumulation, ion exchange, or complexation, which leads to a reduction in interfacial tension (Lal et al. 2018). In the remediation of heavy metals, the concentration of the biosurfactant to be used is a parameter of great importance (Marchant and Banat

2012). This is because the use of high concentrations of biosurfactant induces higher rates of removal of heavy metals, while low concentrations of biosurfactant result in lower rates of removal of these contaminants (Lopes et al. 2021).

5 Biosurfactants in Co-Contaminated Sites Remediation

The techniques used for soil washing act mainly through the solubilization and mobilization of metals, causing the removal of these toxic contaminants, causing the reduction of interfacial tension (Kumar et al. 2015). The mechanism to remove heavy metals contained in contaminated soil occurs through the action of biosurfactants achieved through microbial production.

In the remediation of soil contaminated with heavy metals, two methods are used. To ex situ technique utilization, the contaminated soil is excavated and placed in glass columns for later washing with a biosurfactant solution. In the in situ soil washing technique, drainage pipes and trenches are used to introduce and collect the biosurfactant solution in the soil (Hashim et al. 2011).

Natural surfactants can be applied to remove metals from a small part of the contaminated soil, where the soil is placed in a huge cement mixer, and the biosurfactant–metal complex will be treated to precipitate the biosurfactant, leaving the metal behind. The bond formed between the positively charged metal and the negatively charged surfactant is so strong that the washing water through the soil removes the metal complex of the surfactant from the soil matrix.

In comparison to chemical surfactants such as Tween 80, Sodium Dodecyl Sulfate (SDS), and Triton X-100, natural surfactants have a greater ability to promote the removal of heavy metals through soil washing (Jimoh and Lin 2020). The crucial advantage of the use of biosurfactants in soil washing is the ability to improve the mobility of metal ions, causing the formation of micelles and reducing the interfacial tension. In addition, it can increase soil conductivity, improving plant growth, and this is a useful practice where phytoremediation cannot be used to remediate contaminated environments. The sorption of biosurfactants in the soil is an essential factor for the remediation of metals, the greater sorption in the soil causes the loss and decrease of the efficiency of biosurfactants, making them less effective during the washing process (Singh and Cameotra 2013). Table 1 shows some biosurfactants capable to be used as an alternative solution for bioremediation of polluted environments by heavy metals.

Table 1 Biosurfactant as an alternative solution for bioremediation of polluted environments by heavy metals

Class/type of biosurfactant	Microorganism	Heavy metal removed	References
Fatty Acid Glycoside	<i>Candida tropicalis</i>	Zn, Cu, Pb	Rocha Junior et al. (2019)
Glycolipid	<i>Candida sphaerica</i>	Fe, Zn, Pb	Luna et al. (2016)
Rhamnolipids and saponin	–	Cu, Zn, Cr, Pb, Ni	Tang et al. (2018)
–	<i>Burkholderia</i>	Zn, Pb, Mn, Cd, Cu, As	Yang et al. (2018)
Lipopeptide	<i>Bacillus</i> sp.	Cu, Pb	Saleem et al. (2019)
Surfactin	<i>Bacillus subtilis</i> HIP3	Cr, Fe, Ni, Cu, Zn, Cd, Pb	Md Badrul Hisham et al. (2019)
Rhamnolipid	<i>Pseudomonas</i> sp.	Ni, Cu, Cd	Lee and Kim (2019)
Trehalolipids	<i>Rhodococcus</i> sp.	Co	Narimannejad et al. (2019)
Rhamnolipid	<i>Acidithiobacillus thiooxidans</i> and <i>Acidithiobacillus ferrooxidans</i>	Fe, Zn	Diaz et al. (2015)
–	<i>Bacillus cereus</i>	Cd, Zn, Cu	Wu et al. (2017)
Acidic- and bolaform glycolipid	–	Cu and Zn	Castelein et al. (2021)

Source: Authors

6 Patents of Biosurfactants for Application in the Removal of Heavy Metals

Surfactants of microbial origin, called biosurfactants, are surface-active compounds that play an important role in different industries, and have a wide variety of applications, and can be used in pharmaceutical, cosmetic, cleaning, food, etc. From an environmental point of view, biosurfactants are preferred due to their characteristics of greater biodegradability and because they are obtained from natural sources. However, the processes involving the production of biosurfactants are still very expensive, causing a cost disadvantage compared to the production of synthetic surfactants (López-Prieto et al. 2021).

Due to their promising properties and eco-friendly solution for several industries, the demand for biosurfactants has increased significantly on a global scale. In 2017, estimates of the world market for biosurfactants showed a value of more than \$4 bi and it was predicted to grow to more than \$5 bi dollars in 2022 with an annual growth rate of 5.6%. The global market of biosurfactants extends and prevails from

Table 2 Patents registered to the application of biosurfactants in the removal of heavy metals

Microorganism/ Biosurfactant	Patent n°	Publication date	Authors	Applications
New strains of hydrocarbon-degrading bacteria capable of producing biosurfactants	PI 0519962-0 A2	June 28, 2005	Robin L. Brigmon, Sandra Story, Denis Altman, Christopher J. Berry	Remediation of recalcitrant organics and heavy metals
Rhamnolipid	101948786	January 19, 2011	Xia Wenjie, Dong Hanping, Yu Li, Huang Lixin, Cui Qingfeng	Treatment of heavy metals in wastewater, application to degrading the crude oil, and application to fuel scavenge and oil extraction
Cationic Biosurfactant	3318/DEL/2015	April 21, 2017	Ganesan Sekaran, Paranji Saranya, Panchavarnam Bhavani, Somasundaram Swarnalatha and Asit Baran Mandal	In situ remediation of heavy metal contaminated sites
Rhamnolipid	106077056	November 09, 2016	He Chuan	Remediation method for heavy metal polluted soil in mining areas
Rhamnolipid	107555571	January 09, 2018	Xu Yunli	Treatment process for heavy metal wastewater

Source: Adapted from Silva et al. (2014)

Middle East Africa, the Americas, Asia-Pacific, and Europe to Asia (Ambaye et al. 2021).

Patented applications include the use of biosurfactants to recover crude oil from reservoirs, use as bioemulsifiers to stabilization of hydrocarbons, cleaning of oil-contaminated tankers, recovery of oil from the sludge of oil storage tanks, and, remediation of environments contaminated by heavy metals (Table 2) (Almeida et al. 2016; Silva et al. 2014).

Table 3 summarizes commercial manufacturers of different types of biosurfactants and websites.

7 Conclusion and Future Perspectives

The success of commercializing a biotechnological product largely depends on the economics of its bioprocessing. Currently, the prices of microbial surfactants are not competitive with the prices of chemical surfactants due to high production costs and

Table 3 Producing companies, websites, and types of biosurfactant

Company	Biosurfactants	Websites
Fraunhofer IGB— Alemanha	Cellobiose glycolipids and lipids	https://www.brazil.fraunhofer.com/
AGAE Technologies —USA	Rhamnolipids (R95, an HPLC/MS grade rhamnolipid)	https://www.agaetech.com
TeeGene Biotech— UK	Rhamnolipids and Lipopeptides	https://www.teegene.co.uk/
Jeneil Biosurfactant —USA	Rhamnolipids	https://www.jeneilbiotech.com/ biosurfactants
Allied Carbon Solu- tions (ACS) Ltd— Japan	Sophorolipids	https://www.allied-c-s.co.jp/
Rhamnolipid Com- panies—USA	Rhamnolipids	https://www.bioglyco.com/custom- rhamnolipid-synthesis.html
Saraya Co. Ltd.— Japan	Sophorolipids	https://saraya.world/
BioFuture—Ireland	Rhamnolipids	https://www.biofuture.ie/solutions/ ecocleaning
Logos Technologies —USA	Rhamnolipids	https://www.logostech.net/logos-technolo- gies-introduces-biodegradable-natural-alter- native-to-petroleum-surfactants/
TensioGreen—USA	Rhamnolipids	http://www.tensiogreen.com/
Synthezyme—USA	Sophorolipids	https://members.luxresearchinc.com/ research/profile/SyntheZyme
EcoChem Organics Company—Canada	Rhamnolipids	http://www.ecochem.com/
EnzymeTechnologies —USA	Bacterial biosurfactant (unknown)	https://www.edt-enzymes.com/

Source: Adapted from Almeida et al. (2016)

reduced yields in isolated products. In order to make biosurfactants commercially viable, it will be important to optimize production processes at biological and engineering levels. Advances in research involving the biosurfactant production process have already allowed 10–20 times increase in productivity, despite the need for more depth studies.

As reported in this review, biosurfactants are great candidates to replace synthetic surfactants, and investment in strategies to improve the bioprocessing of these biomolecules is the way to produce biosurfactants on a large scale. Therefore, the studies should be as well applied to the production of new biosurfactants that are able to quickly recover the product and cause the degradation of pollutants.

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Biosurfactants and Their Perspectives for Application in Drug Adsorption



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1 Introduction

The discovery of medicines and the constant evolution of these compounds is undoubtedly one of the most significant advances of humanity. The life expectancy of humans and animals has increased significantly over the years with the help of medicines. Due to population growth and the emergence of new diseases, the consumption of drug compounds is increasing. However, medicines end up reaching the ecosystem through the elimination of urine and feces of users (Rathi et al. 2021; Cunha et al. 2017; Huber et al. 2016), in addition, conventional effluent treatment systems are not efficient in removing these compounds.

The large volumes of domestic and industrial effluents are one of the consequences of economic expansion. It is up to science and technology to minimize the impacts of these effluents that can cause on receiving ecosystems. However, drug detection techniques in water have become more sensitive about the ability to quantify the concentrations of these compounds, warning about the impact of these compounds on the ecosystems. It has already been shown that drugs can cause chronic and acute effects on the ecosystem, especially the marine environment

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(Rathi et al. 2021; Patel et al. 2019). These compounds have characteristics such as endocrine dysfunction activity, high water solubility, persistence, bioaccumulation, and can be potentially carcinogenic and mutagenic (Rathi et al. 2021; Patel et al. 2019; Bonnefille et al. 2018; Noguera-Oviedo and Aga 2016).

The adsorption technique has become increasingly promising and efficient in the removal of drugs present in the liquid phase. Compared with other water treatment technologies, adsorption has advantages such as the simplicity of its equipment, ease of operation, economic from design to the operation process, no sludge formation and no generation of toxic intermediate compounds, the possibility of developing adsorbent materials with different raw materials, and the possibility of reusing adsorbents (Pal and Pal 2019; Afzal et al. 2018; Crini et al. 2018; Sophia and Lima 2018; Crini 2006). The development of adsorbent composites allows the use of distinct raw materials to combine into a new material with properties capable of satisfying the removal of different contaminants.

Biosurfactants are amphiphilic compounds produced by a variety of microorganisms, with the ability to withstand variations in temperature and pH, and which have the same emulsifying and surfactant properties as synthetic surfactants. These properties allow the application of biosurfactants in several areas, including medicine/health, agriculture, environment, and industries (Markande et al. 2021). In this sense, the use of biosurfactants has a high potential to be introduced in the formulation of ecologically appropriate adsorbents for environmental applications (Alshabib and Onaizi 2020; Perez-Ameneiro et al. 2015).

Some studies have been developed to increase the adsorption capacity of drugs with the use of biosurfactants. This area is recent and state of the art is still scarce (Natarajan et al. 2022; Kheradmand et al. 2021; Kumar et al. 2021). Such properties of biosurfactants can assist in the removal of contaminants present in water and soil. However, to understand the molecular interactions involved in these complex mixtures, the real role of biosurfactants needs to be studied. The different chemical structures of biosurfactants and contaminants probably change their interactions and how they organize structurally.

Therefore, this chapter aimed to approach the state-of-the-art use of biosurfactants in the adsorption processes of drugs present in the liquid phase. In this scenario, information on how biosurfactants can help in the process of drug removal in the liquid phase, data from experimental studies, and the possible interactions between biosurfactants and drugs are presented.

2 Characteristics of Biosurfactants

Biosurfactants are amphiphilic molecules with a hydrophilic/polar (head) and a hydrophobic/apolar (tail) part, being produced by bacteria, fungi, and yeasts (Mishra et al. 2021; Schmidt et al. 2021; Drakontis and Amin 2020; Gudiña et al. 2013). Due to the difficulty of microorganisms to use some substances, such as contaminants, as a source of carbon and nutrients, they release biocompounds, including

biosurfactants, to interact with different molecules (Decesaro et al. 2021; Machado et al. 2020). The main characteristics of biosurfactants are low toxicity and biodegradability, so they are considered ecological (Sarubbo et al. 2022; Ambaye et al. 2021; Mishra et al. 2021; Decesaro et al. 2020). In addition, they have several properties, including the ability to reduce surface tension, emulsifying activity, form micelles in the function of their critical micelle concentration (CMC), pH, and temperature tolerance (Sarubbo et al. 2022; Malkapuram et al. 2021; Mishra et al. 2021; Zhu et al. 2021; Drakontis and Amin 2020).

Biosurfactants are presented in aggregates of three types: micelle, lamella, and vesicle (Champion et al. 1995; Vinson et al. 1989). Micelle is a structure formed by several biosurfactants, organized with the nonpolar part facing the inside of the micelle, with the polar groups exposed on the outside (Malkapuram et al. 2021). Biosurfactant monomers can aggregate above a limited concentration to form stable micelles. This concentration is the critical micelle concentration (CMC), which is the minimum concentration required to achieve the lowest surface tension and induce micelle formation (Zhu et al. 2021). From the micelle, two types of aggregates can be formed, the vesicle and the lamella, which can be verified through electron microscope analysis (Shin et al. 2008; Lebrón-Paler et al. 2006; Champion et al. 1995). According to Champion et al. (1995), the change from the micelle to its aggregates occurs through pH variation, the lamella formation occurs mainly at pH 6.0, and the vesicular structure is formed mainly between pH 5.5 and 6.8. The aggregate to be formed in the medium also depends on the size of the polar group and the repulsion force present in the biosurfactant, when larger the group, the greater is the formation of micelles; and the smaller the repulsion force, the greater is the formation of lamella; being the vesicle the intermediate size of aggregates (Shin et al. 2008; Champion et al. 1995). Figure 1 shows the schematic structure of biosurfactants and their types of aggregates.

One of the main characteristics that can interfere with the removal of contaminants from the matrices is the CMC, which can be controlled by pH, temperature, and pressure (Sarubbo et al. 2022; Carolin et al. 2021; Mishra et al. 2021; Mohajeri and Noudeh 2012). Generally, the formation of micelles occurs through hydrophobic interactions and by Van der Waals interactions (Mishra et al. 2021). According to Zhu et al. (2021), the formation of micelles may be the key to the decontamination process because micelles immobilize contaminant molecules in their hydrophobic core for subsequent removal.

There are two major groups of biosurfactants: low molecular weight (50–1000 Da) and high molecular weight (1000–1500 Da) (Malkapuram et al. 2021; Mishra et al. 2021; Van Hamme et al. 2006). Low molecular weight biosurfactants are glycolipids, lipopeptides, phospholipids, and fatty acids (Malkapuram et al. 2021; Mishra et al. 2021) being widely studied. High molecular weight biosurfactants include polymeric polysaccharides and polymeric surfactants (Mishra et al. 2021).

Glycolipids have simple sugars in the hydrophilic group, such as rhamnose, glucose, mannose, and others. In the hydrophobic group, there are saturated and unsaturated lipids (Mishra et al. 2021; Drakontis and Amin 2020). Some examples of

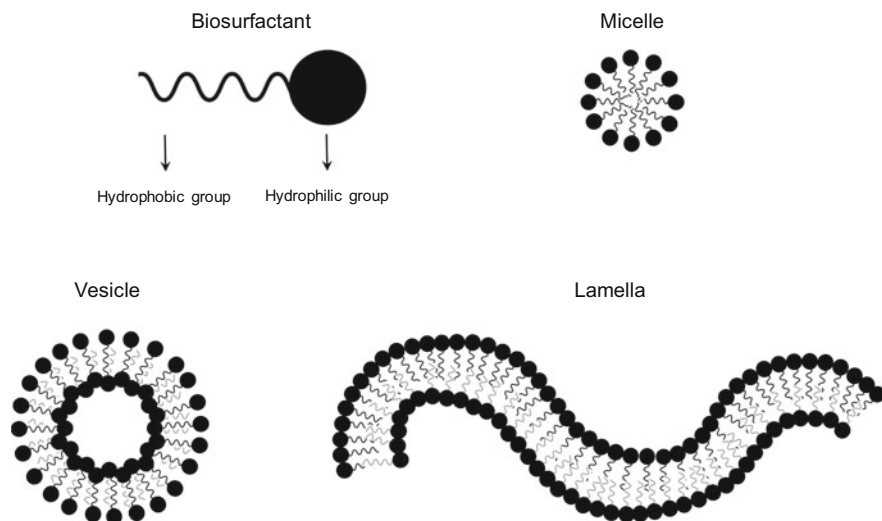


Fig. 1 Schematic representation of biosurfactants and types of aggregates

glycolipids are rhamnolipids, sophorolipids, trehalolipids, and mannosylerythritol (Mishra et al. 2021; Drakontis and Amin 2020). Rhamnolipids have an anionic characteristic due to the hydrophilic carboxylate and rhamnosyl groups, which classify rhamnolipids as mono and di-rhamnolipids. The hydrophobic group is composed of two chains of the 8-carbon alkyl group (Schmidt et al. 2021; Drakontis and Amin 2020; Nguyen and Sabatini 2011).

Lipopeptides have peptides, as this peptide portion is important for the characterization of the biosurfactant as anionic or cationic, and it also has saturated and unsaturated fatty acids as hydrophobic groups (Mishra et al. 2021). Surfactin, polymyxin, viscosine, serrewettin, fengycin, and iturin are examples of lipopeptides (Mishra et al. 2021). Surfactin is an anionic biosurfactant composed of cyclic lipopeptides with seven amino acids in the hydrophilic group and 13–15 carbons in the hydrophobic chain (Drakontis and Amin 2020; Andrade et al. 2017). Due to its anionic charge, surfactin interacts with proteins through electrostatic interactions (Andrade et al. 2017).

Phospholipids are components of bacterial cell membranes and are produced through the absorption of alkanes (Mishra et al. 2021). Fatty acid-based biosurfactants are produced via the biochemical pathway of alkane oxidation (Malkapuram et al. 2021; Mishra et al. 2021).

Polymeric polysaccharides and polymeric surfactants are high molecular weight biosurfactants. According to Mishra et al. (2021), polymeric polysaccharides are long chains of proteins linked to polysaccharides, and polymeric surfactants are natural proteins associated with surfactants produced by some animals, such as frogs (Cooper et al. 2017) and horses (Vance et al. 2013).

Biosurfactants can be obtained by microorganisms from different culture medium, with a wide variety of carbon and nitrogen sources, which are widely studied to reduce production costs, seeking alternatives in residues, mainly agro-industrial (Sarubbo et al. 2022; Machado et al. 2020; Malkapuram et al. 2021; Mishra et al. 2021; Schmidt et al. 2021; Decesaro et al. 2020; Van Hamme et al. 2006). In this way, the composition of biosurfactants can be changed according to the type of producing microorganism and also according to the types of carbon and nitrogen sources used in the culture medium (Sarubbo et al. 2022; Jayalatha and Devatha 2019; Kaskatepe and Yildiz 2016; Nguyen and Sabatini 2011).

Optimizing the culture medium of microorganisms is one of the best alternatives to increase the production of bioproducts, thus, it is necessary to make viable carbon sources for microorganisms, with the balance of nitrogen and micronutrient sources (Sarubbo et al. 2022; Decesaro et al. 2020; Kaskatepe and Yildiz 2016; Najafi et al. 2010). Carbon sources can be divided into three groups, carbohydrates, hydrocarbons, and vegetable oils, in which glycerol, glucose, sucrose, and crude oils stand out as excellent sources of carbon (Decesaro et al. 2021; Decesaro et al. 2020; Kreling et al. 2020; Andrade et al. 2017; Kaskatepe and Yildiz 2016; Chrzanowski et al. 2011). For nitrogen sources, peptone, urea, meat extract, malt extract, sodium nitrate, ammonium nitrate, and ammonium sulfate stand out (Sarubbo et al. 2022; Kaskatepe and Yildiz 2016).

In the literature, the most widespread biosurfactants are rhamnolipid and surfactin (Patowary et al. 2022; Augustyn et al. 2021; Decesaro et al. 2021; Mishra et al. 2021; Abbasi et al. 2020; Decesaro et al. 2020; Machado et al. 2020; Bhosale et al. 2019; Chrzanowski et al. 2011; Cohen et al. 2003; Champion et al. 1995). Different microorganisms can produce the same or similar biosurfactants. The bacterium that stands out in the production of rhamnolipids is *Pseudomonas aeruginosa* (Patowary et al. 2022; Chen et al. 2021; Bhosale et al. 2019; Li et al. 2016). Surfactin is produced mainly by bacteria of the genus *Bacillus* sp. (Schmidt et al. 2021; Decesaro et al. 2020; Liu et al. 2020; Machado et al. 2020).

Biosurfactants have gained prominence in the removal of emerging contaminants, especially drugs. These biomolecules can enhance the removal/biodegradation of contaminants through mobilization, dispersion, solubilization, emulsification, chelation, and adsorption (Carolin et al. 2021; Malkapuram et al. 2021; Zhu et al. 2021; Onaizi 2018; Usman et al. 2016).

3 General Concepts of the Adsorption Technique in the Liquid Phase

The contamination of water by certain substances required new technologies to remove these contaminants present in the liquid phase. In the treatment of water and effluents, adsorption is one of the most efficient techniques used to reduce the levels of toxic compounds present in the ecosystem (Machado et al. 2022; Melara

et al. 2021; Silva et al. 2021; Wang et al. 2020; Li et al. 2016), being one of the most popular methods for removing these contaminants. Its importance stands out as a separation and purification process that has been the subject of studies since the beginning of the twenty-first century due to its technological, biological, and practical applications in industry (Nascimento et al. 2020; Maccabe et al. 1993).

The phenomenon of adsorption results from a combination of several forces involved in physical and chemical adsorption. This phenomenon consists in the mass transfer of a solute present in a fluid phase to the surface of a solid material (Nascimento et al. 2014; Kinniburgh 1986; Ruthven 1984). Therefore, several factors can affect the adsorption process, such as the properties of the adsorbent (solid and insoluble material that processes the adsorption) and the adsorbate (dissolved molecule or particle to be adsorbed), surface area, volume and distribution of pores, properties of the solvent, pH, and temperature of the medium (Humelnicu et al. 2017; Nascimento et al. 2014; Yagub et al. 2014; Piccin et al. 2009).

In addition to the properties mentioned above, others that stand out are the pore density, functional groups present on the surface, and hydrophobicity of the material (Açıkyıldız et al. 2015; Piccin et al. 2009). The properties of the adsorbate depend on some factors, such as molecular size, solubility, polarity, acidity, and/or alkalinity, which selects the species that will have more affinity between adsorbent and adsorbate (Nascimento et al. 2020).

The interaction forces in the adsorption are determined by the bonds between the adsorbent and the adsorbate, divided into two types: physical and chemical adsorption. Chemical adsorption is a type of adsorption considered localized because only some points of the molecules dispersed in the liquid phase are connected with the adsorbent, being limited to bonds only at their active sites (Piccin et al. 2017; Nascimento et al. 2014; Crini and Badot 2008; Cooney 1998). This type of adsorption is stronger than physical adsorption because its mechanism consists in the electron replacement or sharing between the adsorbent and the target molecule (Piccin et al. 2017; Nascimento et al. 2014). On the other hand, physical adsorption occurs from a physical attraction, commonly with Van der Waals interactions, electrostatic interactions, and hydrogen bonds. In this type of adsorption, there are no chemical bonds and no change in the chemical structure of the adsorbent, being called non-localized and possibly reversible (Nascimento et al. 2014; Zuim 2010; Crini and Badot 2008; Cooney 1998).

The pH is an important aspect of adsorption, as it influences the degree of distribution of chemical species (Cooney 1998). The adsorption process can be affected by the surface charges present in the adsorbent, this is because they are related to the characteristics and compositions of its surface, giving rise to the active sites (Piccin et al. 2017; Nascimento et al. 2014; Cooney 1998). To verify the chemical species dissolved in the solution, the zero charge point index (ZCP) can be used, which will infer the presence of positive or negative charges (Newcombe et al. 1993). Therefore, for pH values lower than ZCP, the surface charge is positive, which is favorable to the adsorption of anions. For pH values higher than ZCP, the surface charge is negative, facilitating the adsorption of cations.

In the adsorption isotherms, it is possible to obtain relevant information about the adsorption process, such as the adsorption capacity. When contact occurs between the adsorbent material and the adsorbate, the process occurs until the adsorption equilibrium point is reached (Piccin et al. 2017; Nascimento et al. 2014; Cooney 1998). This is because the ions present in the medium tend to transfer to the surface of the adsorbent material until the liquid phase reaches a constant concentration. When this step occurs, it is defined that the adsorption has reached the equilibrium state and then it is possible to determine the adsorption capacity of the adsorbent.

For this relation, adsorption isotherms are performed at a constant temperature with different dosages of adsorbent or by varying the initial concentration of adsorbate in the liquid phase (Nascimento et al. 2020; Maccabe et al. 1993). Temperature is an important parameter because, through it, the adsorption thermodynamics can be calculated, which is fundamental to verify the spontaneity and nature of the adsorption process. The different behavior of liquid phase adsorption isotherms was classified by Giles et al. (1960), in which the adsorption mechanism is inferred through its shape. Thus, it is possible to obtain information regarding the nature of the adsorption process (Piccin et al. 2017; Giles et al. 1960).

The use of physical-mathematical models of isotherms provides an assessment of the adsorption equilibrium demonstrating the maximum adsorption capacity of an adsorbent (q_{\max}) and relating it to the phenomena described by the models. Some models used in the literature for drug adsorption are the Dubinin-Radushkevich models (Dubinin and Radushkevich 1947), Freundlich (Freundlich 1906), Hill (Hill 1946), Langmuir (Langmuir 1918), Liu (Liu et al. 2003), Redlich-Peterson (Redlich and Peterson 1959), Sips (Sips 1948), and Temkin (Temkin 1941).

In addition, it is also important to evaluate the adsorption rate and what are the mass transfer mechanisms involved (Crini and Badot 2008). Through the adsorption kinetics is possible to obtain the relation of time with the removal of the adsorbate (Dotto et al. 2017; Qiu et al. 2009). The adsorption kinetics involves the mass transfer of one or more components contained in the external medium to the interior of the adsorbent particles, which must migrate through the pores to the innermost region of the particle, being that the diffusion in the pore and on the surface influence directly on adsorption kinetics (Dotto et al. 2017; Qiu et al. 2009).

Adsorption kinetics is generally expressed by curves of adsorption capacity as a function of contact time. For data analysis, mathematical models are used to predict the behaviors involved (Dotto et al. 2017). The fractional order pseudo-reactional kinetic models (Avrami 1939), pseudo-first-order (Lagergreen 1907), pseudo-second-order (Ho and McKay 1999), and Elovich model (Elovich and Larinov 1962) are the most used in the literature for the adjustment of experimental data in the removal of emerging contaminants.

4 Influence of Biosurfactants on Drug Adsorption

The use of biosurfactants in the adsorption technique tends to be an advantageous process. Biosurfactants can be introduced directly in the liquid phase and can also be used in the formation of a composite (Sharma et al. 2021; Kumar et al. 2021). The chemical composition of biosurfactants and their interactions with the adsorbent and the adsorbate are key factors for their application in the adsorption technique. Thus, it is necessary to understand the mechanisms involved in these processes, either with the addition of the biosurfactant in the composite formulation or as an auxiliary in the medium to increase the bioavailability of the contaminant. Table 1 summarizes the studies observed concerning the removal of drugs by adsorption using adsorbent materials with biosurfactants in their composition.

Biosurfactants can act as a spacing agent between layers of the base material and improve the stability of the material (Kheradmand et al. 2021). They can also affect surface charges and hydrophilicity/hydrophobicity, and reduce the surface and interfacial tension of the adsorbent with the aqueous system containing the contaminant (Natarajan et al. 2022). All these advantages contribute to successful adsorption, obtaining adsorption capacities between 55 and 200 mg/g according to Table 1, involving different interactions between the composites with biosurfactants and the drugs.

This was also verified by the study realized by Kumar et al. (2021), who developed carbon-based composites from tropical fruit residues (precursor of biochar) using biosurfactant and Fe_2O_3 as activators. These materials were able to improve the precursor surface porosity ($\text{GMAC} > \text{GAC} > \text{AC}$), consequently increasing the adsorption of diclofenac (77.51, 55.86, and 20.87 mg/g, respectively). In the activation process, the materials are impregnated into the precursor for reaction and partial depolymerization of hemicellulose and lignin. This provides some elasticity and decreases mechanical strength, making the particle swell. Then, the thermal process was carried out for dehydration and condensation of the components. Porosity was increased by the protection of some additional cross-links induced by the presence of metallic ionic species and biosurfactant functional groups (Kumar et al. 2021; Molina-Sabio and Rodriguez-Reinoso 2004).

In the study by Sharma et al. (2021), they sought to understand the reaction mechanisms involved in the synthesis of a material through the sol-gel method, using surfactin produced by *Bacillus subtilis* and a silica precursor (tetraethyl orthosilicate, TEOS). The interaction between the biosurfactant and TEOS occurred in the presence of aqueous NH_4OH , H_2O , and $\text{C}_2\text{H}_5\text{OH}$, to generate the complexed molecule of Si–O–surfactin. After this, these molecules bonded to each other to generate a spherical molecule composed of numerous complexes based on Si–O–surfactin. The main interactions with surfactin were through its hydrophilic part. Already Augustyn et al. (2021) verified, through the zeta potential, the occurrence of Van der Waals interactions between surfactin and activated carbon, being directly affected by the pH and the concentration of the biosurfactant.

Table 1 Types of adsorbents with biosurfactants and their operational conditions in the adsorption of drugs through liquid phase assays

Adsorbent	Drug	Operating conditions			q _{max} (mg/g)	Interactions	Reference
		pH	Temperature (°C)	Concentration (mg/L)			
Chitosan-encapsulated magnetic nanoparticles coated with rhamnolipids	Paracetamol	5	30	20	96.35 ^a	Chemical adsorption	Natarajan et al. (2022)
Magnetic rhamnolipid layered double hydroxide nanocomposite	Ibuprofen	5	25	80	200.09 ^a	Physical adsorption, electrostatic interaction, hydrogen bond and anion exchange	Kheradmand et al. (2021)
Glycolipid supported magnetic activated carbon (GMAC)	Diclofenac	5	–	10	77.51 ^a	–	Kumar et al. (2021)
Glycolipid supported activated carbon (GAC)					55.86 ^a	–	
Activated carbon (AC)					20.87 ^a	–	

^aLangmuir isothermal model

Thus, understanding the active sites available for interactions with the target molecules is necessary, as their use must also be justified by the improvement in physical and chemical characteristics to involve advances in materials science, in addition to the sustainability of processes.

Perez-Ameneiro et al. (2015) produced a lignocellulosic composite with the addition of a natural lipopeptide biosurfactant for the treatment of effluents. The presence of biosurfactant improved the homogenization and quality of the emulsion in the composite formulation step, in addition to resulting in a rougher, rounded, compact, and better-emulsified sphere. Thus, the adsorption of contaminants can be achieved through the formation of an emulsified biosurfactant coating around the adsorbents, and the functional groups present in the adsorbent can also increase the bond formation and adsorption capacity (Zhu et al. 2021).

Zhu et al. (2013) developed a lipopeptide-modified clay adsorbent (Na-montmorillonite) for heavy metal removal, which proved to be efficient for this purpose. This was due to the formation of complexes with the free metal ions in the solution through coordinated bonds or ionic bonds, with the O and N-rich ligands of the biosurfactant.

In the liquid phase, biosurfactants have the ability to interact with different substances due to their amphiphilic nature and can act in different ways to assist in the drug removal process. These interaction mechanisms are, often, described in studies related to drug degradation. Table 2 presents some studies that approach the degradation of drugs by biosurfactants and microorganisms.

In liquid phase, biosurfactants improve surface interactions between polar and nonpolar substances (Carolin et al. 2021; Sarubbo et al. 2015). It is known that the hydrophobic part of biosurfactants interacts with nonpolar compounds, while the hydrophilic part interacts with water at the interface (Malkapuram et al. 2021; Li and Chen 2009). Through these interactions, there is an increase in the bioavailability of the hydrophobic organic compound, making its removal or degradation possible (Malkapuram et al. 2021; Sarubbo et al. 2015). The surfactants from microorganisms are generally more efficient than chemical surfactants in reducing the surface tension of fluid–fluid interfaces (Onaizi 2018; Sarubbo et al. 2015). Thus, they are agents of interest to increase the bioavailability of contaminants and, consequently, in their removal (Malkapuram et al. 2021; Varjani and Upasani 2017; Usman et al. 2016).

Mobilization is one of the ways to improve the bioavailability of contaminants. This process occurs when the concentration of the biosurfactant is below its CMC (Malkapuram et al. 2021; Usman et al. 2016). On the other hand, solubilization occurs when the concentration of the biosurfactant is greater than the CMC through the formation of micelles (Carolin et al. 2021; Usman et al. 2016; Li and Chen 2009). The hydrophobic part of the contaminant is linked inside the micelle, and the hydrophilic part is linked with the liquid phase (Carolin et al. 2021). Thus, the increasing concentration of biosurfactants facilitates the complexation of the micelle with contaminants, improving their solubilization (Zhu et al. 2021).

Some biosurfactants are highlighted in terms of their ability to decontaminate by increasing the emulsification of contaminants in the liquid phase (Zhu et al. 2021; Onaizi 2018). Emulsification consists of dispersing hydrophobic organic compounds

Table 2 Types of biosurfactants and operational conditions in the biodegradation of drugs

	Drug	Operating conditions			Removal (%)	Time	Reference
		pH	Temperature (°C)	Concentration			
Biosurfactant/microorganism	Norfloxacin	–	30	7.5 mg/L	76	26 d	Jalowiecki et al. (2017)
Lipopeptides produced by three species of <i>Bacillus subtilis</i>	Triclosan	–	–	0.356 mg/L of domestic effluent	100	16 h	Jayalatha and Devatha (2019)
Lipopeptide produced by <i>Bacillus licheniformis</i>	Tetracycline	–	–	10 mg/L	84.60	120 h	Liu et al. (2020)
<i>Bacillus clausii</i> with the addition of rhamnolipid	Oxytetracycline	–	–		100	96 h	
	Chlortetracycline	–	–		100	24 h	
	Tetracycline	–	–		83.70	24 h	
	Oxytetracycline	–	–		100	24 h	
<i>Bacillus clausii</i> with the addition of surfactin	Chlortetracycline	–	–		100	72 h	
	Tetracycline	7.12	37		–	–	
Co-culture of surfactin-producing <i>Bacillus clausii</i> and <i>Bacillus amyloliquefaciens</i>	Oxytetracycline				76.60	–	
	Chlortetracycline				88.90	–	
	Triclosan	–	–	30 µg/g of sediment	93.87	56 d	Guo et al. (2016)
Bacterial community ^a with di-rhamnolipid (di-RL)					49.47		
Bacterial community ^a with mono-rhamnolipid (mono-RL)					76.03		
Bacterial community ^a with crude rhamnolipid (RL)					89.53		
Bacterial community ^a with crude rhamnolipid (RL)		8–9	35	< 90 µg/g of sediment			

^aIndigenous microorganisms in water-sediment systems

in the aqueous phase as tiny droplets that can be increased by the presence of biosurfactants in the mixture (Malkapuram et al. 2021; Varjani and Upasani 2017). High molecular weight biosurfactants are considered more efficient emulsifiers, as they interact with surfaces and stabilize dispersions from one liquid into another (Kreling et al. 2020; Varjani and Upasani 2017; Markande et al. 2013). Thus, the stability of a dispersion phase can be analyzed through the emulsification activity (Kreling et al. 2020).

It is known that several factors can interfere with the adsorption and biodegradation processes, such as pH and temperature. The electrostatic behavior, defined through the pH, will occur through the positive (H^+) or negative (OH^-) charges present on the surfaces of the adsorbent and the adsorbate. This behavior can make the process attractive or repulsive between molecules. The ionization constant of drugs must be considered to favor the adsorptive process. In addition, the main methods of separation and/or recovery of biosurfactants are carried out through precipitation due to changes in pH (Decesaro et al. 2021; Sharma et al. 2021; Machado et al. 2020; Kreling et al. 2020). In this way, surface precipitation of the contaminant/surfactant complex can be a removal mechanism.

On the other hand, changes in the pH of the solution can affect the morphology of biosurfactants (Wu et al. 2015). As an example of this, rhamnolipid changes its morphology from lamellar to vesicular and, later, to micelles with increasing pH (Champion et al. 1995). This biosurfactant is characterized as a weak acid and with increasing pH, its hydrophilic part becomes more charged (Alshabib and Onaizi 2020; Wu et al. 2015), also influencing the morphological arrangement of the biosurfactant.

Change in the pH can also affect the relationship between the contaminant and the sediment. At higher pHs, there is more organic matter dissolved in the liquid phase (Guo et al. 2016; Wu et al. 2015). This also ends up influencing the attraction that the contaminant may have for the sediment as a function of its dissociation constant (K_D), justifying the changes in the bioavailability of contaminants at different pH values. Under alkaline conditions, the sediment becomes more hydrophilic and the dissolved organic matter can act as an adsorption medium (Wu et al. 2015).

Temperature affects diffusion velocity of the adsorbate molecules and the adsorption thermodynamics (adsorption capacity). Thus, in Tables 1 and 2, the temperatures observed ranged from 25 to 37 °C. In addition to that, the temperature influences the amount of contaminant that will stay adsorbed (Guo et al. 2016). This parameter will also influence the microbiological communities present in the aqueous medium, remaining in the environment the most favorable for a given condition and affecting the degradation of the contaminant through direct effects on enzymatic activity (Pettersson and Bâath 2003).

According to Zhu et al. (2021), temperature affects the organization of micelles during their formation process. It is associated with the Krafft point, which is defined as the minimum temperature for surfactant compounds to form micelles (Zhu et al. 2021; Vauter-Giongo and Bales 2003; Hirata et al. 1996; Krafft 1899). In addition, temperature can influence the type of bonds that can prevail in the formation of micelles. Hydrophobic interactions increase in intensity with increasing temperature

(Mohajeri and Noudeh 2012; Chandler 2005), while electrostatic interactions are favored at low temperatures (Zhu et al. 2021) and hydrogen bonds are impaired with increasing temperature (Mohajeri and Noudeh 2012; She et al. 2012). In this way, temperature plays an important role in the formation and stabilization of micelles, and how they will interact with the contaminants.

The formation of micelles promotes desorption, diffusion, and dissolution of contaminants in the liquid phase through improved micellar decontamination (Zhu et al. 2021), which facilitates removal by different processes such as bioremediation, phytoremediation, microbial degradation, adsorption, precipitation, filtration, and among others (Natarajan et al. 2022; Sonowal et al. 2022; Decesaro et al. 2021; Liu et al. 2020; Machado et al. 2020). Micelles can also act in the transport of catalysts, such as nanoparticles, for various decontamination processes (Zhu et al. 2021).

The formation of micelles with different functional groups and charges on their surface stands out because of excellent chelating and adsorbing properties (Zhu et al. 2021). With this, the contaminants' interactions with the surface of the micelle form a complex mixture, these interactions can be of different types, such as ionic and electrostatic (Rastogi and Kumar 2021; Zhu et al. 2021; Zhu et al. 2013). Biosurfactant micelles can interact with inorganic contaminants, forming complexes that can be retained and removed (Zhu et al. 2021). One example is the formation of complexes by the interaction of the metal and the biosurfactant/micelles, this interaction occurs through the ion exchange of the negative ligands present on the surface of the micelle and with the metallic ions. This mechanism is increased when the activity of the solution phase of metal ions is reduced, according to Le Chatelier's principle (Srivastava et al. 2021; Rastogi and Kumar 2020; Atkins and Paula 2006).

The physicochemical properties of biosurfactants (for example, surface and interfacial tension and their CMC values) and their micellar formation depend on their type, their structure (geometry of the hydrophilic part and length of the hydrophobic part), and loaded groupings (Zhu et al. 2021). Consequently, they influence the formation of different conformations and behaviors of micelles, such as their size, shape change, and number of micellar aggregates (Zhu et al. 2021; Rastogi and Kumar 2020). In this way, the surface area of the micelle and the number of binding sites, which facilitate or determine interactions with contaminants, are directly affected (Zhu et al. 2021; Rastogi and Kumar 2020).

Alshabib and Onaizi (2020) studied the effect of rhamnolipid biosurfactant as an additive for the enzymatic remediation of bisphenol A by lactase. The rhamnolipid showed itself efficient to increase the enzymatic removal rate, due to the impediment of the access of free radicals/polymeric products formed in the active sites of the laccase, minimizing the loss of the enzymatic activity. This effect was found at concentrations below the CMC of the biosurfactant, in its pre-micellar form. However, the removal of bisphenol A decreased with the increase of rhamnolipid concentration, still below the CMC, possibly due to undesirable interactions with the enzyme and/or with the contaminant, forming aggregates with the biosurfactant molecules, preventing degradation by lactase.

Biosurfactants can replace the higher energy molecules at the interface, decreasing the free energy of the system and, finally, acting to reduce the surface and

interfacial tension of the two phases (Carolin et al. 2021; Zhu et al. 2021; Sarubbo et al. 2015). Thus, the effectiveness of removal processes using biosurfactants can be measured through the maximum reduction of surface tension (Haidar et al. 2020; Varjani and Upasani 2017).

5 Conclusion and Future Perspectives

New noble applications for biosurfactants are discovered, linked to the need for more sustainable products and processes to ensure the preservation of natural resources. The use of biosurfactants in drug adsorption processes in liquid phase is a topic to be filled in knowledge gaps. Few studies applied biocompounds for this purpose, obtaining satisfactory and improved results with their use, as was the case of rhamnolipid and surfactin. In this process, several significant factors are involved, and biosurfactants can act in different ways, such as spacing agents, in the reduction of surface and interfacial tension, mobilization, dispersion, solubilization, emulsification, chelation, and adsorption. In this way, the use of biosurfactants and operational agents remains challenging.

Thus, through this systematic review, it is confirmed that biosurfactants have several properties of interest in the process of drug removal in liquid phase. These can be inserted with the contaminant or in formulations of new adsorbent materials. The possible interactions of biosurfactants with drugs and other contaminants may be strongly related to the formation and morphology of micelles, and to the functional groups favorable for chemical interactions, increasing bioavailability and, consequently, the removal of substances. Physical interactions also help in this process. In addition, operating conditions such as pH and temperature must be taken into account to achieve efficient removal of emerging contaminants.

The development of adsorbent composites with biosurfactants allows their use in other areas, such as the development of sensors, membranes, catalysts, recovery of a molecule of interest, flotation agents, drug loading and delivery.

Acknowledgments The authors thank the Coordination of Superior Level Staff Improvement (CAPES—Financing Code 001), National Council for Scientific and Technological Development (CNPQ - Project Code 140541/2021-7), Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS) and the University of Passo Fundo (UPF).

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Role of Biosurfactants in Promoting Biodegradation in Waste Treatment



Brian Gidudu and Evans M. N. Chirwa

1 Introduction

The development of new products or the increase in production of goods in the food industry, agriculture sector, and oil industry to meet the world demand increases waste generation. Depending on the method of waste management, waste disposal can increase the carbon footprint and may contain inorganic and organic constituents that are considered toxic to the environment. For instance, benzene, toluene, ethylbenzene, and xylenes (BTEX), polycyclic aromatic hydrocarbons (PAHs) and petroleum hydrocarbons, and phenols are common constituents of organic wastes (Wang et al. 2022a, b, c). Most of these pollutants are hazardous and toxic to the environment because of their tendency to beget detrimental effects on plants, animals, water resources, and land (Gupta and Pathak 2020). The International Agency for Research on Cancer classifies BTEX compounds as carcinogens. It goes further to classify ethylbenzene as an IIB carcinogen, xylenes and toluene as Group III neurotoxins, and benzene as a Group I carcinogen (Leili et al. 2017). Therefore, attempts are always made to prevent these persistent and hazardous compounds from reaching the environment or remediating contaminated sites to protect the environment from extensive degradation.

Bioremediation is often an economically viable option for removing organic pollutants from waste. Bioremediation involves the partial or full biodegradation of pollutants by microorganisms (Sajadi Bami et al. 2022). Since it does not involve further environmental degradation by introducing foreign hazardous compounds, bioremediation is considered a sustainable waste treatment solution. However, bioremediation is a slow process and recalcitrant pollutants such as PAHs cannot easily be degraded by bacteria because of the complex structures that make them

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highly hydrophobic and insoluble (Pourfadakari et al. 2019). High hydrophobicity and insolubility decrease the bioavailability of the pollutants to the microbes, thereby affecting the biodegradation process. Biosurfactants are introduced during bioremediation to increase the bioavailability and biodegradation rate of hydrophobic organic compounds (Gidudu and Chirwa 2020b). Biosurfactants can increase the rate of biodegradation by increasing the bioavailability of the hydrophobic organic pollutants by 5–20%, depending on the type of biosurfactant applied (Chauhan et al. 2008). When synthetic surfactants are used to increase bioavailability, they must be applied independently from the bacteria to avoid inhibition of the growth of the microbes. But when it comes to biosurfactants, a single bacterium can be used to simultaneously produce biosurfactants as it degrades the pollutants (Bezza and Chirwa 2017). Biosurfactants are also sustainable and viable solutions since they have high biodegradability, diversity, emulsification potential, selectivity, and low toxicity. Moreover, biosurfactants can also be used effectively in varying salinity, pH, and temperature (Bezza and Chirwa 2015). The role of biosurfactants in aiding the degradation of priority pollutants in waste has been described extensively in this chapter.

2 Waste Management

Because of human activities, solid and liquid wastes are generated daily. Waste generation increases with an increase in the population and economic activity of a place. The production of waste rich in organic and inorganic constituents adversely affects water, air, and human health (Husain Khan et al. 2022). The waste management hierarchy of waste reduction, recycling, reuse, recovery, and disposal is usually considered to protect the environment. Much as it is easier to contain and manage waste that is well collected and separated, it can be challenging to remediate sites that have been contaminated with pollutants contained in different waste streams. The best technology for managing a specific waste stream is based on cost-effectiveness, durable operation, waste chemistry, reuse and discharge plans, space availability, and by-products (Singh et al. 2017).

Different types of waste usually contain four kinds of pollutants, i.e., xenobiotic organic compounds, heavy metals, inorganic macro components, and degradable organic matter (Ma et al. 2022). Some of the waste streams contain a combination of all the pollutants but waste streams containing xenobiotic organic compounds and degradable organic matter may contain PAHs, BTEX compounds, oils, dyes, and phenols (Wang et al. 2022a, b, c).

An example of waste containing xenobiotic organic compounds and degradable organic matter is petrochemical waste. Petrochemical waste is usually composed of heavy metals, asphaltenes, rusts (resulting from oxidation of storage and transportation surfaces), and heavy hydrocarbons, depending on the crude oil source (Pazoki and Hasanidarabadi 2017). Petrochemical waste mainly comes in the form of oil sludge composed of aqueous waste, contaminated solids, hydrocarbon wastes, spent catalysts, and inorganic wastes (Islam 2015).

On a micro level, oil sludge can be composed of slop oil emission solids, waste oils/solvents, tank bottoms and desalting sludge which are components of hydrocarbon waste. It may also be composed of hydroprocessing catalysts, spent inorganic clays and fluid cracking catalysts which are components of spent catalysts. Furthermore, sludge is often composed of spent caustic, waste amines and spent acids which are components of chemical waste. If the sludge has contaminated soils, it is likely to have waste coke, waste sulphur and heat exchanger cleaning sludge.

PAHs and BTEX constituents in petrochemical waste render it hazardous because it may beget mutagenic, teratogenic and mutagenic effects on humans, animals and plants (Singh et al. 2017). Therefore, any waste such as petrochemical waste containing PAHs and BTEX is classified as hazardous waste because of the presence of priority pollutants.

2.1 Toxicity of Waste Containing Hazardous Pollutants

Some of the major pollutants in waste are polycyclic aromatic hydrocarbons, BTEX compounds, and all kinds of emerging contaminants. However, PAHs are the most toxic component of waste containing hydrocarbons. In addition, PAHs have different levels of toxicity depending on their structure. PAHs can also combine with other compounds in the environment to form compounds of higher toxic potency than the original compounds.

In aquatic systems, aquatic species such as fish and coral reefs suffer from endocrine disruptions, gill abnormalities, osmoregulatory imbalances, reduced growth, embryo deformities, uncontrolled cell growth, hepatic tumours, increased mortality, immunotoxicity and gills deformation (Snyder et al. 2015).

Acute human exposure to PAHs causes diarrhoea, confusion, vomiting, nausea, eye exasperation, dermal soreness and irritation (Patel et al. 2020). Victims of exposure to benzo(a)pyrene, naphthalene and anthracene may experience dermal sensitization and irritation and thrombotic effects, especially in patients already having lung and heart diseases (Imam et al. 2022). The chronic effects experienced are cancer, lung impairment, impaired immune response, renal and liver cirrhosis, respiratory tract issues and eye cataracts (Lawal 2017).

Birds may undergo reduced growth and infertility in early and adult stages, whereas deformities and the death of embryos are seen in embryos (Waszak et al. 2021).

3 Bioremediation in Waste Management and Treatment

Bioremediation through techniques such as composting is considered a viable and sustainable method for managing industrial, municipal and commercial waste (Husain Khan et al. 2022). Biological waste management techniques like

bioremediation have low greenhouse gas emissions, require less monitoring, and produce leachate that does not require treatment (Ayilara et al. 2022). In bioremediation, microorganisms such as bacteria and fungi with good catabolic abilities are used to break down organic pollutants to restore polluted mixtures and environments. Methods such as bio-slurry treatment, land treatment, and composting/biopile are used to break down contaminants in contaminated media (Hu et al. 2013). This is achieved when indigenous microorganisms or more efficient degraders are introduced into the contaminated media to remove the pollutants efficiently. Methods such as bioaugmentation, biostimulation and bioventilation are employed to attain efficient degradation of the contaminants (Wu et al. 2016).

In bioaugmentation, efficient degraders or genetically engineered degraders are introduced into contaminated media to enhance degradation (Pugazhendi et al. 2022); In biostimulation, the environmental conditions of the media are adjusted to trigger the growth of existing microorganisms by changing the limiting factors such as electron acceptors and nutrients to increase the biodegradation of the contaminants (Popoola et al. 2022). In bioventilation, the rate of biodegradation is improved by injecting oxygen to enhance the growth of aerobic organisms (Kayastha et al. 2022). The rate of total mineralization of organic compounds such as hydrocarbons is proportional to the concentration of the compound. On a micro level, alkanes are the most highly degradable, followed by branched alkanes, low molecular weight aromatics, and cyclic alkanes in descending order.

3.1 Land Treatment

In land treatment, wastewater is directed to a constructed wetland where microbial degradation occurs (Ludang et al. 2022). In contrast to wastewater treatment, solid waste is combined with soil such that the contaminants can be removed by microbial degradation, evaporation, or photodegradation (Hejazi et al. 2003). To attain high removal of the contaminant, the rate of application of the contaminated media is regulated together with pH, moisture, fertilizer, and aeration (Abdelhafeez et al. 2022). Land treatment is commonly used because of its low capital investment. It is easy to engineer and customize, has low energy consumption, and can treat large volumes of contaminated media (Abdelhafeez et al. 2022). However, land farming is time consuming and requires an extensive land area where the waste can be displayed. A time range of 6 months–2 years or longer may be needed to attain substantial contaminant removal because the conditions are usually uncontrolled to enable the efficient breakdown of the pollutants (Khan et al. 2004). The method also leads to air pollution by emission of volatile organic compounds and is greatly affected by weather (Bhattacharyya and Shekdar 2003).

3.2 Composting/Biopile

In biopile, organic waste or contaminated media is collected into windrows (2–4 m) for the organic pollutants to be broken down by indigenous microorganisms (Scopetani et al. 2022). The efficient removal of the pollutants is enforced by adjusting the moisture content, blowing air, and adding nutrients achieved by manipulating the carbon, nitrogen, and phosphorus ratio (Wang et al. 2022a, b, c). This method requires less land than land farming and is more environmentally friendly since the volatile organic compounds can be controlled by auxiliary collection units (Wu et al. 2022). It is also easy to engineer and customize for particular conditions, but the treatment capacity is lower than land treatment.

3.3 Bio-slurry Treatment

In bio-slurry treatment, contaminated media is mixed with water 10–60% w/v to dissolve the pollutants into an aqueous phase in which the microbes can easily transform into less toxic intermediates such as carbon dioxide, water, organic acids, or aldehydes (Avona et al. 2022). Reactors such as the vertical tank and rotating drum equipped with mixing components are used to enhance degradation by increasing the contact between microorganisms and pollutants. The technology is effective and requires a small land area compared to land treatment and composting (Hu et al. 2013). But the method is not suitable for media with clay mixtures and produces volatile compounds. It also requires dewatering of the mixtures after treatment.

4 Microbial Degradation of Organic Pollutants Found in Waste

Bioremediation uses bacteria and their products, such as enzymes and biosurfactants, to mineralize organic compounds. In aerobic conditions, organic compounds can be transformed into water and carbon dioxide, whereas in anaerobic conditions, organic compounds are converted to methane (Haritash 2020). Degradation of pollutants during bioremediation can achieve positive results in eliminating hazardous wastes, but some pollutants such as PAHs are persistent, ubiquitous, and hydrophobic, making them difficult to degrade. Many microorganisms, mainly bacteria, fungi, and yeast, have the genetic makeup to aid in the breakdown of organic compounds. More than 200 species of bacteria are known as potential degraders of petrochemical hydrocarbons in both anaerobic and aerobic conditions (Singh et al. 2017). Bacteria can degrade most hydrocarbons including PAHs, but fungi simply transform most aromatics co-metabolically to less toxic products as they cannot utilize PAHs for

metabolic purposes (Ismail et al. 2022). Bacteria species such as *Rhodococcus*, *Pseudomonas*, *Mycobacterium* and *Bacillus* are known hydrocarbon degraders. *Trametes versicolor*, *Aspergillus* sp., *Pleurotus ostreatus* and *Phanerochaete chrysosporium* are some examples of fungi species involved in the degradation of organic compounds (Imam et al. 2022). Biodegradation of pollutants is affected by environmental conditions, structure complexity of the substrate, catabolic potential of the microbes, and the diversity of species of the microbes (Imam et al. 2022).

Starting with the easiest to degrade, hydrocarbons are arranged as n-alkanes, branched-chain alkanes, branched alkenes, low molecular weight n-alkyl aromatics, monoaromatics, cyclic alkanes, PAHs and asphaltenes (Singh et al. 2017). But PAHs are regarded as the priority pollutants of concern because of their complexity, toxicity and persistence in the environment. PAHs are categorized as lower molecular weight (LMW) and high molecular weight (HMW) PAHs depending on whether they have less than four benzene rings or more than three benzene rings, respectively (Premnath et al. 2021). HMW PAHs have lower aqueous solubility, are highly hydrophobic and have higher melting points than LMW PAHs (Ismail et al. 2022). In addition, HMW PAHs are recalcitrant hydrocarbons, so they are not easily degraded by bacteria. Examples of LMW PAHs are phenanthrene, anthracene and naphthalene, whereas examples of HMW PAHs are pyrene, triphenylene, chrysene and benzo[a]pyrene, amongst others.

Bacteria are regarded as the best degrading microorganisms because of their catabolic potential, diversity, adaptability and vitality. Bacteria can efficiently break down hydrocarbons into water and carbon dioxide as they use the energy and carbon obtained for the growth and maintenance of the cells. Both Gram-negative and Gram-positive bacteria can effectively break down hydrocarbons (Ismail et al. 2022). But Gram-positive bacteria are mostly reported as more efficient degraders of HMW PAHs (Subashchandrabose et al. 2019). *Mycobacterium*, *Corynebacterium*, *Actinobacterium* and *Rhodococcus* are widely reported as Gram-positive bacteria used during remediation. On the other hand, *Pseudomonas*, *Stenotrophomonas maltophilia*, *Sphingomonas* and *Sphingobacterium* are the most extensively reported Gram-negative bacteria used in bioremediation. Besides concentration of the pollutant, the degradation of hydrocarbons may also depend on the bacteria's ability to produce metabolites and the bioavailability of the pollutant (Ismail et al. 2022). To degrade recalcitrant hydrocarbons, bacteria need enzymes such as oxygenases or emulsifying agents such as biosurfactants to accelerate the degradation process (Xia et al. 2019). The use of more than one culture for degradation purposes offers broad enzymatic capability, leading to more efficient degradation than pure cultures. Table 1 presents different bacterial species and the hydrocarbons they are known to degrade.

Table 1 Bacteria used in the degradation of hydrocarbon pollutants

Bacteria species	Hydrocarbon species
<i>Acinetobacter</i> sp.	C ₁₀ –C ₃₀ alkanes
<i>Acinetobacter</i> and <i>Caulobacter</i> , <i>Candida maltose</i> , <i>Rhodococcus</i> , <i>Burkholderia</i> , <i>Yarrowia lipolytica</i> , <i>Candida tropicalis</i> , <i>Pseudomonas</i> and <i>Mycobacterium</i>	C ₅ –C ₁₆ fatty acids, alkylbenzenes, cycloalkanes, and alkanes
<i>Methylocystis</i> , <i>Methylocella</i> , <i>Methylococcus</i> , <i>Methylomonas</i> and <i>Methylosinus</i>	C ₁ –C ₈ cycloalkanes, alkanes and alkenes
<i>Pseudomonas</i>	Salicylate
<i>Rhodococcus</i> and <i>Mycobacterium</i>	C ₁₀ –C ₃₀ alkanes
<i>Rhodococcus</i>	C ₅ –C ₁₆ fatty acids, alkylbenzenes, cycloalkanes, and alkanes
<i>Haemophilus</i> and <i>Pseudomonas</i> species	Phenanthrene
<i>Pseudomonas</i>	Plasmid naphthalene
<i>Ralstonia</i> , <i>Rhodococcus</i> and <i>Pseudomonas</i>	Aromatic hydrocarbons, e.g. xylene, benzene, ethylbenzene and toluene

5 The Processes Involved in the Biodegradation of Hydrocarbons

During degradation, oxygen is required in installing a hydroxy group into a hydrocarbon (hydroxylation), the insertion of oxygen atoms into dihydroxylated compounds (ring cleavage) and terminal electron acceptance in the aerobic degradation of hydrocarbons. Anaerobic degradation is mainly based on reductive reactions (Imam et al. 2022). Microorganisms take different degradation pathways to degrade different types of hydrocarbon pollutants (Ismail et al. 2022). But the mineralization of organic pollutants under aerobic conditions mainly has three stages (Das and Chandran 2011): (1) The process starts with the activation and introduction of oxygen in the organic compound using the oxygenase enzyme as the catalyst. The bacteria also incorporate the peroxidase enzyme to decompose hydrogen peroxide produced as a by-product of the reaction involving oxygen. (2) Organic pollutants are then biotransformed into intermediates of central intermediary metabolism. At this point, the tricarboxylic acid cycle (TCA) is a dominant process that facilitates the breakdown of the organic pollutants for the cells to harvest the energy needed for growth. (3) This is followed by biosynthesis of the cell biomass from precursor metabolites such as acetyl-CoA.

The process starts with the degradation of large molecules of organic pollutants into a 2-carbon acetyl coenzyme A (acetyl-CoA). Acetyl-CoA reacts with oxaloacetate to release coenzyme A as it forms citrate. In succession, the citrate is then arranged to form isocitrate, alpha-ketoglutarate is formed after isocitrate losses as a carbon dioxide molecule, another molecule is lost by alpha-ketoglutarate to form succinyl CoA, with the enzymes as catalysts, succinyl CoA is converted to succinate, Fumarate is formed from the oxidation of succinate, malate is then formed from the

hydration of fumarate, and lastly, oxaloacetate is formed from the oxidation of malate. On every cycle turn, oxaloacetate and two carbon dioxide molecules are formed. In every cycle FADH_2 (Flavin adenine dinucleotide) and NADH (Nicotinamide adenine dinucleotide + hydrogen) molecules formed from FAD and NAD^+ transfer their energy to the electron transfer chain to allow cellular respiration. Other processes such as biosurfactant production or the attachment of microbial cells to the substrate may occur at this point.

Hydrocarbons are also degraded under anaerobic conditions. Some pollutants are found in waste or environments with limited oxygen conditions, such as in groundwater and soil. In anaerobic degradation, intermediates such as benzoyl-CoA are used in the degradation process. In the degradation of BTEX compounds such as ethylbenzene, toluene and benzene, benzoyl-CoA is produced as a common intermediate. Benzoyl-CoA contains substituents that can withdraw electrons hence creating an effect that facilitates electron transfer to the ring of the PAH (Singh et al. 2017). Substituents such as carboxy thioester create the electron withdrawing effect in this process. At last, the intermediate benzoyl-CoA gets transformed into acetyl-CoA as it shrinks.

5.1 Factors Affecting the Rate of Degradation

Bioremediation is generally a slow process, but favourable conditions favour the full mineralization of the pollutants. The degradation of pollutants involves the generation of enzymes for catalyzed catabolism, changes in the degraders' genetic construct and the introduction of competent microorganisms for effective degradation. The effective decomposition of pollutants is also affected by nutrients, pH, oxygen, temperature and other factors are described below (Wei et al. 2021).

Temperature: Low temperatures reduce the enzymatic activating rate of microbes leading to low degradation (Bisht et al. 2015). Bacteria are classified based on their ability to grow under specific temperatures as thermophiles if they grow in temperatures higher than 45°C , mesophiles if they grow in temperatures between 44°C and 20°C and psychrophiles if they grow in temperatures less than 20°C (Wang et al. 2022a, b, c). In the soil environment, maximum degradation is achieved in the range of $40\text{--}30^\circ\text{C}$. In a freshwater environment, maximum degradation is achieved in the range of $30\text{--}20^\circ\text{C}$, whereas in marine environments, microbes efficiently degrade if the temperatures are in the range of $20\text{--}15^\circ\text{C}$ (Das and Chandran 2011). Below 10°C , bacteria microbial activity reduces immensely, but any increase in temperature increases adsorption, bioavailability and subsequent biodegradation (Ismail et al. 2022).

Oxygen: The presence of oxygen in the degradation environment determines whether the process will be aerobic or anaerobic. The degradation of hydrocarbons often happens in aerobic environments, but degradation can also occur in oxygen-limited environments like aquifers and marine sediments (Singh et al. 2017). In aerobic degradation of hydrocarbons, oxygen is a central component of the initial

oxidation of the hydrocarbon by monoxygenase and di-oxygenase enzymes. In the absence of oxygen, acceptors of electrons such as sulphate, ferrous and nitrate ions would be required to oxidize the PAH. Oxygen in soil environments varies depending on soil moisture content, the ability of soil microbes to consume soil oxygen and the amount of usable hydrocarbons, which can lead to the depletion of oxygen (Haritash and Kaushik, 2009). Petrochemical pollutants like xylene, toluene, naphthalene, acenaphthene, 1,3 dimethylbenzene and benzene have all been degraded in anaerobic environments, but higher degradation is only reported in aerobic conditions (Al-Hawash et al. 2018).

Nutrients: To obtain effective biodegradation, nutrients such as nitrogen, carbon, iron, oxygen, phosphorus and hydrogen are needed (Abdelhafeez et al. 2022). In environments where degradation happens, organic pollutants act as the main source of carbon, whilst water acts as the main source of hydrogen and oxygen (Kalantary et al. 2014). The absence of sufficient nutrients affects degradation, but excess nutrients may also inhibit biodegradation. For example, the availability of the pollutants used as a carbon source in high concentrations may alter the cell membrane structure of the bacteria, leading to ineffective degradation (Zafra et al. 2015). This is why the decomposition of short-chain alkanes with carbon constituents ranging from C₉ to C₁₁ is impeded because hydrocarbons dissolve the cellular membrane of the bacteria. Furthermore, the degradation of long-chain alkanes with carbon ranging from C₁₉ to C₂₅ may also be inhibited because of the low solubility and stable solid nature of alkanes.

Salinity: High salinity may decrease the degradation of petrochemical hydrocarbons if it reduces the metabolic rate of the microbes besides affecting microbial growth and diversity in mixed culture environments (Qin et al. 2012). At times enzyme activation is necessary for effective degradation may also be affected significantly (Ebadi et al. 2017).

pH: Microbial degradation is affected by pH if it affects enzyme activity, catalytic reaction balance and cell membrane transport (Gidudu and Chirwa 2020b). In very alkaline and acidic conditions, the degradation of organic pollutants is affected by the low growth of microbes. Neutral to alkaline pH is reported to favour the growth of heterotrophic bacteria. Bacteria such as *Pseudomonas aeruginosa* has been reported to effectively degrade hydrocarbons at a pH of 7–8 (Gidudu and Chirwa 2020a). The complete degradation of recalcitrant hydrocarbons like octadecane and naphthalene has been reported at a pH of 6.5–8 (Al-Hawash et al. 2018).

Concentration and nature of the pollutant: The initial concentration of the organic pollutant affects the degradation process. High concentrations of the pollutant affect the bioremediation process mainly because the bacteria can be affected by the interactions of the cell with the toxins of the organic pollutants (Rabani et al. 2020). Besides pollutant concentration, the nature of the pollutant such as the number of benzene rings in PAHs, affects the degradation process. HMW PAHs with a high number of benzene rings are more difficult to degrade than LMW PAHs (Ismail et al. 2022).

Bioavailability of pollutants: Hydrocarbon pollutants such as PAHs are hydrophobic organic pollutants with little solubility in water and high resistance to

photocatalytic and biological breakdown (Lawal 2017). Solubility, interfacial tension, capillary pressure and viscosity are some of the factors that affect substrate bioavailability. High interfacial tension, capillary pressure and viscosity decrease the bioavailability of contaminants (Cipullo et al. 2018). Furthermore, when hydrocarbons end up in the environment, they strongly bind to solid particles making it very difficult for the bacteria to degrade (Gidudu and Chirwa 2020b). Bioavailability depends on the movement of the pollutants in the aqueous bulk phase and the general mass transfer of the hydrocarbon. Bioavailability can be increased by increasing temperature. Temperature can reduce capillary pressure, interfacial tension, viscosity and hydrophobicity of the organic pollutants (Wang et al. 2022a, b, c).

But surfactants can also resolve this problem by increasing the availability of the hydrophobic compounds 5–20 folds hence enhancing the rate of degradation. Surfactants such as Triton X100, Brij 35 and Tween 80 have been used in enhancing bioremediation. Different types of surfactants affect the degradation process differently. Biosurfactants produced by microbes have come up as potential replacements for synthetic surfactants.

6 Role of Surfactants in the Degradation of Organic Pollutants

A surfactant is a surface-active molecule containing hydrophilic and hydrophobic groups that allow it to adsorb at interfaces of a solution (oil or water phase) and a different phase (solids or gases) as micelles (Nakama 2017). The hydrophobic group comprises an alkyl chain with 8–22 carbons and dislikes water. The hydrophilic group has functional groups that allow it to have an affinity to water (Kubicki et al. 2019). These properties enable the emulsification and demulsification of water–oil–solid mixtures when the hydrophobic group of the molecule gathers at the interfaces of the solution (oil) and a different phase (solids or gases), whilst the hydrophilic group increases the solubility of the hydrocarbons (oil) in the water phase. These properties are why surfactants or biosurfactants are also used in oil recovery from oil wells.

6.1 Classification and Properties of Surfactants

Surfactants can be classified depending on whether they are made synthetically or by microorganisms (Mondal et al. 2019). Surfactants are categorized into anionic or ionic depending on whether they carry a positive or negative charge. Ionic surfactants can be subclassified as cationic, anionic and amphoteric/zwitterionic surfactants if their hydrophilic group disassociates into cations, anions or both cations and anions, respectively (Boulakradeche et al. 2015).

The hydrophilic groups of ionic surfactants are made up of quaternary ammonium ($-R_4N^+$), sulphobetaine ($-N(CH_3)_2C_3H_6SO_3^-$), carboxybetaine ($-NR_2CH_2COO^-$), sulphonate (SO_3^-), sulphate ($-OSO_3^-$) and carboxylate ($-COO^-$). Polyoxyethylene, glyceryl, or sorbitol groups usually make up the hydrophilic groups of anionic surfactants (Nakama 2017). The hydrophobic group comprises CH_2 groups connected in continuous alkyl chains consisting of 4–18 CH_2 groups and an end group of CH_3 (Free 2016). Much as synthetic surfactants are categorized according to their polar groups, biosurfactants are classified according to their producing organisms and chemical structure (Sajadi Bami et al. 2022). The hydrophilic groups of biosurfactants are made up of carbohydrates, cyclic peptides, amino acids, carboxylic acids, alcohols or phosphates. In contrast, the hydrophobic group is made up of long-chain fatty acids, α -alkyl- β -hydroxyl fatty acids or hydroxyl fatty acids (Mulligan 2009).

Surfactants are also classified according to their solubility based on whether they are soluble in water (hydrophilic) or lipids (lipophilic). Most ionic surfactants are hydrophilic, but non-ionic surfactants can either be lipophilic or be hydrophilic depending on the capacity of their lipophilic and hydrophilic groups. The lipophilic group attracts oil, whilst the hydrophilic group attracts water (hydrophilic–lipophilic balance (HLB)) (Nakama 2017). Making use of the HLB scale, a range of 3.5–6 shows that the surfactant can be used in the creation of W/O (water/oil) emulsions, whilst an HLB ranging from 8 to 20 indicates that the surfactant can be used in the formation of O/W (oil/water) emulsions (Zheng et al. 2015).

Since surfactants can reduce interfacial and surface tension of liquids, solids and gases, they are used to enhance the decomposition of organic pollutants (Gidudu and Chirwa 2020b). The commonly used surfactants are Tween 80, Triton x-100, Afonic 1412–7, Corexit 9527 and sodium dodecyl sulphate (SDS). The application of surfactants for enhancing oil recovery and environmental remediation is fast, efficient and can aid in the treatment of large volumes of contaminated media. But surfactants are costly and are toxic to the environment (Gudina et al. 2015). Hence, biosurfactants have been proposed as a potential replacement for synthetically produced surfactants because of their lower toxicity, high biodegradability, diversity, demulsification potential and selectivity (Mulligan 2021). Biosurfactants can also be used effectively in varying salinity, pH and temperature (Bezza and Chirwa 2015).

6.2 Toxicity of Surfactants and Biosurfactants

Studies have shown that biosurfactants are less toxic than synthetic surfactants (Abalos et al. 2004). A couple of previous studies have reported that biosurfactants exhibit low toxicity, but some exhibit strong antimicrobial activity (Sarubbo et al. 2013). But in general, not much has been reported about the toxicity of biosurfactants to plants and microorganisms in the environment (Santos et al. 2017). It is claimed that surfactants affect microbes in two different ways by either

disrupting the cell membranes due to the interactions between the surfactant and the lipid components of the cell or the reaction of the surfactant with the cell protein required for cell functioning (Lima et al. 2011). Cationic surfactants are most toxic at neutral pH, whilst anionic surfactants are highly toxic below 7 (Lima et al. 2011).

6.3 Types of Biosurfactants and Biosurfactant Producing Microorganisms

Biosurfactants are amphiphilic compounds containing hydrophilic and lipophilic groups. Biosurfactants have surface-active properties that allow them to emulsify and reduce surface tension and interfacial tension. Compared to synthetic surfactants, some biosurfactants have lower critical micelle concentrations than synthetic surfactants (Sajadi Bami et al. 2022).

Biosurfactants are classified into five major groups: glycolipids, fatty acids/neutral lipids, lipopolysaccharides, phospholipids and lipopeptides (Mondal et al. 2019). The characteristics and nature of biosurfactants vary depending on the producer organism. Various microorganisms, including bacteria, fungi and yeasts, produce biosurfactants (Gudina et al. 2015). *Rhodococcus*, *Bacillus*, *Halomonas*, *Arthrobacter*, *Pseudomonas*, *Acinetobacter* and *Enterobacter* are the most widely studied biosurfactant-producing bacteria (Mondal et al. 2019). Table 2 shows the different types of biosurfactants produced by different species of bacteria. The classification of the biosurfactant depends on the fatty acid length, which allows specifications of the biosurfactants as per their respective congeners. In addition, the fatty acid length or the specification of the congener varies depending on the strain responsible for producing that biosurfactant and the carbon source used in the production process (Wang et al. 2007).

Bacteria are great producers of biosurfactants with high molecular weight (HMW) and low molecular weight (LMW) properties. HMW biosurfactants such as liposan, alasan, biodispersan and emulsan produced by bacteria are excellent emulsifiers, whilst LMW biosurfactants can reduce surface tension and interfacial tension between different phases of different polarity in liquids, solids and gases (Sajadi Bami et al. 2022). Examples of LMW biosurfactants are glycolipids, sophorolipids and trehalose lipids. These are made up of long-chain fatty acids or disaccharides that are acylated with hydroxy fatty acids. They may also comprise carbohydrates attached to long-chain lipopeptides or aliphatic acids (Ron and Rosenberg 2002). Examples of HMW biosurfactants are lipopolysaccharides, polysaccharides, lipoproteins, proteins or complex mixtures of these biopolymers.

The main difference between HMW biosurfactants and LMW biosurfactants is that HMW biosurfactants prevent the coalescence of oil droplets in W/O or O/W emulsions since they can bind to the oil droplet surfaces whilst LMW biosurfactants lower the surface tension and interfacial tension between oil-water droplets. This creates the difference between bioemulsifiers and biosurfactants where

Table 2 Microorganisms are known for producing biosurfactants and their respective critical micelle concentrations and tension reduction potential as adopted from Souza et al. (2014), Uzoigwe et al. (2015) and Das and Chandran (2011)

Biosurfactant classification	Biosurfactants	Microorganisms	Critical Micelle Concentration	Interfacial Tension (mN/m)	Surface tension (mN/m)
Lipopeptides and lipoproteins	Surfactin,	<i>Bacillus subtilis</i>	23–160	1	27–32
	Subtilisin	<i>Bacillus subtilis</i>	–	–	–
	Polymyxins	<i>Bacillus polymyxa</i>	–	–	–
	Peptide-lipid	<i>Bacillus licheniformis</i>	12–20	0.1–0.3	27
	Viscosin		150	–	26.5
Glycolipids	Trehalolipids	<i>Mycobacterium sp.</i>	0.3	15	38
	Trehalolipids	<i>N. erythropolis</i>	20	3.5	30
	Rhamnolipids	<i>Pseudomonas aeruginosa</i>		0.25	29
	Rhamnolipids	<i>Pseudomonas Sp.</i>	0.1–10	1	25–30
	Sophorolipids	<i>Candida bombicola</i>	–	–	–
	Rhamnolipids	<i>Pseudomonas fluorescens</i>	150	–	26.5
	Sophorolipids	<i>T. bombicola</i>	–	1.8	33
	Sophorolipids	<i>T. apicola</i>	–	0.9	30
Fatty acids, neutral lipids and phospholipids	Fatty acids	<i>C. lepus</i>	150	2	30
	Neutral lipids	<i>N. erythropolis</i>	–	3	32
Polymeric surfactants	Carbohydrate-protein-lipid		10	–	27
	Emulsan, biodispersan	<i>A. calcoaceticus</i>	–	–	–
	Liposan	<i>C. lipolytica</i>	–	–	–
	Protein PA		–	–	–
Particulate biosurfactants	Vesicles and fimbriae	<i>A. calcoaceticus</i>	–	–	–

bioemulsifiers allow the emulsification of immiscible phases but not necessarily through surface and interfacial tension reduction. In contrast, biosurfactants are mainly characterized by the ability to reduce interfacial and surface tension between droplets of different phases (Uzoigwe et al. 2015).

In some studies, synthetic surfactants have been used to increase bioavailability but biosurfactants are more effective, especially for hydrophobic substances such as petrochemical hydrocarbons. But much as the application of biosurfactants enhances the biodegradation process, if biosurfactants are added in concentrations that facilitate the generation of micellar substrates, the bioremediation process may be

compromised (Makkar and Rockne 2003). When synthetic surfactants are used to increase bioavailability, they have to be applied independently from the bacteria to avoid inhibition of the growth of the microbes. When it comes to biosurfactants, a single bacterium can be used to simultaneously produce biosurfactants as it degrades the pollutants (Chauhan et al. 2008). The addition of exogenously produced biosurfactants enhances the degradation of hydrocarbons, including ring hydrocarbons. Biosurfactants are highly stable and can withstand high temperatures and salt concentrations. Biosurfactants are either produced as intracellular molecules bound on the cells or extracellular substances. Cell-bound biosurfactants aid in the passage of substrates through the membrane to aid in the biodegradation process. On the other hand, extracellular biosurfactants emulsify the substrates to increase their bioavailability.

Biosurfactants increase the bioavailability of pollutants by increasing the solubility of hydrophobic compounds. The increase in solubility is an effect of reducing surface and interfacial tension. The reduction in surface and interfacial tension increases the surface area of hydrocarbons which makes them available to the microbes (Kreling et al. 2020). Biosurfactants improve the rate of degradation of the pollutants by enhancing their solubility and controlling the interaction between bacterial cells and hydrophobic contaminants. The enhancement of biodegradation by the application of biosurfactants can be explained in three different steps:

- (a) In the presence of non-aqueous-phase liquid organics, interfacial tension between aqueous and non-aqueous phases is reduced due to the dispersion of non-aqueous liquid organics. The reduction in interfacial tension increases the area of contact enhancing the mobilization of sorbed liquid-phase contaminants and the mass transport of the pollutants to the aqueous phase.
- (b) The surface tension of the solid particle-pore water is reduced due to the increase in the solubility of the pollutants. Solubility increases due to the increase in biosurfactant concentration leading to the formation of micelles around hydrophobic organic pollutants.
- (c) Expulsion of the pollutants from the solid matrix due to the interactions between the pollutant and the biosurfactant and the interaction of the solid particles with the biosurfactant. The expulsions are due to the interactions of the single biosurfactant molecule with the contaminant, the sorbed contaminant with the biosurfactant, and the swelling of the organic matrix due to the reduction of surface and interfacial tension releasing the entrapped pollutant.

Biosurfactants can emulsify or demulsify mixtures of substances. Therefore, the application of biosurfactants as an enhancement for bioremediation depends on the biosurfactant's capacity to enhance the dissolution and desorption of compounds in the matrix to obtain improved biodegradation (Gidudu and Chirwa 2020b). Biosurfactants adsorb at the interface of the hydrocarbons and water to facilitate the solubilization and micellization of the hydrocarbon. The increase in solubilization increases the mobility of the pollutant making them susceptible to biodegradation. There is a direct relationship between emulsification/demulsification activity of the biosurfactant, biosurfactant production capacity of the organism, cell surface

hydrophobicity and hydrocarbon biodegradation (Hassanshahian 2014). Organisms with highly hydrophobic cells and high biosurfactant-producing potential are likely to be great degraders (Hassanshahian 2014).

For instance, applying a lipopeptide biosurfactant produced by *Bacillus subtilis*, as reported by Bezza and Chirwa (2015), led to the degradation of motor oil up to 82% in 18 days of incubation. In another study, a mixture of 11 rhamnolipid congeners was reported to enhance the degradation of organic content in crude oil by up to 91% in 35 days (Cameotra and Singh 2008). Gidudu and Chirwa (2020a) varied the application of different biosurfactant concentrations of 28 g/L, 56 g/L and 84 g/L to remediation of oil-contaminated soil in an electrokinetic cell. It was reported that the highest oil recovery of 83% was obtained by 56 g/L, whilst the highest degradation of the hydrocarbons was obtained when 84 g/L were added to the cell.

Abalos et al. (2004) reported an improvement in the decomposition of petroleum hydrocarbons in crude oil from 32% to 61% in 10 days after adding a rhamnolipid biosurfactant. The bacterial degradation of C₁₃-C₂₁, C₂₂-C₃₁ and C₃₂-C₄₀ in sludge containing oil and grease was reported as 83–98%, 80–85% and 57–73%, respectively, in 56 days after the addition of a rhamnolipid biosurfactant (Rahman et al. 2004). Moldes et al. (2011) also reported that in the soil containing 70,000 mg of hydrocarbons/kg of soil, 58.6–62.8% of octane was removed in 15 days, and 78% was removed in 30 days after the addition of a biosurfactant produced by *Lactobacillus pentosus*. In the absence of biosurfactants, only 1.2–24% of octane was removed.

The fungal strain *Scedosporium* sp. ZYY was combined with an *Acinetobacter* sp. Y2 biosurfactant producing strain for the degradation of pollutants in crude oil (Atakpa et al. 2022). The production of a biosurfactant by *Acinetobacter* sp. Y2 increased the degradation of total petroleum hydrocarbons from 23.36% to 58.61%.

A rhizobacterium that can produce a biosurfactant was isolated from Malaysian native bulrush *Scirpus grossus* to degrade crude oil sludge (Sharuddin et al. 2021). The highest degradation of 39.7% was achieved with the aid of the biosurfactant produced by *Lysinibacillus* sp. strain.

A *Pseudomonas putida* strain isolated from marine sediments with the ability to produce biosurfactants and degrade hydrocarbons produced a rhamnolipid biosurfactant that enhanced the degradation of fluoranthene, benzo(k)fluoranthene, acenaphthylene, phenanthrene, benzo(b)fluoranthene, chrysene, benzo(a)-anthracene, anthracene and pyrene in residues from oil extraction (Martínez-Toledo et al. 2022).

7 Conclusion

Biosurfactants play a central role in degrading hydrophobic and persistent organic pollutants found in waste. By reducing surface and interfacial tension between the substrate and solids or water, the substrate is solubilized. The increase in

solubilization increases the surface area and the mobility of the pollutant, thereby increasing the bioavailability of the pollutant to the microbes. The increase in bioavailability due to the application of biosurfactants increases the degradation of organic pollutants in different waste streams. In the future, biosurfactants should be adopted for the remediation of sites polluted with organic wastes where indigenous organisms can be used to produce biosurfactants in situ. In cases where there is a lack of great indigenous degraders and biosurfactant-producing organisms, bioaugmentation can be adopted together with biostimulation to enhance degradation.

Authorship and contributions

Brian Gidudu: Writing original draft, Reviewing and editing. Evans M.N. Chirwa: Conceptualization, Reviewing and editing, Funding acquisition.

Acknowledgments We acknowledge the National Research Foundation (NRF) of South Africa (<https://www.nrf.ac.za/>), University of Pretoria (<https://www.up.ac.za/>), Rand Water (<https://www.randwater.co.za/Pages/Home.aspx>) and the Water Research Commission (<http://www.wrc.org.za/>) as our funders and partners.

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Role of Biosurfactants in Agriculture Management



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1 Introduction

The growing trends of human population, industrialization, and urbanization have been associated with the emergence of various challenges in the form of water scarcity, shrinkage of agricultural land, soil degradation, environmental pollution, and reduction of crop productivity due to severe plant infections. The impact of climate adversity could likely create severe food shortages and food insecurity in the upcoming years owing to a direct relation between crop yield and biophysical stresses (Elgar et al. 2021). Besides rapid development of innovative cropping practices, most of the agriculture management strategies rely on the application of synthetic agrochemicals and pesticides. The extensive applications of these chemicals have negative effects on the ecosystem and pose serious health repercussions for all life forms (Wang et al. 2022; Parra-Arroyo et al. 2022). Considering these implications, there is a great push to create agriculture and environmental sustainability by replacing synthetics with natural products. Biosurfactants are one of the emerging biochemicals having excellent interfacial and antimicrobial properties. These features make them one of the most relevant molecules for soil and agriculture applications (Gayathiri et al. 2022; Dutta and Bhatnagar 2022). Technically, biosurfactants are organic surface-active molecules produced by a variety of bacteria, actinomycetes, yeast, and filamentous fungi during their growth on hydrocarbon substrates (Ślizewska et al. 2022; Chakraborty et al. 2015). These molecules contain hydrophilic and hydrophobic ends enabling them to concentrate at the interfaces for reducing surface and interfacial tensions between the two immiscible systems (Sharma et al. 2016). They have an excellent tendency to enhance solubility

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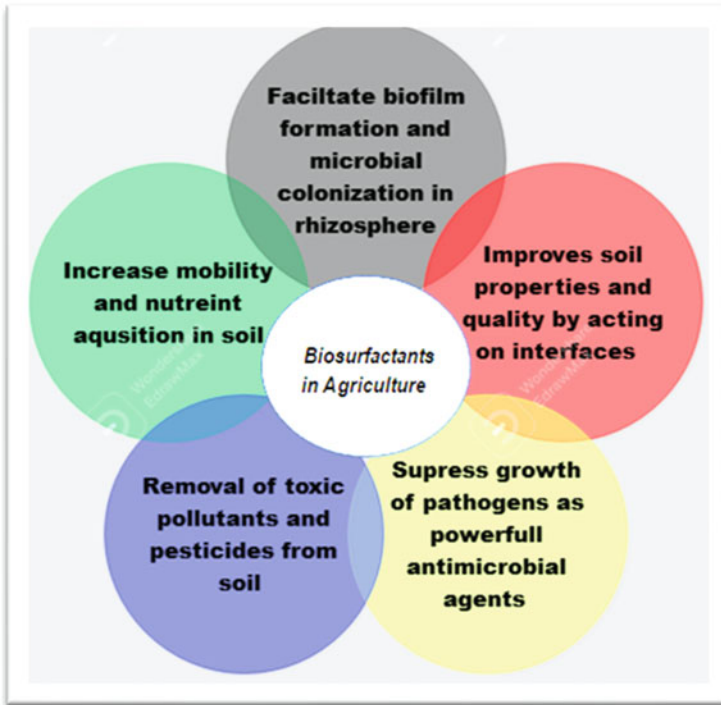


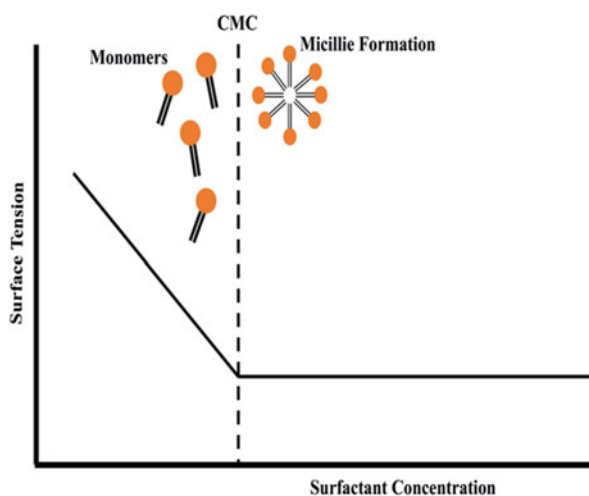
Fig. 1 Role of biosurfactants in agriculture

of hydrophobic chemicals and micronutrients in the aqueous medium in order to improve their mobility and bioavailability (Revathi et al. 2022; Malkapuram et al. 2021). They offer several advantages over synthetic surfactants which include higher activity at lower critical micelle concentration (cmc), biodegradability, great chemical and structural diversity, resistance toward hostile environmental conditions, ease of production using agriculture and industrial waste and potential for use in wide-ranging industrial applications (Vu and Mulligan 2022; Parthipan et al. 2022). Currently, biosurfactants find applications in agriculture, food, cosmetics, petroleum and environmental industries as natural detergents, foaming, wetting, dispersing, and emulsifying agents (Mouafo et al. 2022; Sarubbo et al. 2022). The sustainable nature and versatile properties encourage their use in agriculture sector as antimicrobial agents to control plant infections. In agriculture soils, biosurfactants improve mobility and solubility of the micronutrients via metal–surfactant complex formation making them readily available for plant uptake (Kumari 2022). On the basis of unique properties and chemical diversity, biosurfactants are now becoming an important part of agricultural formulations and capturing space in agri-business as solubility enhancers, emulsifiers and antimicrobial agents. Beneficial roles of biosurfactants in agriculture are highlighted in Fig. 1.

2 Unique Properties of Biosurfactants

Biosurfactants exhibit astonishing chemical diversity and a myriad of superior physical, chemical, and biological properties as compared to synthetic surfactants. Since most of the biosurfactants' properties come into play with the formation of discrete micelles, it is considered imperative to understand surfactants self-assembly and micellization phenomenon. Biosurfactants, like chemical surfactants, are comprised of hydrophilic and hydrophobic moieties. Dual polarity of biosurfactant molecules enables them to display unique phase behavior and properties in the aqueous system (López-Prieto et al. 2022). In aqueous solution, surfactant monomer exists alone at concentrations lower than *cmc* and accumulates at air–water interfaces, transforming from free to aggregation state. At higher concentrations, surfactant monomers tend to self-assemble by non-covalent interactions governing the synthesis of various potent thermodynamically stable supramolecular aggregates. These surfactant aggregates are termed as micelles and the corresponding minimum surfactant concentration is termed as critical micelle concentration (*cmc*) (Aboelkhair et al. 2022). The geometry of the surfactant micelles is the function of surfactant concentration, counterions, temperature, and critical packing parameters (Glikman et al. 2022; Chen and Lee 2022). In solution phases, size, shape, and surfactant micelles structure have been considered the most important attributes to understand their physiochemical properties and possible area of applications. In a surfactant-water system, micelles are designed by packing hydrophobic tails within the micelle core and orientation of hydrophilic heads toward the aqueous environment (Figs. 2 and 3). Self-assembly of the surfactant molecules at or above critical micelle concentration is the principle move operating behind the emergence of amplified surfactants properties. At critical micelle concentration or slightly above the *cmc*, surfactants molecules self-assembles into spherical, ellipsoid, or cylindrical

Fig. 2 Micelle formation at *cmc* of biosurfactants



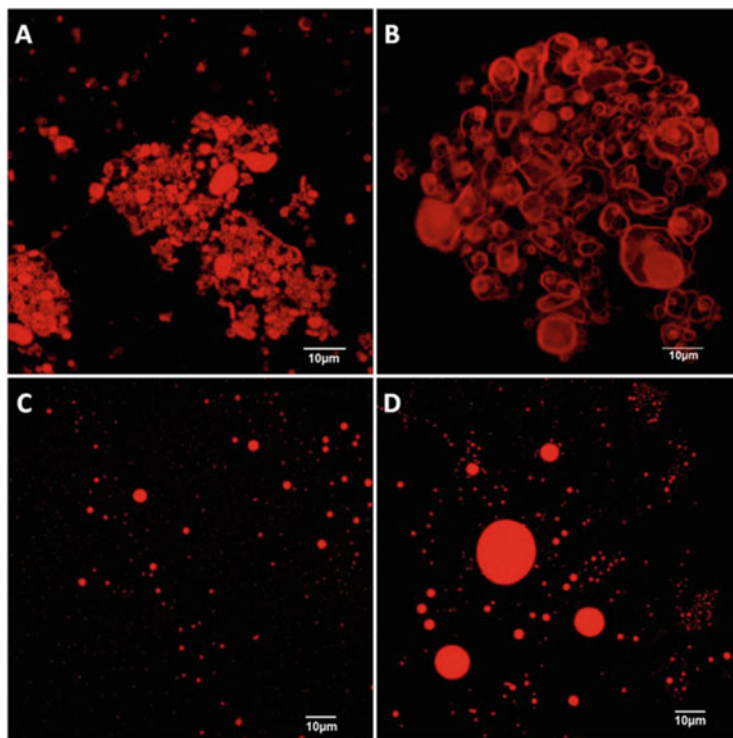


Fig. 3 Self-assembled structures of biosurfactants, (a, b) Crude rhamnolipid mixture, (c) Micelles of RL-1, (d) Micelles of RL-2 in NaCl solution (Rodrigues et al. 2017)

shapes (Mallik and Banerjee 2022). On higher concentrations, the synthesis of more entangled suprastructures of the micelles are formed with unique features. This phase behavior of the surfactants is responsible for a noticeable change in surface and interfacial tension, adsorption, detergency, density, and spatial charge distribution at air–water interfaces (Onuzulike et al. 2022; Kaga et al. 2022; Barzic 2022; Somoza-Coutiño et al. 2020). Therefore, concentration-dependent surfactants self-assembly and micellization processes have received considerable attraction in the surface science and technology. The surfactants micelle formation is presented in the figs. 2 and 3.

Rhamnolipid (RL) is a best-known sugar–lipid conjugate biosurfactant produced by different strains of the bacterium *Pseudomonas aeruginosa*. Being a natural amphiphile, RL displays exceptional physiochemical properties and phase behavior. The phase behavior of the RL molecules has been investigated in finer detail suggesting the synthesis of various forms of micelles such as spherical, disk, or rod like in the solution at critical micelle concentration (Fig. 3a–d). Chen et al. 2010 observed a concentration-dependent self-aggregation of the RL-1 and RL-2 congeners in which RL-1 molecules showed stronger partitioning efficiency than the RL-2 molecules owing to the greater packing constraints encountered by dirhamnose

headgroup. Moreover, in a dilute solution, both RL species tend to form small globular micelle aggregates (Zhang et al. 2020). On increasing surfactant concentration, RL-1 developed planar structures whereas; RL-2 remains predominantly globular. Rehman et al. 2021, reported biosynthesis of a mixture of two rhamnolipid variants (RL-1 and RL-2) by *P. aeruginosa* having the capability of reducing surface tension of the water to 29 mN/m at a cmc of 40 mg/L. They observed RL micelles of an average of 350 nm in diameter which increased to 700 nm in size at a concentration higher than the cmc. The influence of surfactants concentrations on phase transition has also been observed in sophorolipids biosurfactants produced by *Candida bombicola* and *Meyerozyma* sp. (Akanji et al. 2021; Fontoura et al. 2020). In case of sophorolipids (SL), lactonic forms make small unilamellar vesicles at low surfactant concentrations which grow to larger unilamellar vesicle structures at higher concentrations. However, in contrast, the acidic SL molecules form smaller globular micelles at lower concentrations with the possibility of coexistence of lamellar and vesicular forms at comparatively higher surfactants concentration (Baccile et al. 2022; Kleinen et al. 2022). Interestingly, lactonic SL congeners display more complex phase behavior than the acidic sophorolipids probably due to a relatively larger headgroup (Penfold et al. 2011). These properties have also been observed in other classes of biosurfactants. Surfactin is a member of non-ribosomal lipopeptide biosurfactants containing seven amino acids chain (heptapeptide) attached to β -hydroxy fatty acid chain with 13–16 carbons. Members of the genus *Bacillus* including *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus tequilensis*, *Bacillus mojavensis*, and *Bacillus amyloliquefaciens* are well known to produce surfactin and related surface-active lipopeptides (Galitskaya et al. 2022; Liu et al. 2022a, 2022b; Kraigher et al. 2022; Lilge et al. 2022; Cortés-Camargo et al. 2021). Surfactin is considered as one of the most prominent and powerful surface-active lipopeptide with excellent antimicrobial activity. With cmc value as low as 20 mg/L, surfactin can reduce the surface tension up to 24 mN/m and interfacial tension of oil–water emulsion to 1.5 mN/m. These features are associated with surfactin's ability to make peculiar microstructures at different interfaces (Sagisaka et al. 2021). The heptapeptide moiety of the surfactin molecule adopts horse-saddle topology with β -sheet conformation because of the inward movement of the fatty acid tail into the peptide core that drives longitudinal packing of surfactin molecules in the form of monolayer aggregates (Hutchinson 2019). Further, growth of the micelle aggregates such as spherical, rod like, bilayers, and vesicles are attributed to the possible interaction among side chains of the molecules at a relatively slow pace (Cui et al. 2009). Similar observations have also been reported for other lipopeptides such as lichenysin, iturin, fungicin, and mycosubtilin. Regardless of the ionic and nonionic nature of the biosurfactants, the self-assembly and micelle formation is derived from surrounding chemical environment such as pH, temperature, centurions, and surfactant concentration (Larsson et al. 2022; Vu et al. 2021). Extensive research is carried out to investigate the structure–function relationship, supramolecular architecture, design principles, and biophysical properties of biosurfactants. The rapid discovery of finer details of aforementioned aspects has changed the traditional outlook of bioamphiphiles. Biosurfactants have the ability to bind various

metal ions and hydrophobic materials and can be used for the bioremediation of metals and hydrophobic contaminants (Sonowal et al. 2022). They show better hydrophobic–lipophilic balance (HLB) as compared to the synthetic surfactants thus exhibiting excellent solubilization and emulsification activities (Saranraj et al. 2022). Rhamnolipid serves as a potent emulsifier and solubilizing agent for various hydrophobic substrates because of its suitable HLB value as compared to the SDS, Tween 80, and some other industrial surfactants. Emulsions formed by biosurfactants are stable and have the capability of reducing electrostatic interactions at interfaces (Cavalero and Cooper 2003). Biosurfactants show higher stability under acidic and alkaline pH, higher salt concentrations, and retain their activity even at elevated temperatures. The glycolipid and lipopeptide biosurfactants extracted from *Burkholderia* sp., *P. aeruginosa*, *Candida bombicola*, and *Bacillus subtilis* maintained significant emulsification activity between 4–100°C, 2–10 pH, and salt concentrations of 2–7% (Ali et al. 2021). This aspect of biosurfactants has been of particular interest when considering their functional stability under harsh operational conditions. For example, surfactant flooding has been a promising approach to enhance oil recovery where they alter interfacial tension and wettability of the reservoir in order to mobilize trapped crude oil (Hirasaki and Zhang 2004). Surfactant flooding is an expensive method owing to the high chemical cost, in addition to poor performance of the chemical surfactants in harsh reservoir conditions. Many researchers have recommended the use of biosurfactants like surfactin, sophorolipids, rhamnolipids, and trehalolipids as a potential replacement to the chemical regime for the crude oil recovery, biodesulfurization processes, control of agriculture pathogens, and rehabilitation of pesticides contaminated agriculture soils (Onwe et al. 2022; Yang et al. 2020; Fenibo et al. 2019). Rhamnolipids produced by *P. aeruginosa* demonstrated recovery of about 50 % of the trapped oil after two months of the recovery process due to its high emulsification activity (Câmara et al. 2019). Similarly, sophorolipids from *Candida bombicola* ATCC 22214 produced stable emulsion with different hydrophobic substrates at 15% NaCl, 2–12 pH, and up to 100°C (Elshafie et al. 2015). The SPLs flooding were able to mobilize the resident crude oil from the well with a recovery rate of 27 %. *Bacillus licheniformis* L20, capable of producing lipopeptide biosurfactants, was able to withstand the hostile reservoir conditions and showed a 20% increase in crude oil recovery (Liu et al. 2022a, 2022b). On a similar mechanistic ground, biosurfactants enhance the solubility of the contaminants including hydrophobic materials by making stable microemulsions in the soil for improving mobility of contaminants including polychlorinated biphenyls. According to emerging scientific data, biosynthesis of surfactants by soil-dwelling microbes play an essential role in establishing soil–microbe interactions, enhancing nutrients exchange and bioavailability of complex hydrocarbons, bioremediation of organic pollutants, and competitive inhibition of plant pathogens for better plant growth and productivity (Singh et al. 2018; Nielsen et al. 2006). Despite the availability of high-resolution molecular approaches for the description of soil microbial communities and characterization of metabolomes with distinctive biochemical activities, our current understanding with reference to the role of biosurfactants in soil ecosystems is imperfect. Therefore, it is

important to obtain the finer details of the individual metabolic performance of soil microbes to understand their future role in environmental and agriculture sustainability.

3 Role of Biosurfactants in Biofilm Formation and Root Colonization

Soil is a complex matrix of living and nonliving regimes where multiple interfaces interact with each other. Most of the biochemical trade between the plants and soil is influenced and regulated by the soil rhizosphere microbiomes. Biosurfactant-producing microorganisms are regarded as an important component of the soil ecosystem because synthesis of biosurfactants assists microbial colonization to the plant root surfaces and establishment of beneficial rhizomicrobiome (Sang and Kim 2012). The production of biosurfactants promotes attachment of bacteria with the plant roots and establishes species richness by means of biofilm development (Ward 2010). The aggregation and colonization of the bacterial communities around the root surfaces is a complex process involving various physiochemical factors including pH, presence of beneficial root exudates, availability of micronutrients, and progressive interactions of plant and microbial genomes (Yuan et al. 2015; Van Aarle et al. 2002). During past years, most of the agriculture research has been focused to investigate the root–microbe interactions resulted in advancing out current understanding of symbiotic networking in the rhizosphere for sharing of beneficial traits. The radial distribution of microbial communities around the root surfaces is dictated primarily by the availability of chemical attractants present in the rhizosphere (Compant et al. 2005). Furthermore, mobility and attachment of the soil bacteria are greatly facilitated by the synthesis of biosurfactants (Ibrar et al. 2022). Some previous studies have provided great insights for an accurate and deeper understanding of the underlying factors responsible for the species abundance, growth, and proliferation of microorganisms at root–soil interfaces. Pioneer work in this direction was carried out by Newman and Watson (1977) who proposed a theoretical framework of microbial population dynamics in the rhizosphere. Dupuy and Silk 2016, developed a mathematical model to investigate interactions between bacteria and the root surface for understanding bacterial growth and adhesion on the root surfaces. The adhesion of bacteria on the rhizoplane is considered as an intricate multistage process where initially soil bacteria move toward the root surfaces from the bulk soil through passive motion or active transport (Huang et al. 2022). The strength of bacterial association with the root surface is greatly influenced by surface charges, cell size, and ionic strength of the soil solution (Rossez et al. 2014). On approaching the target site, fimbriae, flagella, and surface proteins mediate initial bacteria binding at the root surface. During this stage, the bacterial cells adhesion is reversible, however, with the passage of time bacteria start secreting biopolymers such as polysaccharides and biosurfactants causing irreversible adherence of the

bacteria to the rhizoplane). The extracellular biopolymers act as “molecular glue” for maintaining cell-to-cell contact and bacterial aggregation forming discrete microcolonies at the root surface (Das 2022). The progressive molecular communication between the root colonizers then comes into play and transforms these microcolonies into mature biofilm. As previously stated biosurfactant production is critical for the establishment of microbial communication and biofilm formation. Another attractive model to understand the mechanism of bacterial mobility, surface attachment, and biofilm formation is based on the bacterial synthesis of diffusible autoinducer signals via Quorum sensing networks (QSN). According to the emerging evidences, a strong link between quorum sensing, biofilm formation, and production of biosurfactants has been established (Awdhesh Kumar Mishra and Kodiveri Muthukaliannan 2022). The *Las*, *rhl*, and *lux*-based QS systems have been investigated in different Gram-negative bacteria including *Agrobacterium tumefaciens*, *Pseudomonas aeruginosa*, and *Vibrio fischeri* (Bouyahya et al. 2022; Wang et al. 2008; Duan and Surette 2007). The biosynthesis of rhamnolipids is also dictated by the *Las* and *rhl* QS molecules in certain *Pseudomonas* strains. It is documented that production of RLs regulates various physiological events in *P. aeruginosa* such as motility, cell–cell communication, differentiation, substrate accession, and biofilm development (Blunt et al. 2022; Meliani and Bensoltane 2015). The colonization of *P. aeruginosa* on the root surfaces is navigated by twitching motility and swarming mobility of the bacterium which involves cell elongation, hyperflagellation, and cellular differentiation (Ortiz-Castro et al. 2014; Steindler et al. 2009). Rhamnolipids functions in the swarming mobility of the bacterium because it acts as both surface wetting and chemotaxis stimuli. RLs molecules are imperative for developing water channels in mature biofilm (Davey et al. 2003). This assumption is supported by the fact that *rhl* mutant *P. aeruginosa*, lacking the ability to produce rhamnolipid, was unable to construct defined microcolonies and water channels in the biofilm. In contrast, overproduction of RLs steers disruption of biofilm, restricts bacterial aggregation, and refrains co-aggregation of genetically different bacteria in the preexisting biofilm matrix (Davey et al. 2003). The cell dispersal and inhibition of pathogens aggregation in the biofilms are also associated with the antimicrobial and dispersal activity of the biosurfactants (Pamp and Tolker-Nielsen 2007). Many reports suggested the active role of lipopeptide biosurfactants produced by *Bacillus subtilis* in biofilm formation (Arnaouteli et al. 2021; Morikawa 2006). The surfactin acts as a signaling molecule to regulate biofilm formation in *Bacillus subtilis* using quorum sensing pathway (Aleti et al. 2016). In *Rhizobium* spp. the swarming mobility and root surface colonization have been directly related to biosurfactants (Primo et al. 2015). Production of low molecular weight lipopeptide biosurfactants has been reported for *Azospirillum* isolates. On the contrary note, surfactin-deficient *Bacillus* strains lack the ability to colonize on the phylloplane (Luo et al. 2015). Similarly, surfactin-deficient *Bacillus subtilis* generates defective biofilm and poor root colonization activity (Bais et al. 2004).

4 Potential of Biosurfactants as Antifungal Agents

Plant infections and productivity losses are among the most important global issues considering the current population growth rate index. During the recent years, a continued decline in agriculture output has been recorded due to changing climate, deterioration of the soil habitats, plant infections and chemical acquired resistance in the pathogens (Newell et al. 2010; Dordas 2008). Generally, about 20% of yield losses are attributed to the invading phytopathogens, therefore, new insights should be taken into account for developing future plant protection strategies with least negative effects (Zou et al. 2018; Wu et al. 2019). The use of soil microorganisms as catalysts for effective pest management and promoting plant growth have been among the contemporary approaches being successfully implemented across the globe (Sinha et al. 2010). The biocontrol activity expressed by most of these microbial strains is arbitrated through the production of diverse antimicrobial compounds including biosurfactants. In various biocontrol studies biosurfactants like rhamnolipids, sophorolipids, trehalolipid, fengycin, surfactin, iturin, and mycosubtilin have been investigated (Théâtre et al. 2022; Rasiya and Sebastian 2021; Horng et al. 2019; Fan et al. 2017). The biocontrol efficacy of the rhamnolipid biosurfactants is well documented in the literature against a broad range of agriculture pathogens. Rhamnolipids showed determinant antimicrobial activity against damping-off of cucumber disease caused by *Phytophthora capsici* (Kruijt et al. 2009). In another study, RL molecules were reported for inhibition of *P. capsici* spore germination and suppression of fungal cellular growth at low concentrations as compared to the commercial antimicrobial agents. Similarly, rhamnolipids RL-DS9 and RL-R95 showed an antifungal effect against *Colletotrichum falcatum* with an 86% decline in the spore germination rate and up to 83% inhibition of the fungal growth possibility because of disruption of fungal membrane (Shu et al. 2021). RL concentration of $10 \mu\text{g ml}^{-1}$ was proved effective for zoospores lysis and higher concentrations up to $25 \mu\text{g ml}^{-1}$ caused the significant collapse of the fungal zoospores. The rhamnolipid was equally affected against phytophthora blight and *Colletotrichum orbiculare*-mediated leaf infections in cucumber plants (Kim et al. 2000). The cell-free supernatant from *Pseudomonas aeruginosa* ZJU211 containing two congeners of rhamnolipids, RL-1 and RL-2, showed higher activity against cellular growth of various plant pathogens belonging to *Oomycetes*, *Ascomycota*, and *Mucor* spp. The RL-2 demonstrated more potent antifungal activity against metalaxyl-resistant *Oomycetes* as compared to RL-1 probably due to the more charged polar headgroup of the molecules (Sha et al. 2012). A mixture of mono and di-Rhamnolipid from *P. aeruginosa* from mangrove sediments demonstrated strong antagonistic action against tomato wild disease caused by *Fusarium oxysporum* at 200 $\mu\text{g/L}$ concentration (Deepika et al. 2015). Sophorolipids, a potent class of glycolipids biosurfactants, express great potency to restrict spore germination and hyphal growth in *Phytophthora*, *Aspergillus*, *Botrytis*, and *Fusarium* species (Valotteau et al. 2017; Yuan et al. 2015). Mannosylerythritol lipids (MELs) are another important glycolipid biosurfactants produced during the resting

cell stage by different yeast strains. Yoshida et al reported the inhibition of conidial germination of various phytopathogenic fungi with the application of MELs biosurfactant including *Collectotrichum dematium*, *Glomerella cingulata*, and *magnaporthe grisea*, and suggested MEL as a novel alternative to the chemical pesticides (Yoshida et al. 2015).

Genus *Bacillus* has remarkable metabolic potential of producing fascinating diversity of antibacterial and antifungal lipopeptides including surfactin, iturin, and fengycin with the possibility of application as natural biological control agents. On average 4–5% of the *Bacillus* genome is associated with secondary metabolites production and can generate more than twenty chemically distinct antimicrobial compounds (Stein 2005). These lipopeptides display excellent surface, interfacial and antagonistic properties both at laboratory and field scales making them attractive molecules for developing innovative pest management strategies (Eeman et al. 2009; Ongena and Jacques 2008). In this pursuit, various *Bacillus* strains have been tested for their fungicidal action including *B. subtilis*, *B. amyloliquefaciens*, and *B. pumilis* (Tran et al. 2022; Medeot et al. 2020). Surfactin is a model surface active cyclic lipopeptide of *Bacillus subtilis* that has been studied in great detail for structure–function relationship and commercial applications. At the cellular level, its biosynthesis is associated with various physiological events giving an obvious competitive advantage to the producing bacteria. The biosynthesis of surfactin helps bacterial colonization, biofilm development, and enhance bioavailability of complex hydrocarbons in agriculture and forest soils. Surfactin also facilitates attachment of the bacteria with the root surfaces to establish beneficial interactions between plants and root microbiome (Bais et al. 2004). The lipopeptide biosurfactants are produced with an exciting diversity of chemicals that influence bacterial survival in the presence of various plant pathogens. Interestingly, a single strain of the bacteria has the metabolic potential to produce homologous series of related compounds having different physiochemical and biological activities forming a mixed micelle system in the aqueous environment. Because of this feature, biosurfactants are of particular interest for agriculture and biomedical applications particularly for controlling plant and human infections. Production of structurally distant biosurfactants is a perfect example of evolutionary optimization allowing microorganisms to interact with multiple interfaces at a time and perform dynamic role in the ecosystem. The biosynthesis of surfactin and related cyclic lipopeptides has been investigated in *Bacillus subtilis*. They are produced as heptapeptides linked with fatty acid chains via a non-ribosomal peptide synthetase platform in which multi-modular enzymes are structured as complex NRPS assembly lines. The structural intricacy of non-ribosomal peptide synthetase is responsible for conferring variation among the cyclic lipopeptides like surfactin with respect to amino acid sequence, length, and branched chain fatty acids (Ongena and Jacques 2008). Due to the chemical heterogeneity of the surfactins, these biosurfactants show excellent antimicrobial action against the resistant plant pathogens Krishnan et al. (2019) have reported the antifungal activity of the surfactin produced from *Brevibacillus brevis* against *Fusarium moniliforme* with significant suppression of fungal growth, DNA, and protein damage. Various other researchers have demonstrated the biolytic effect of

fengycin and iturin family of lipopeptides. Fengycin, a cyclic lipopeptide by *Bacillus subtilis*, provides excellent protection against *Rhizoctonia* disease. Likewise, iturin produced by *B. amyloliquifaciens* has been used for controlling infections of *R. solani*. Lipopeptides belong to fengycin family usually composed of decapeptides interlinked with a β -hydroxy fatty acid chain of 13–19 carbons (Hamley et al. 2013; Caulier et al. 2019). Interest in fengycin has been growing for its prominent antifungal properties against filamentous fungi (Deleu et al. 2008). Iturin family of LPs are heptapeptides attached with a β -amino fatty acid of varying carbon chain lengths of C14–C18, and also exhibit strong antifungal activity against various plant pathogens (Maget-Dana and Peypoux 1994). The synergistic activity of different cyclic-LPs has been demonstrated using a mixture of surfactin, iturin, and fengycin against apple scab caused by *Venturia inaequalis*. The results revealed that antifungal activity of these lipopeptide mixtures was equivalent to the tebuconazole, a commercially available antifungal agent (Desmyttere et al. 2019; Ongena and Jacques 2008).

5 Mechanism for Antimicrobial Action

In recent years, biosurfactants have received a renewed attraction as an alternative to man-made chemicals for their powerful antimicrobial properties, low toxicity, and better performance under different chemical environments (Marchant and Banat 2012). With respect to their antimicrobial action, various mechanisms have been proposed for the inhibition of pathogenic bacteria and fungi by using biosurfactants. Since most of the literature is confined to glycolipid and lipopeptide biosurfactants, therefore, their mechanism of antimicrobial action will be discussed in this chapter. Generally, glycolipids are characterized by the presence of different sugar molecules attached to fatty acids of varying length and branching (Poirier et al. 2022; Al-Fadhli et al. 2006). The mechanism of action of different glycolipids has been described in the literature with more focus on rhamnolipids and sophorolipids. Glycolipids have the ability to damage the microbial cell membrane with varying complexities. The incorporation of the rhamnolipid molecules induces structural changes by forming rhamnolipid-enriched domains within the unit membranes. These observations reflected that penetration of the RL molecules into the hydrophobic core of plasma membrane leads to a structural discrepancy with the phospholipid's acyl chain and thus disrupts supramolecular bilayer assembly (Sánchez et al. 2009). The finer insight of the possible interaction of rhamnolipids revealed that insertion of RL molecules into the plasma membrane yield lateral phase separation into rhamnolipid-rich and rhamnolipid-poor domains. These RL induce morphological changes decrease multilamellar arrangement of the cell membranes with a significant increase in the thickening of bilayer and increase in interlamellar distances (Shao et al. 2017; Ben Ghorbal Salma et al. 2022; Hadi et al. 2022). This particular disordering effect has been associated with the higher concentration of the rhamnolipids. Another possible explanation of this effect could be the dehydration effect imposed by higher

concentrations of RL molecules. The dominating antimicrobial effect of RL-2 is linked with the additional rhamnose units found at the hydrophilic end of di-rhamnolipid. At the membrane–water interfaces, RL-2 exhibits a higher tendency to establish hydrogen bonding with the surrounding water thus reducing the interactions of water with the membrane phospholipid headgroup and altering the membrane fluidity (Euston et al. 2021). The rhamnolipids also affects the permeability of the biological membranes at RL–lipid ratio of 1:1 causing leakage of the cellular fluids. A similar effect has been elucidated for other glycolipid biosurfactants including trehalolipid (Arathi et al. 2021). The RL shows a limited tendency to bind with the membrane proteins and decipher much lower protein denaturing efficacy in vitro. However, they can influence lysis of the membrane protein via activation of cellular proteases. Besides scarcity of the data, fascinating details are emerging to provide better insight into protein modulating activity of the rhamnolipid in particular and glycolipids in general. In case of lipopeptide biosurfactants, they are secondary metabolites having strong antimicrobial activity. It is proposed that LPs induce their fungicidal effect either by blocking the membrane transport or by creating holes in the cell membranes of the fungal pathogens. Some *Bacillus* lipopeptides are known to cause disintegration of internal cellular structures of the fungal cells (Wu et al. 2019; Nawaz et al. 2018). It is demonstrated that lipopeptide biosurfactants change permeability of the membranes, structural modification in the phospholipid bilayer architecture, and modification of membrane proteins (Zihahirwa et al., 2017). Besides the emergence of exciting evidences, there are certain gray areas in understanding the interaction of biosurfactants with membranes bonded proteins. Table 1 enlists different types of biosurfactants, their sources and probable mode of action.

6 Role of Biosurfactants in Nutrient Bioavailability in the Soil

Nutrient deficiency and availability in the soil have a direct relation with species richness, distribution, plant health, and ecosystem productivity, both in the natural and agricultural settings. In general terms, bulk soil contains sufficient quantities of nutrients which are mostly present in the bound state with organic and inorganic compounds. Because of their restricted mobility and solubility in the soil matrix, the concentration of these nutrients in the rhizosphere remains lower than what is required for healthy plant growth and development. Plants deploy various strategies to access unavailable nutrients; one of the most prominent is the recruitment of appropriate root colonizing microorganisms. Since most of the chemical trade between plants and soil is carried out in the rhizosphere, rhizosphere microbiology has gained significant interest in recent years. It has been demonstrated that the availability of nutrients in the rhizosphere is dictated by synergistic effects of soil properties, plant characteristics, and root–microbe interactions. Many soil dwelling

Table 1 Biosurfactants producing microorganisms, their proposed mode of action and target pathogens

S. No.	Biosurfactants	Producing strain	Active against	Mode of action	References
1	Surfactin	<i>Bacillus subtilis</i> , <i>Bacillus BS5</i> , <i>amyloliquefaciens B128</i> , <i>Bacillus circulans ATCC 4513</i> .	<i>Byssoschlamys fulva</i> , <i>Fusarium moniliforme</i> , <i>Clostridium perfringens</i> , <i>Brachyspira hyodysenteriae</i> , <i>Bacillus. hyodysenteriae</i>	Membrane perturbation, decreased microbial resistance, disruption of cell morphology and growth	(Tran et al. 2022; Horng et al. 2019; Horng et al. 2019; Sekhon et al. 2011; Hsieh et al. 2004)
2	Fengycin	<i>Bacillus subtilis</i> 9407, <i>Bacillus amyloliquefaciens MEP₂18</i>	<i>Xanthomonas axonopodis</i> sp. <i>Vesicatoria</i> , <i>Pseudomonas aeruginosa PA01</i> , <i>Botryosphaeria dothidea</i>	Suppression of biofilm formation, membrane charge imbalances, and alteration in membrane permeability channels leading to pore formation and cell death	(Panchbhai 2022; Tran et al. 2022; Medeot et al. 2020; Fan et al. 2017)
3	Iturin	<i>Bacillus subtilis</i> CS 93, <i>Bacillus subtilis</i> B-3, <i>Bacillus pumilus HY 1</i> , <i>Bacillus cereus</i>	<i>Penicillium crustosum</i> , <i>Botryosphaeria</i> sp., <i>Lasiodiplodia theobromae</i>	Destabilization of membrane sterols leading to vanished cell membrane integrity and loss of functionality	(Théâtre et al. 2022; Tran et al. 2022; Rasiya and Sebastian 2021; Cerqueira et al. 2012)
4	Mycosubtilin	<i>Bacillus subtilis</i> BBG100, <i>B. subtilis</i> LBS1	<i>Paecilomyces variotti</i> , <i>Byssoschlamys fulva</i> , <i>Candida krusei</i> ,	Lysis of yeast spheroplasts, disintegration of cell membrane, denaturation of lipids, RNA, and proteins leading to altered metabolism	(Khatoon et al. 2022; Kourmentza et al. 2021)
5	Bacillomycin	<i>Bacillus amyloliquefaciens</i> , <i>Bacillus subtilis</i> BBK-1	<i>Fusarium solani</i> , <i>Erysiphe cichoracearum</i> , <i>Candida albicans</i> , <i>Helminthosporium turcicum</i>	Cell damage via accumulation of reactive oxygen species, morphological changes in cell wall, plasma membrane changes, altered gene expression and phosphorylation leading to cell death	(Lin et al. 2022; Adetunji et al. 2022; Gu et al. 2017; Roongsawang et al. 2002)

(continued)

Table 1 (continued)

S. No.	Biosurfactants	Producing strain	Active against	Mode of action	References
6	Viscosin	<i>Pseudomonas fluorescens</i> SBW25, <i>Pseudomonas fluorescens Pf0-7</i>	<i>Batrachochytriumden drobatidis</i> , <i>Aspergillus fumigates</i>	Alteration in membrane permeabilization due to Glu2/Gln2 structural variations leading to cell dysfunctioning	Doole et al. 2022; Sastoque-Cala et al. 2010;
7	Rhamnolipid	<i>Pseudomonas aeruginosa</i> , <i>Acinetobacter junii</i> , <i>Rhodococcus fascians</i> , <i>Enterobacter cloacae</i>	<i>Micrococcus luteus</i> , <i>Staphylococcus epidermidis</i> , <i>Serratia methicillin-resistant</i> <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Mycobacterium phlei</i>	Insertion of acyl tails in cell membrane, cytoplasmic leakage, membrane pore formation resulting in loss of metabolites and cell function	(Curiel-Maciél et al. 2021; Abbasi et al. 2012; Darvishi et al. 2011 Gesheva et al. 2010)
8	Sophorolipid	<i>Candida bombicola</i> , <i>Rhodococcus wratislaviensis</i> , <i>Stenotrophomonas maltophilia</i>	<i>Enterococcus faecalis</i> , <i>Pseudomonas aeruginosa</i> , <i>Pseudomonas luteola</i> , <i>Vibrio fluvialis</i>	Extravasation of cytoplasmic contents, decreased antibiotic resistance, inhibition of biofilm formation, and damaging bacterial envelope	(Cerqueira et al. 2012; Tuleva et al. 2008)
9	Mannosylerythritol lipid (MEL)	<i>Candida antarctica</i> , <i>Pseudozyma</i> sp., <i>Ustilago</i> sp.	<i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas</i> sp.	Membrane alterations by the action of Lipid-B component of MEL, altered genetic expression affecting cell differentiation and leading to cell death	(Bhangale et al. 2022; Coelho et al. 2020; Arutchehvi et al. 2008)
10	Lipopeptide	<i>Bacillus subtilis</i> SPB1, <i>Serratia marcescens</i> ; <i>Acinetobacter</i> sp. D3-2.	<i>Bacillus cinerea</i> , <i>Zymoseptoria tritici</i> , <i>Xanthomonas arboricola</i> pv. <i>pruni</i> , <i>Xanthomonas campestris</i> pv. <i>Vesicatoria</i>	Antimicrobial action due to the activity of serrawettin Stephensiolides, membrane destabilization, and leakage via micelle aggregation resulting in cell lysis	(Ledger et al. 2022; Chen et al. 2012; Roldán-Carrillo et al. 2011)

microorganisms are known to produce a wide array of biosurfactants and create micelles of different sizes, shapes, and geometrical forms in the aqueous system. Micelle formation is the fundamental property of all types of surface active agents including biosurfactants. These mixed micelles show a higher tendency to interact with various physical and biological interfaces where they can resolve issues of restricted mobility, poor solubility, and limited mass transfer of organic and inorganic substrates (Mishra et al. 2021; Sponza and Gok 2011). Besides the emergence of fascinating details, interaction of biosurfactants with soil minerals is largely unexplained. However, studies on surfactants–metal interactions have greatly helped to understand the possible mechanism of their transport in the soil matrix.

Phosphate, potassium, iron, zinc, and copper are among the most frequently cited nutrients facing limited mobility in soils. Besides high prevalence in the bulk soil, plant available fraction and their relative concentration in the soil solution is not sufficient to cater to the physiological needs of the plants (Rengel 2001). Acquisition and utilization efficiencies of soil nutrients can be improved by the action of biosurfactants-producing bacteria (Banat et al. 2010). Biosurfactants act as *micro-manipulators* thereby enhancing solubility of the unavailalbe nutrients using their micelle formation properties (Pacwa-Plociniczak et al. 2011). Rhamnolipids have been studied in great detail for their structure–function relationship, phase behavior, and performance under various chemical backgrounds. In order to perform at solid–liquid interfaces, RL molecules tend to organize into highly ordered structures (micelles) based on self-assembly parameters. In an aqueous environment, micelles adopt a spherical shape by packing hydrophobic tails inward forming a micelle core and hydrophilic heads facing water. With this orientation, they can reduce the surface and interfacial tension between the two phases thereby enhancing solubility and mobility of organic and inorganic materials. RLs, because of its anionic nature, have the ability to make complexes with positively charged metals (cations) in the soil and aqueous solution, therefore, play important role in their bioavailability to the plants (Mnif and Ghribi 2015; Sun et al. 2021; Liu et al. 2018). The hydrophilic surfaces of the RL micelles are negatively charged and provide attractive electrostatic interaction with positively charged metal ions. As a result of strong electrostatic attractions, the bound nutrients are released from the organic matter and clay particles and get attached to the surfactants. The rhamnolipid shows excellent copper ions chelating activity which is comparable with that of synthetic surfactants. The immobilizing rate of copper ions in RL solution was found to be 70% according to the study by Cieřla et al. (2018). The aggregation state of RL is significantly affected by the presence of K⁺ ions and lowered copper fixation in rhamnolipid solution. However, in contrast, RL showed comparatively lower metal complexion activity as compared to EDTA. This could be attributed to the higher proton-donating efficiency of EDTA (Hemlata et al. 2015). The monorhamnosyl (RL-1) and dirhamnosyl rhamnolipids (RL-2) form lipophilic complexes with Zn and improve transport and uptake by *Triticum durum* roots (Stacey et al. 2008). The acidic sphorolipids can chelate copper ions by making biosurfactant–metal complex at varying pH, possibly due to steric availability of its both headgroups. Because of the aforementioned, acidic sphorolipid is capable of sequestering Cu ions, provided,

when these cations are available at the sulfide surfaces. Apart from Cu, sophorolipid showed a similar trend of complexation with other metals including Ca, Mg, Al, and Fe (Dhar et al. 2021). The measurement of zeta potential provided a mechanistic understanding of the metal removal by the surfactants. The rhamnolipid and surfactin showed efficient removal of organically bound copper whereas, sophorolipid removed carbonate and oxide-bound zinc with better efficacy. The metal removal action of the biosurfactants is associated with surfactant sorption onto the soil surfaces and complexes with metals. These surfactant-bound metals are then released into soil solution where they are taken up by plant roots (Mulligan et al. 2001; Sheng et al. 2008). The ability of biosurfactants to enhance the solubility of metals and trace elements has important implications in modern agriculture (Bezza et al. 2015). In case of saline soils, biosurfactants have the capability to sequester soil-bound nutrients and enhance their bioavailability (Rufino et al. 2011; Gregory 2006; Pacwa-Plociniczak et al. 2011). In most of the cases role of biosurfactants is restricted to the bioremediation of metal-contaminated soils. Despite the huge volume of biosurfactant research that exists today, only a small fraction is dedicated to nutrient mobility and availability in agriculture soil. The effect of different soil ingredients on the self-assembly and micellization of biosurfactants, the determination of effective surfactant dose for improving the mobility of different soil nutrients and unlocking molecular communication between plant and microbes, are some of the potential research areas that could translate into more productive agriculture systems.

7 Biosurfactants in Pesticide Degradation and Soil Rehabilitation

Emergence of invasive pests and plant pathogens is posing serious threats to food security and safety. In order to provide food to the billions of humans, chemical pesticides are being used indiscriminately all over the world as the most prevailing pest and pathogen control strategy. The excessive use of pesticides in the agriculture products both at pre- and post-harvesting stages has been associated soil, water, and food contamination leading to serious health repercussions and ecological deterioration. Pesticides are among the most recalcitrant chemicals owing to their complex structure and longer persistence in the soil (Bose et al. 2021). Due to their proven role in inducing cellular toxicities in the form of cancer progression, promoting mutagens, and causing various systemic disorders, pesticides are placed at the top of the priority pollutants list. Therefore, reclamation of the pesticides contaminated has been considered one of prime concern with reference to agriculture and environmental sustainability. Biosurfactant-based remediation methods have been gaining considerable attraction in the preview of their cost-effectiveness and excellent performance for on-site degradation of pesticides and other xenobiotics. In the soil, biosurfactants application enhances solubilization of the toxic pesticides,

making them biologically available for microbes. These microbes then assimilate pesticides as a source of carbon, nitrogen, and phosphate in order to satisfy their cellular needs (Lamilla et al. 2021). During the past few years reports on biosurfactants in pesticide degradation is exceedingly growing. Because of lower cmc and higher aggregation number, biosurfactants maintain supremacy over the synthetic regimes besides having less cellular yield. As a result of micelle formation at relatively low cmc, biosurfactants are more effective in solubilization of organic pollutants than chemical surfactants. Pesticides are hydrophobic chemicals having a higher tendency to bind with soil organic matter. Their adsorption onto the soil surfaces negatively regulates soil physiochemical and biological properties. The role of biosurfactants in the bioremediation of hydrophobic organic contaminants (HOCs) has been highlighted by many researchers. Emerging models have provided detailed insight into the mechanism of surfactant–hydrocarbons interactions in an aqueous medium. In contaminated soils, the addition of biosurfactants below *cmc* concentrations improves desorption of hydrophobic contaminants in the aqueous phase because of hydrophobic interactions between surfactant monomers and organics. On increasing concentration, biosurfactant molecules accumulate at interfaces and reduce interfacial tension (Santos et al. 2016). At surfactant concentration above cmc, micelles encapsulates hydrophobic contaminants into micelle core owing to competition between micelles and soil particles. The entrapment within the surfactants micelles improve aqueous solubilization and mobility of the HOCs and assists contaminant remediation (Lamichhane et al. 2017). The possible mode of action of biosurfactants in the mobilization of nutrients and HOC is given in Fig. 4a, b.

In order to release attached pesticides from soil particles, surfactant monomers act on soil interfaces and alter the surface and interfacial tension thereby facilitates their detachment. This process is termed as desorption. These molecules are then moved into the liquid phase where surfactant micelles encase hydrophobic pesticides within their hydrophobic micelle core and improve their solubilization. Finally, the micelle-entrapped pesticide molecules are delivered to the surface of microbial cells, from where these molecules enter the cell and are degraded by the action of microbial enzymes (Rasheed et al. 2020; Twigg et al. 2019; Banat et al. 2010). The action of biosurfactants thus makes the contaminants biologically available to the microorganisms and enhances the soil remediation process. Other interactions such as ion exchange, electrostatic interaction, and precipitation-dissolution are also involved in the surfactant-mediated pesticide bioremediation. A number of previous reports highlighted the role of microbial surfactants in accelerating the solubility and biodegradation of wide-ranging toxic contaminants including pesticides. Rhamnolipid is considered as a model biosurfactant system widely used in laboratory investigations and field-scale pesticide bioremediation projects (Banat et al. 2010; Varjani and Upasani 2017). It has been highlighted that RL monomers accumulate at soil–oil interface at hypo-cmc concentration causing electrostatic repulsion between soil particles and RL hydrophilic head groups, ensuing desorption of hydrophobic organics from soil. The role of biosurfactants in the biodegradation

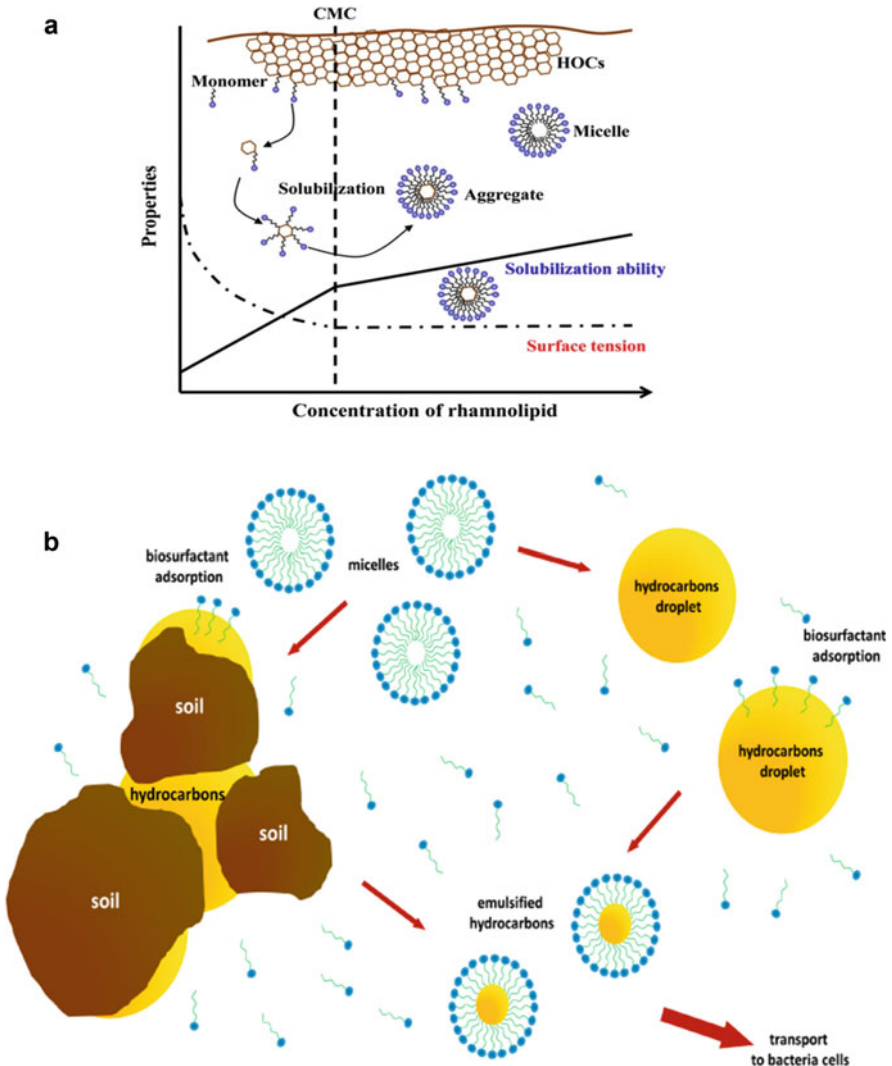


Fig. 4 (a) Possible mechanisms of action of biosurfactants below and above cmc for the removal of organic contaminants. (b) Biosurfactants induced transport and bioavailability of contaminants to the bacterial cells (Zeng et al. 2018; Kaczorek et al. 2018)

of pesticides with their mode of action and microbial sources has been listed in Table 2.

RL has been tested for enhancing solubilization and bioremediation of commercial pesticides including chlorpyrifos, trifluralin, endosulfan, coumaphos, and atrazine (Tan and Li 2018; García-Reyes et al. 2018). RLs show enhanced solubilization of various alkanes, polyaromatic hydrocarbons, and complex polychlorinated biphenyls (Maia et al., 2019). [Wattanaphon](#) and co-workers reported the RL biosurfactant

Table 2 Degradation potential of biologically produced biosurfactants, along with their proposed mechanism

S. no.	Producing microorganism	Biosurfactant type	Target pesticide	Possible mode of action	References
1	<i>Rhodococcus</i> , <i>Pseudomonas</i> sp., <i>Arthrobacter globiformis</i> , <i>Lysinibacillus sphaericus</i>	Rhamnolipid	Cypermethrin, Endosulfan, DDT, Quinalphos, Carbendazim Chloropyrifos, Hexachlorocyclohexane (HCH), Difenoconazole	Degradation via organophosphate hydrolase, monooxygenase, and dioxygenase production and transesterification of pesticide, partitioning kinetics resulting in degradation of pesticide	(Satapute and Jogaiah 2022; Lamilla et al. 2021; Niu et al. 2019; Pérez-Armendáriz et al. 2019; de Andrade et al. 2016)
2	<i>Bacillus subtilis</i> , <i>Paenibacillus</i> sp., <i>Actinomyces</i>	Lipopeptide	Endosulfan, HCH,	Decrease in surface tension, desorption, solubilization, and uptake by microbes, utilization as a carbon, nitrogen, and phosphorus source in metabolism	(Drakontis and Amin 2020; Marcelino et al. 2019; Bhatt et al. 2019; Jimoh and Lin 2019)
3	<i>Pseudomonas</i> sp., <i>Burkholderia cenocepacia</i> sp.	Glycolipid	Methyl parathion, parathion	Utilization of p-nitro phenol as carbon source, solubilization, increasing surface area for microbial degradation and uptake mechanisms	(Schultz and Rosado 2020; Patowary et al. 2017 Fernández-López et al. 2017)
4	<i>Rhodococcus</i> sp.	Trehalolipid	DDT	Contact angle changes, micelle formation, and solubilization lead to pesticide degradation	(Bhatt et al. 2021; Bages-Estopa et al. 2018, Egorova et al. 2017)
5	<i>Serratia</i> sp.	Lipoproteins	Nicosulfuron, organic pollutants	Desorption from soil surface, enhancing bioavailability, mineralization, and emulsification of pesticide	(Wang et al. 2022; de Andrade et al. 2016; Zhang et al. 2012)
6	<i>Pseudomonas</i> sp.	Exopolysaccharides	2,4-Dichlorophenoxyacetic acid	Increasing surface area for microbial degradation, utilization as carbon, phosphorus, and nitrogen source	(Fitch et al. 2022; Karlapudi et al. 2018a, 2018b; Han et al. 2015)

from *B. cenocepacia* significantly improves the solubility of methyl parathion, ethyl parathion, and trifluralin in aqueous medium at 2 mg/L concentration. The RL exhibited much higher solubilization activity than commercial surfactant (Tween80) and showed the same activity as SDS under neutral pH. RL and SDS and anionic surfactants hence their activity may coincide under given reaction conditions (Mata-Sandoval et al. 2002). The biosurfactants' performance is a function of micelle formation. It is believed that the concentration above the cmc value facilitates a high micelle formation rate that leads to the improved solubility of the hydrophobic contaminants (Ahn et al. 2010). In case of α -, β -endosulfan, and γ -hexachlorocyclohexane, rhamnolipid produced from *Lysinibacillus sphaericus* enhanced many-fold solubilization of these pesticides at a concentration of 90 mg/L as reported by Gaur et al. 2019. The RL molecules can improve the solubility of chlorinated pesticides such as triclosan and endosulfan (Guo et al. 2016; García-Reyes et al. 2018). Crude RL improved the aqueous solubility of chlorpyrifos 15-folds and enhanced biodegradation efficiency of the mixed bacterial culture by up to 30% after 6 days (Singh et al. 2016). The biodegradation of trifluralin increased by up to 35% in the soil treated with RL (Bai et al. 2017). The improved solubilization of complex hydrophobic compounds is associated with the surfactants ability to make micelles of different sizes and shapes. The rhamnolipid and sophorolipid biosurfactants mixed micelles in the aqueous system improved the solubility of petroleum hydrocarbons and some other hydrophobic materials. It is also suggested that production of glycolipid biosurfactants increased the cell surface hydrophobicity of *P. aeruginosa* and *Meyerozyma* stains leading to 91% and 87% biodegradation of complex petroleum hydrocarbons, respectively. In addition, crude extract of RL mixture produced stable oil–water emulsions (Ramla Rehman et al. 2021). Other classes of biosurfactants can also enhance solubilization and degradation of different pesticides. The cyclic lipopeptides (Surfactin) from *Bacillus subtilis* enhance the degradation of endosulfan in aqueous and soil conditions (Landa-Faz et al., 2022). The surfactin-producing *Lysinibacillus* strain resulted in 90% difenoconazole biodegradation under laboratory conditions (Satapute and Jogaiah 2022). *Lysinibacillus sphaericus* IITR51 showed the capability to produce thermostable biosurfactant that can solubilize hydrophobic pesticides such as HCH (hexachlorocyclohexane) and endosulfan (Manickam et al. 2012; Gaur et al. 2019). Based on the surface activity, antimicrobial action, micelle forming properties, foaming, wettability, partitioning efficiency, and operational stability, biosurfactants are emerging as a potential biochemical alternative for agro-industrial application and catalyst for improving the quality of agriculture soils.

8 Conclusion and Future Prospects

Biosurfactants are becoming one of the most important biotechnological products both in terms of their properties and spectrum of commercial applications. The better efficacy of the biosurfactants under varying chemical environments makes them

promising candidates for ecological and agricultural sustainability. Based on the emerging scientific evidences, biosurfactants present various technical advantages and certain limitations of high volumetric production. One of the most important challenges is the purity of the biosurfactants since a single microbial strain produces a series of structurally related compounds with different properties. With this metabolic efficiency, the experimental results are sometimes confusing specifically when these molecules are required for a target-specific biomedical application. In agricultural soils, biosurfactant-producing microorganisms are an important component of the soil ecology, soil microbiomes, and rhizosphere. In rhizosphere biosurfactants producing microbes colonize the root surfaces and facilitate nutrient transport and acquisition, establish co-aggregation of the microbial communities, biofilm formation, developing healthy plant–microbe interactions, mineralizing complex hydrocarbons, pathogen inhibition, and promoting better plant growth and immunity. However, the exact role of antimicrobial agents yet remains elucidative and requires further investigation in order to demonstrate their real agricultural potential as eco-responsible alternatives. In addition, biosurfactant-producing microorganisms degrade pesticides, HOCs, and other contaminants to improve soil quality and health. Today, only a small fraction of biosurfactants research is dedicated to nutrient mobility and availability in agriculture soil. The effect of different soil ingredients on the self-assembly and micellization of biosurfactants, determination of effective surfactant dose for improving mobility of different soil nutrients and unlocking molecular communication between plant–microbes, are some of the potential research areas that could translate into more productive agriculture systems. Despite the availability of high-resolution molecular approaches for the description of soil microbial communities and characterization of metabolomes with distinctive biochemical activities, our current understanding of biosurfactants role in soil ecosystems is lacking. Therefore, it is concluded that unlocking functional traits and metabolic performance of soil microbiomes associated with plant roots could be very helpful for developing more productive and sustainable agriculture systems.

9 Commercial Resources of Biosurfactants

1. AGAE Technologies—USA (Rhamnolipid biosurfactants production) <https://www.agaetech.com/>
2. Allied Carbon Solutions (ACS) Ltd—Japan (Sophorolipids) <https://www.allied-c-s.co.jp/english-site>
3. BioFuture—Ireland (Rhamnolipid biosurfactants) <https://biofuture.ie/>
4. EcoChem Organics Company—Canada (Rhamnolipid biosurfactants) <http://www.biochemica.co.uk/>
5. Ecover Eco-Surfactant—Belgium (Sophorolipids) <https://www.ecover.com/>
6. Fraunhofer IGB—Germany (Glycolipid and cellobiose lipid biosurfactants production) <https://www.igb.fraunhofer.de/>

7. Rhamnolipid Companies—USA (Rhamnolipid biosurfactants) <http://rhamnolipid.com/>
8. Saraya Co. Ltd.—Japan (Sophorolipid biosurfactants) <http://worldwide.saraya.com/>
9. Synthezyme—USA (Sophorolipid biosurfactants) <http://www.synthezyme.com/index.html>
10. TeeGene Biotech—UK (Lipopeptides and Rhamnolipids) <http://www.teegene.co.uk/>

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Biosurfactants and Their Benefits for Seeds



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1 Introduction

Currently, agricultural productivity is a challenge around the world to answer the growing need for human food. In the current scenario, achieving sustainable agriculture is ideal, so the use of eco-friendly substances becomes a viable strategy, as is the case with the use of biosurfactants, which can be used in agriculture in the process of eliminating phytopathogens and can also be related to increased bioavailability of nutrients for plant and microorganisms. In addition, the application of biosurfactants for the remediation of agricultural soils improves soil quality and favors seeds for better plant growth (Gayathiri et al. 2022).

Biosurfactants can probably replace synthetic surfactants used in the formulation of agricultural products. The use of biosurfactants promises to be a valuable tool to reduce the use of agrochemicals, as these compounds can contribute to crops without producing toxicity to the environment. Furthermore, their potential as bioremediation agents can contribute to improving the health of soil systems by increasing the solubility of harmful and remaining pesticides, which can make them available for biodegradation by other microorganisms (Sharma et al. 2022).

For agricultural applications, biosurfactants can be inserted into the crop production chain at different points. In general, direct inoculation into the soil is widely

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used to provide a greater amount of inoculant in the soil or in the case of brittle seeds, however, due to the need for a large amount of inoculant, this alternative is not economically viable (Vosátka et al. 2012). Another possible method is the inoculation of plants by immersion of roots and leaves, however, these techniques demand plant nursery preparation and also the application of large amounts of inoculant (Rocha et al. 2019). In contrast, the seed inoculation process can be an economically viable alternative in providing inoculants on a large scale (O'Callaghan 2016), providing the target crop inoculant, ensuring close contact from germination between the plant and the inoculant (Philippot et al. 2013).

The use of biosurfactants before germination has different beneficial effects on the seeds, including antifungal, antibacterial, and antiviral properties (Mulligan 2005), the ability to facilitate absorption of biogenic substances by plants (such as the nutrient phosphorus) (Mukherjee et al. 2006), and it can also increase the solubility and spray level when applied in conjunction with other chemical plant protection products (Mata-Sandoval et al. 2000). Furthermore, the stimulation in the pre-sowing stage with biosurfactants aims to accelerate seed germination. Therefore, the application of biosurfactants in seeds aims to increase the resistance to diseases caused by phytopathogens and provide the initial dose of nutrients after germination, in the development of seedlings (Krawczyńska et al. 2012).

Among the possible applications of biosurfactants in agriculture, the potential of biosurfactants in seed protection and as a plant biostimulant has been investigated. Seed inoculation has been highlighted, as it is considered an economical and accurate method to provide inoculants of microbial origin (Ehsanfar and Modarres-Sanavy 2005; O'Callaghan 2016), for increasingly used to improve seed quality, and seed germination, and also manages to improve plant parameters on a contaminated base (Mukherjee et al. 2006).

Seed protection, therefore, is a logical target for agricultural practices, where treatment with biosurfactants is expected to protect seeds from toxic compounds existing in the soil and increase the vigor of planted seeds. In this chapter, the different ways that biosurfactants contribute to the seed protection and fertility of the soil will be addressed, a summary of these ways is described in Fig. 1.

2 Antimicrobial Properties

Phytopathogens cause enormous economic losses worldwide, with agricultural damage reaching up to 40%, depending on crop conditions (Savary et al. 2019). Therefore, to combat diseases and pests, new sustainable techniques have been explored in agriculture. Biosurfactants have shown promise as biocontrol agents against pathogenic microorganisms that affect crops. Biosurfactants serve as an ecologically correct tool to control pathogens and have the protective function of endophytic organisms that benefit plants (Singh and Rale 2022).

Among the existing microbial biosurfactants, rhamnolipids and lipopeptides are highlighted in terms of antimicrobial activity, since they are the most studied for

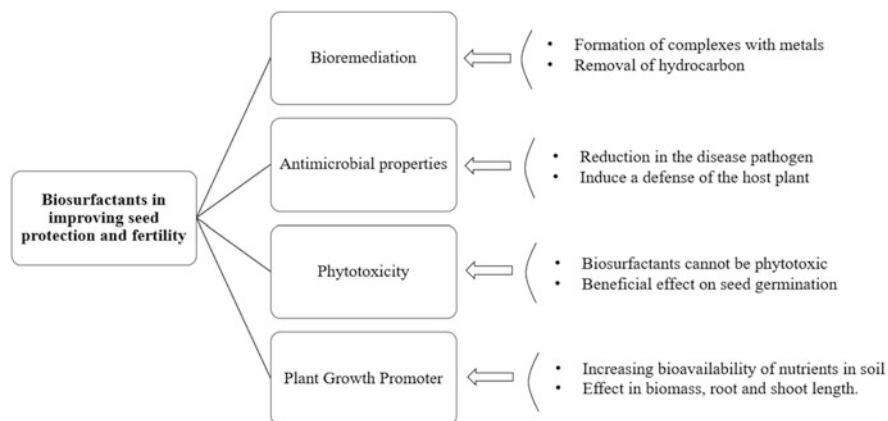


Fig. 1 Different ways that biosurfactants contribute to seeds and the caused effects

agricultural applications, in addition to being effective and economically profitable for application in crops (Crouzet et al. 2020).

Glycolipids, consisting of a carbohydrate fraction coupled to fatty acids, have different possible structures and are characterized by having the capacity to reduce interfacial and surface tension. The most popular glycolipids consist of rhamnolipids, trehalolipids, mannosylerythritol lipids, and cellobiose lipids. Which receive great attention because they can be used as biopesticides, in the control of plant diseases and improvement of the health of the same ones (Mnif and Ghribi 2016).

Rhamnolipid demonstrates a broad range of activities on fungi and bacteria (mostly Gram-negative) because the active compounds present in rhamnolipid biosurfactants have in breaking and destroying the biological membranes of microorganisms causing death (Haba et al. 2014; Oluwaseun et al. 2017). Due to their amphiphilic nature, glycolipids can interact directly with the plasma membranes of microorganisms (Otzen 2017). Another effect of this type of biosurfactant is to affect mycelial cells, which can destabilize or even cause cell lysis (Crouzet et al. 2020).

Lipopeptide biosurfactants consist of a lipid tail coupled to an oligopeptide. They can be produced by fungi and bacteria and have proven antimicrobial, cytotoxic, antitumor, and surfactant activities (Raaijmakers et al. 2010). The antimicrobial activity of this type of biosurfactant via direct inhibition of phytopathogens has already been evidenced in several studies, which indicate that lipopeptides can compromise the stability of the cell membrane of microorganisms, which results in cytoplasmic content leakage, death of fungal hyphae or inhibition of the germination process of spores of resistant pathogens (Gong et al. 2015; Pérez-García et al. 2011; Qian et al. 2016). These biosurfactants are also capable of acting and can act directly or indirectly on different intracellular structures and contribute to the alteration of microbial cell function (Qi et al. 2010).

The protection of the biosurfactant lipopeptide produced by *Bacillus licheniformis* in fava beans was studied by Akladious et al. (2019), fava bean seeds (*Vicia faba* cv. Nubaria 1 and cv. Sakha 1) were treated with biosurfactants and exposed to the fungus *Rhizoctonia solani*, and the minimization of the root rot disease was measured. The most frequency of the disease (62.11%) in bean plants was reported in untreated Nubaria 1 cultivars, while in the same plant treated with biosurfactant the disease incidence reduced to only 20%. In the tests carried out with the seed of cv. Sakha 1, the disease incidence in untreated plants (only with fungus inoculation) was 38.93%, and for those that received treatment with biosurfactant inoculation, the incidence was 16.51%. Such reduction observed in the development of root rot in plants in which the seeds were treated with the biosurfactant can be correlated with the presence of biological compounds that can induce the host plant's defense mechanism (Velho et al. 2011).

In this same study, the morphological characteristics were evaluated: the number of leaves and branches shoot and root lengths, stem diameter, and fresh and dry weights of shoots and roots. In the seeds with the application of biosurfactant occur a significant increase in all growth criteria of infected and healthy plants, when compared with untreated seed, that is, the biosurfactant, in addition to contributing to the protection of the seed against the pathogenic fungus, also contributes to the growth and development of the plant.

In the work of Khare and Arora (2021) the di-rhamnolipid biosurfactant produced by *Pseudomonas guariconensis* LE3 was presented as a biocontrol agent with antifungal activity against *Macrophomina phaseolina*, the main agent of coal rot in several crops, responsible for large losses in the agricultural area and causes infections in plant seeds (Malathi and Doraisamy 2004). Samples treated with biosurfactant showed a 54.95% decreased occurrence of charcoal rot disease of sunflower, so the application of biosurfactant in the seed protected so that with the development of the plant there was a reduction in the disease caused by a pathogenic fungus.

The pathogenic fungus *X. oryzae*, which causes one of the most devastating diseases of rice (Ou 1985) was studied in the work of Shalini et al. (2017), being a seed-born pathogen and a worldwide threat to rice cultivars, these authors studied the application of the glycolipid biosurfactant from the endophytic bacterium *Acinetobacter* sp. ACMS25 against the fungus, and the ability of *X. oryzae* to survive on the surface of rice seed treated with the biosurfactant. In vitro assays demonstrated that the biosurfactant was capable to decrease the growth percentage of the fungus *X. oryzae* by 38.4%. In the tests with the seeds, with the inoculation of *X. oryzae*, a significant decrease was observed in the percentage of germination, and the length of the shoot and root. On the other hand, when the seeds were treated with a 3% biosurfactant, germination was improved by 91.1%, root length by 9.1 cm, and shoot length by 19.8 cm. In addition, the treatment with biosurfactant in the seeds was capable to afford 76.9% of protection against diseases.

The in vivo efficacy of rhamnolipid B from *Pseudomonas aeruginosa* strain B5 for the control of phytophthora blight in pepper plants was checked in the work of Kim et al. (2000). With increasing the amount of rhamnolipid B used, phytophthora

disease was remarkably reduced in the first branches of pepper plants. These authors confirmed the protective effect of biosurfactants by submitting pepper seeds to biosurfactants before and after inoculation of the fungus *Phytophthora capsici*, was observed that before inoculation the application of rhamnolipid B was more effective, the concentration of 500 mg.ml^{-1} protected the pepper plants from the phytophthora pest.

One of the most important effects of biosurfactants is associated with their antimicrobial activity. In this perception, biosurfactants are an effective and sustainable option to replace the use of synthetic pesticides, providing protection to the seed and ensuring its fertility to produce healthy plants, due to their antimicrobial effect or inducing the plant's defense against pathogenic microorganisms that affect crops and cause agricultural losses. Figure 2 shows a summary of the antimicrobial activity of biosurfactants and their effects on the microorganisms, as well as the effect on the induction of plant defense, which will also be discussed in the next item.

3 Stimulation of Plant Immunity

It is reported that plants can develop complex defense mechanisms related to increased resistance to phytopathogens, such as ion fluxes, phosphorylation cascades, and increased retention of reactive oxygen species (ROS), causing responses related to plant defense (Bigéard et al. 2015; Garcia-Brugger et al. 2006). This increase in resistance to pathogens is related to the synthesis of elicitor substances by the plant itself or synthesized by microorganisms (Schellenberger et al. 2019).

The role of microorganisms in stimulating plant immunity by different systems may be related to the synthesis of biosurfactants (Crouzet et al. 2020; Vatsa et al. 2010). Accordingly, the induction of innate plant immunity by biosurfactants is related to seed quality, where plant defense mechanisms are activated, protecting the seed which contributes to the healthy development of the plant.

In addition to direct antagonism by antimicrobial activity, some beneficial bacteria, as well as the biosurfactant produced by them, can protect plants indirectly through stimulation of inducible defense systems, through the mechanism called "induced systemic resistance" (ISR), which makes them more resistant plants and less susceptible to the attack of pathogens. Such induction of greater defensive capacity can be systemic since the treatment of roots and seeds can trigger protective effects on the aerial fraction of the plants (Ongena et al. 2007).

Possibly the biosurfactants lipopeptides are capable of causing disorders in the plant plasma membrane and, as a consequence, they can stimulate a cascade of molecular events that are responsible for activating the defense mechanisms of the plant (Schellenberger et al. 2019). Biosurfactants such as surfactin, a Cyclic lipopeptides biosurfactant, on the other hand, act in the initiation of plant defenses to result in the triggering of systemic resistance (Debois et al. 2015; Ongena et al. 2007). Several studies prove the effectiveness of surfactin as an inducer of ISR (induced systemic resistance). Cawoy et al. (2014), when studying several strains of

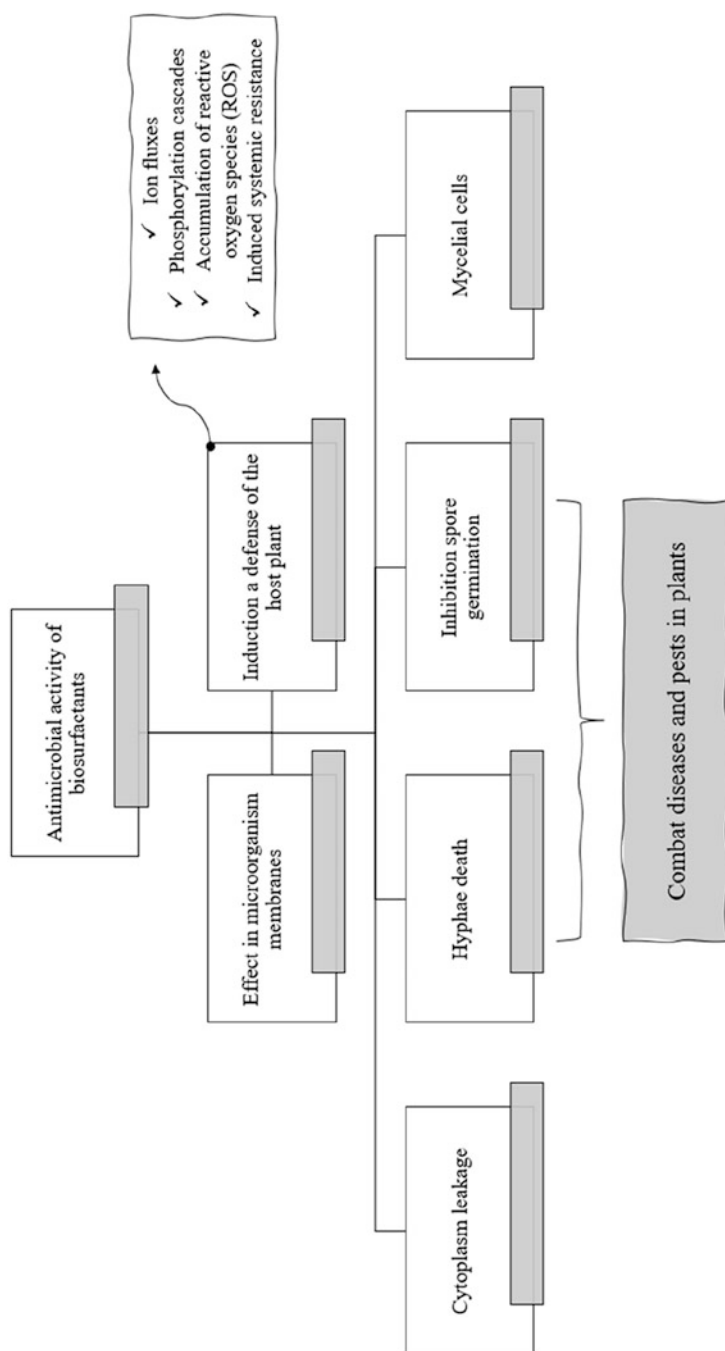


Fig. 2 Antimicrobial activity of biosurfactants and effects on the microorganisms

Bacillus sp., observed a strong correlation between the induction of defense mechanisms and how much surfactin the different strains can produce., enhancing the function of surfactin as an ISR inducer. In the work of Waewthongrak et al. (2014), surfacing from *Penicillium digitatum* stimulates defense responses that are responsible for producing signal molecules for ISR activation. The reduction of *Sclerotium rolfsii* disease was evidenced in *Arachis hypogaea* when pre-treated with the biosurfactant surfactin (Rodríguez et al. 2018).

Rhamnolipids biosurfactants, in the process of induction of defense genes, can stimulate genes of proteins related to pathogenesis and genes involved in the biosynthesis of oxylipins (Jasmonic acid, an important regulatory signaling molecule of various physiological processes in plants) and phytoalexins (antimicrobial molecule) (Varnier et al. 2009). Furthermore, the stimulation of plant defense by rhamnolipids can be done in another way, like phosphorylation cascade, calcium influx, and generation of reactive oxygen species (ROS). These responses are triggered by the presence of biosurfactant rhamnolipids that play a key role in the transduction of the vine plant defense signal (Monnier et al. 2018; Varnier et al. 2009).

Sanchez et al. (2012) proved that rhamnolipids biosurfactants from *Pseudomonas aeruginosa* produce an immune response in the Arabidopsis plant (*Arabidopsis thaliana*) due to the accumulation of signaling molecules, as well as the activation of the defense gene. In this study, the stimulation of the immune response by the biosurfactant participated in the resistance of the studied plant against the pathogenic microorganisms *Pseudomonas syringae* pv tomato, *Botrytis cinerea*, and *Hyaloperonospora arabidopsidis*. Resistance mediated by biosurfactants involves different signaling pathways, salicylic acid has a central role in resistance against all microorganisms mediated by the biosurfactant tested. For the microorganisms *H. arabidopsidis* and *P. syringae* pv tomato, ethylene is involved in the induced resistance, while for the fungus *B. cinerea* jasmonic acid is essential to evidence the resistance of the plant against this pathogen.

In the work of El-Sheshtawy et al. (2022), the toxic effects of heavy metals on the growth and quality of lettuce (*Lactuca sativa*) derived from seeds treated with biosurfactants from *B. megaterium* strain HHA was determined. Treatment of lettuce seeds with 100 mM of lead (Pb) and arsenic (Ar) significantly reduced the vegetative parameters. The biosurfactant from *B. megaterium* significantly increased the concentration of proline and enzymes with antioxidant activity, and a significant reduction in lipid peroxidation, H₂O₂, and O₂. Plants with a high concentration of proline react to the presence of heavy metals and act as protective cells, so in seeds treated with the biosurfactant, the plant's immune system was activated and the increase in proline may have worked with a hydroxyl radical scavenger and a singlet oxygen scavenger, reducing the adverse effects of Reactive Oxygen Species, and contributing to the plant growth.

4 Bioremediation Properties

One of the applications of biosurfactants that can affect seed protection and seedling fertility is the bioremediation of soils contaminated with heavy metals or also by the presence of hydrocarbons. The remediation method involves soil decontamination, employing a mechanical and/or chemical technique that applies liquids to extract pollutants from soils (Dermont et al. 2008). It is considered one of the most promising ecological solutions among those available for removing metals and hydrocarbon contaminants. Soil decontaminations with surfactants attempt to solubilize soil contaminants, which negatively interferes with the physical and chemical structure of soils, which limits the reuse (Hong et al. 2002), so the biosurfactant emerges as a removal strategy in the remediation process, removing only the contaminant from polluted soil and restoring soil health for potential future planting. Therefore, biosurfactants, when compared with synthetic surfactants, have great advantages for use in soils, seeds, and plants, due to their reduced toxicity, facilitated production, biodegradability, and possible reuse (Kilic et al. 2011).

Some studies show that trace metals have high compatibility with biosurfactants when compared to other compounds that are generally present in contaminated soil (Chakraborty and Das 2014; Juwarkar et al. 2007). The heavy metal desorption process in contaminated soils is aided by biosurfactants in different ways (Das et al. 2017). According to Le-Chatelier's principle, complexation of free metals in a solution can occur, reducing the activity of the metal's solution phase, which results in the promotion of desorption (Chakraborty and Das 2014). Another way biosurfactants manage to reduce interfacial stress is by aggregating at the solid–liquid interface (Miller 1995). In addition, biosurfactant micelles can also remove metal ions from the rhizosphere (Macías-Almazán et al. 2020). Therefore, the capacity of biosurfactants to associate with metals is essential for the bioremediation process of contaminated soils and contribute to soil fertility and seedling fertility.

In the work of Singh and Cameotra (2013) the biosurfactant produced by strain A21, identified as *B. subtilis* and isolated from the rhizosphere of *Parthenium hysterophorus*, was tested in a seed germination experiment, carried out through the cultivation of Indian mustard seeds (*Brassica juncea*) in two different soils, one metal contaminated soil and the other in the same soil after washing with the lipopeptide biosurfactant solution. Soil washed with biosurfactant showed total germination of mustard seed (100%), and this fact was not observed in the seeds present in the soil washed only with water, where the seeds did not germinate. Therefore, the biosurfactant used in this study was able to decontaminate the soil and contribute to the protection of the seed, through the process of removal of a considerable concentration of hydrocarbons and heavy metals from the soil.

Soil pollution by petroleum oil is a very common incident, and the presence of this hydrocarbon in the soil can induce high changes in physical and chemical characteristics, which results in an unfavorable effect on several events in plants. The constituents of petroleum contribute by reducing the number of nutrients, water, and oxygen available in the soil, resulting in reduced seed germination, therefore,

due to the presence of highly toxic hydrocarbons, the use of soil contaminated by petroleum oil is not recommended for agricultural practices (Nogueira et al. 2011).

Das and Kumar (2016) studied the effect on *W. somnifera* seeds prepared with biosurfactant produced by *Pseudomonas* sp. growing in soil contaminated with petroleum oil. As a result, these authors found that the percentages of seeds germination were influenced by the treatment of seeds with the biosurfactant. For the treated seeds, the germination percentage was about 81.33%, in a medium containing 10 ml of petroleum oil, on the other hand, this percentage was 68%, for seeds not treated at the same concentration of petroleum oil.

The effect of contamination with petroleum compounds in the soil is related to the unfavorable effect on the water content in the plant tissues, causing a water deficit in plants that grow in contaminated soil (Hawrot-Paw et al. 2015). In the case of seeds, occur changes in water–air relations in the soil, result in the appearance of an impermeable film with oil properties that surrounds the seeds, which interferes with proper germination (Adam and Duncan 2002; Hawrot-Paw et al. 2015; Ziółkowska and Wyszowski 2010).

5 Non-phytotoxicity of Biosurfactants

The existence of organic and inorganic toxic compounds in the soil negatively influences the growth of plant cells (Kaushik and Sharma 2012; Patil et al. 2009). Seed germination tests have been used to quantify the negative effect of these compounds on plants, as they have a low implementation cost and quick results (Tiquia et al. 1996). Several works have studied the toxicity of the biosurfactant in the process of seed germination, in addition to the possible changes that it may cause in other vital parameters of plant growth (Diniz Rufino et al. 2014; Luna et al. 2013). Normally, tests performed on plants usually study the effects on the germination process of seeds, root growth, vigor index, and seedling elongation (Fletcher 1991).

For the evaluation of the toxicity of biosurfactants referring to the different species of existing plants, the germination index (GI) is a method with high sensitivity, because it combines seed germination and seedling growth (embryo already developed but still closed in seed). A high germination index value is considered an indication of low toxicity (Tiquia et al. 1996) and is a clear answer to the protection of the seed using biosurfactants, wherein the application of biosurfactants in seeds, the higher the germination index, the greater the effective protection and increase in the fertility of this compound on different plant species. Table 1 lists studies on the effect of biosurfactants on seed germination and the initial development of some plant species.

Table 1 List of manuscripts, with respective information about species that produce the biosurfactant, their chemical nature, target species, and main effects

Manuscript	Authors	producing species	Biosurfactant	Target species	Benefit for the target species
Production and structural characterization of <i>Lactobacillus helveticus</i> -derived biosurfactant	Sharma et al. (2014)	<i>Lactobacillus helveticus</i> MRTL91	Glycolipid	<i>Brassica nigra</i> <i>Triticum aestivum</i>	Root elongation and the germination and vigor indices
Glycerol as a substrate for the production of biosurfactant by <i>Pseudomonas aeruginosa</i> UCP0992	Silva et al. (2010)	<i>P. aeruginosa</i> UCP 0992	Glycolipid	<i>Brassica oleracea</i>	The seed germination index and seed germination and root elongation
Analysis of biosurfactants produced by bacteria growing on textile sludge and their toxicity evaluation for environmental application	Singh et al. (2020)	<i>Stenotrophomonas</i> sp. BAB-6435 <i>Brevis bacillus</i> brevis BAB-6437	Glycolipid Lipopptide	–	Antimicrobial activity
Bioremediation of soil contaminated with crude oil: Evaluation of rhamnolipid addition for toxicity and biodegradation efficiency	Millioli et al. (2007)	–	Rhamnolipid	<i>Lactuca sativa</i>	Seed germination index
<i>Pseudomonas</i> sp. BUP6, a novel isolate from Malabari goat produces an efficient rhamnolipid-type biosurfactant	Priji et al. (2017)	<i>Pseudomonas</i> sp. BUP6	Rhamnolipid	Rice and green grass	Inhibition of the growth and adhesion of the pathogens <i>E. coli</i> and <i>S. aureus</i> increase in the germination rate
Characterization and optimization of a rhamnolipid from <i>Pseudomonas aeruginosa</i> C1501 with novel biosurfactant activities	Oluwaseun et al. (2017)	<i>Pseudomonas aeruginosa</i> C1501	Rhamnolipid	<i>Sorghum bicolor</i> <i>Solanum lycopersicum</i> <i>Triticum aestivum</i> <i>Vigna unguiculata</i> <i>Capsum annum</i>	Antimicrobial activity
Characterization, surface properties, and biological activity of a biosurfactant	Luna et al. (2013)	<i>Candida sphaerica</i> UCP 0995	Glycolipid	<i>Brassica oleracea</i>	Seed protection

produced from industrial waste by <i>Candida sphaerica</i> UCP0995 for application in the petroleum industry	Diniz Rufino et al. (2014)	<i>Candida lipolytica</i> UCP 0988	Lipopeptide	<i>Solanum gilo</i> <i>Lactuca sativa</i> <i>Brassica oleracea</i>	Seedling development
Characterization and properties of the biosurfactant produced by <i>Candida lipolytica</i> UCP 0988	Araújo et al. (2019)	<i>Serratia marcescens</i> UCP 1549	–	<i>Brassica oleracea</i>	Germination index and seedling development
Sustainable biosurfactant produced by <i>Serratia marcescens</i> UCP 1549 and its suitability for agricultural and marine bioremediation applications	Singh and Rathore (2019)	<i>Brevisbaccillus brevis</i> BAB-643	Lipopeptide	Wheat and pepper	Seed germination, biomass, specific leaf weight, chlorophyll content, soluble sugar, protein content, and ascorbic acid in the analyzed plants
Impact assessment of azulene and chromium on growth and metabolites of wheat and chili cultivars under biosurfactant augmentation	Santos et al. (2019)	<i>Streptomyces</i> sp. DPUA1566	Lipoprotein	<i>Lactuca sativa</i> <i>Brassica oleracea</i>	Germination index and root elongation
Production of a new lipoprotein biosurfactant by <i>Streptomyces</i> sp. DPUA1566 isolated from lichens collected in the Brazilian Amazon using agroindustry wastes					

6 Potent Plant Growth Promoter

Biosurfactant application can play an interesting role in the direct promotion of plant growth due to the increase in nutrient availability for them (Khare and Arora 2021). The effect of biosurfactants on plant growth has been studied by some authors, and studies show that biosurfactants can indirectly interfere with plant growth, by making hydrophobic compounds more bioavailable for use by soil-dwelling microorganisms (Khan et al. 2014; Marchut-Mikolajczyk et al. 2018; Saraf et al. 2014). A summary of the application of biosurfactants in promoting plant growth is shown in Fig. 3.

Studies prove that the addition of surfactants to the soil causes a considerable increase in the concentration of micronutrients available in the soil (Kumar et al. 2021). When studying the effect of rhamnolipid biosurfactant on plants, Stacey et al. (2008) inoculated bread wheat (*Triticum aestivum* L. cv. BDME10) and durum wheat seeds (*Triticum turgidum* L. durum cv. Balcali2000) in soils containing different concentrations of biosurfactants and a concentration of 2 mg/kg of Zinc (trace element necessary for plant growth). As a result of this study, the authors found that the use of 2 mg of rhamnolipid/kg of soil increased dry matter production of bread and durum wheat. In addition, with the application of 4 mg/kg of biosurfactant, Zn concentrations present in wheat shoots increased. Therefore, the biosurfactant rhamnolipid had a favorable effect on soil fertility and plant growth, facilitating the uptake or translocation of Zinc by the wheat plant.

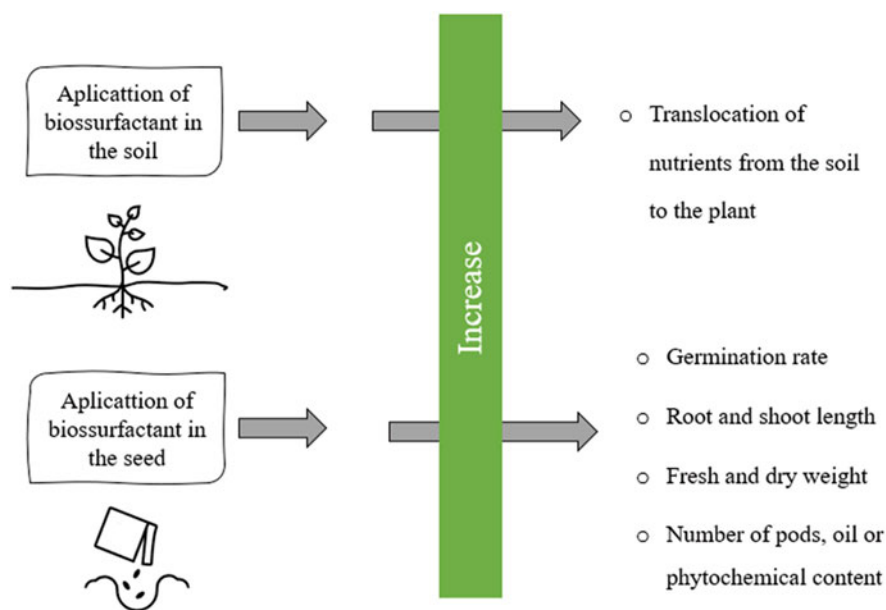


Fig. 3 Application of biosurfactants in promoting plant growth

Another study that evaluated plant growth-promoting by biosurfactants was carried out by Adnan et al. (2018), in which pepper seeds were treated with the biosurfactant produced by the endophytic fungus *Xylaria regalis*. In this work, the morphological characteristics of the plants (weight, root, and shoot length) and the chlorophyll, nitrogen, and phosphorus concentrations (vital nutrients for plants) were measured to indicate the ability of *X. regalis* biosurfactant to ensure the soil fertility and enhance plant growth. Seed treatment had the effect of increasing the length of the pepper seedlings, which resulted in easier plant growth. There was a significant improvement in the chlorophyll, nitrogen, and phosphorus contents when compared to control plants.

The Di-rhamnolipid biosurfactant produced by *Pseudomonas guariconensis* LE3 was studied by Khare and Arora (2021), the application of biosurfactant in seeds of sunflower variety “Swati” caused 174.73% enhancement of Root-Adhered Soil and Root Tissue, in addition, 40.51% increase in seed yield in comparison to control. Root-adhered soil is related to the formation of the environment where plants immediately start to absorb water and nutrients for growth (Vurukonda et al. 2016). Therefore, the application of di-rhamnolipid biosurfactant contributed to a better seed development environment, increasing fertility, and promoting plant growth.

In the work of Mishra et al. (2020), Varuna-T56 variety of *Brassica juncea* (L.) seeds were treated with a rhamnolipid biosurfactant produced by *Pseudomonas putida* BSP9. The application of the biosurfactant (2%), when compared to the control (without application), contributed to a better germination rate (69.4%), a greater length of different plant structures such as the root (74%) and shoot (66.6%), in relation to weight, there was also an increase in fresh mass (28.6%) and dry mass (44.3%), there was also a positive interference in the number of pods (50.1%), and by finally, there was an increase in the content of oil (18.6%), flavonoid (64%) and chlorophyll (71.7%). So, the application of biosurfactants in the seeds conferred added benefits to the plants.

The glycolipid biosurfactant produced by *Bacillus pumilus* 2A was tested in the work of Marchut-Mikołajczyk et al. (2021), on plant growth by applying 0.2% of a biosurfactant solution and comparing it with the control (without biosurfactant) on seeds *Phaseolus vulgaris* L. (beans), *Raphanus* L. (radish), *Beta vulgaris* L. (beet), the authors found a fourfold increase in the growth of bean and radish plants, and with the application of biosurfactant in beet seeds, the plant doubled in size when compared with the untreated control.

Lipopeptide biosurfactants from *Bacillus subtilis* SNW3 were tested in the work of Umar et al. (2021), on tomato (*Solanum Lycopersicum*), pea (*Pisum sativum*), chili pepper (*Capsicum annuum*), and lettuce (*Lactuca sativa*) seeds for plant morphological characteristics such as length shoot, root length, and dry mass, as well as the percentage of seed germination. The germination of all tested seeds was higher in treatments containing 0.7 mg/m of the tested biosurfactant, with emphasis on the increase in the percentage of germination for chili pepper seeds (139%). All plants that emerged from seeds treated with the lipopeptide biosurfactant showed higher biomass, root elongation, and increased root development.

The objective of the work by El-Sheshtawy et al. (2022), was to study the impact of biosurfactant lipopeptide produced by *B. megaterium* strain HHAM on the growth (leaves, root, length) of lettuce (*Lactuca sativa*). All vegetative parameters (leaves, root length) had a significant increase with the treatment of lettuce seeds with a high concentration of biosurfactant, so the treatment with biosurfactant contributed to the plant growth.

7 Synergistic Action of Biosurfactants

Biosurfactants by themselves have already presented numerous advantages that have already been discussed in this chapter, but many studies have been carried out when using biosurfactants together with other compounds, to obtain better results in bioremediation processes or the fight against pathogenic microorganisms (Joshi-Navare and Prabhune 2013; Rahman et al. 2002). Biosurfactants can offer a synergistic effect in the concomitant application of other compounds, which can lead to improved seed protection and fertility, either by increasing antimicrobial activity or soil health.

Takemoto et al. (2010) studied two compounds together: rhamnolipids and lipopeptinopeptide syringomycin E (SRE), and showed greater inhibitory activities of the mixture when compared to the application of SRE alone against fungal species associated with grapes at the germination stage *Aspergillus japonicus*, *Cladosporium cladosporioides*, *Curvularia brachyspora*, *Greeneria uvicola*, *Nigrospora sphaerica*, *Trichoderma* sp., *Penicillium sclerotiorum*, and *P. thomii*. The isolated biosurfactant was also tested, but it did not affect the conidia growth of these fungi. On the other hand, the exposure of the mixture between the biosurfactant and the SRE reached 50% death of conidia in the germination of all these tested fungal species, with a concentration three times lower than that tested with the SRE alone. Thus, the results demonstrate that the fungicidal properties of SRE became even more lethal with the mixture of the biosurfactant for a wide range of fungi that normally attack grape seeds.

In the work by Yan et al. (2015), biosurfactant rhamnolipids from *P. aeruginosa* ZJU-211 were tested against *A. alternata* of cherry tomatoes (*Lycopersicon esculentum*). The application of rhamnolipids alone was not effective in inhibiting *A. alternata* cherry tomato infections, but when combined with laurel essential oil, a significant reduction in the concentration necessary for antifungal activity, and better impact on the decrease of *A. alternata* in cherry tomatoes, when compared to the application of the essential oil alone. After 4 days of incubation, the combined treatment of biosurfactant and essential oil reduced the pathogen *A. alternata* to 43%.

Two metabolites with antimicrobial activity produced by *Pseudomonas aeruginosa* PNA1, rhamnolipids biosurfactants and phenazines, were studied in the work of Perneel et al. (2008) and were tested in the biological control of *Pythium splendens* on beans (*Phaseolus vulgaris* L) and *Pythium myriotylum* on cocoyam

(*Xanthosoma sagittifolium* L Schott). The authors used a *Pseudomonas aeruginosa* PNA1 mutant that did not produce rhamnolipids and another that was deficient in phenazine, and observed that when using the mutants separately, they did not obtain any suppressive effect of the disease despite each mutant producing an antimicrobial compound (rhamnolipids or phenazines). On the other hand, when the mutants were placed together in the soil in contact with the seeds, the biocontrol activity is the same as that found for the *Pseudomonas aeruginosa* wild-type strain, with the significant reduction of the increase of the mycelium of *P. myriotylum*, thus the synergistic effect in the control of *Pythium* spp. was observed for the use of biosurfactants and phenazines.

The use of biosurfactants and other compounds was used as methods to increase the biodegradation rates of hydrocarbons in soil contaminated with gasoline in the work of (Rahman et al. 2002). The study used the rhamnolipid biosurfactant produced by *Pseudomonas* sp. DS10–129 in synergy with organic residues poultry litter and coconut fiber (to make aeration easier and increase soil water retention) and also in the presence of bacterial consortium containing the strains—*Micrococcus* sp. GS2–22, *Flavobacterium* sp. DS5–73, *Bacillus* sp. DS6–86, *Pseudomonas* sp. DS10–129, and *Corynebacterium* sp. GS5–66. This entire mixture was tested for *Phaseolus aureus* RoxB growth parameters, including chlorophyll content, seed generation, shoot, and root length. The application of the bacterial consortium in the soil contributed to the degradation of About 78% of the hydrocarbons in 60 days, biosurfactants, and organic residues, at concentrations of 1%. In addition, by using the mixture of metabolites, the maximum percentage of seed germination, chlorophyll content, and important plant lengths, such as the root and shoot, were increased. The absence of biosurfactants resulted in a lower percentage for all parameters analyzed. Therefore, all tested additives had significant positive effects on the bioremediation process in the contaminated soil, seed germination, and growth of the plant.

In the work of Joshi-Navare and Prabhune (2013), biosurfactants from *Candida bombicola* ATCC 22214 were tested together with tetracycline to evaluate the effect against the pathogenic bacterium *Staphylococcus aureus* ATCC-29737, and also together with the antibiotic cefaclor against *E. coli* ATCC 8739. For *Staphylococcus aureus* strain, a total inhibition was observed before 4 hours of exposure with the application of the biosurfactant with tetracycline, whereas tetracycline alone could not completely inhibit the growth of the microorganism after 6 h. The inhibition caused by exposing the bacteria to the mixture was approximately 25% higher compared to the application of the biosurfactant alone. For the Gram-negative bacterium *E. coli*, the mixture of biosurfactant and cefaclor after 2 hours of inhibition approximately 48% more inhibition was observed compared to cefaclor alone. Biosurfactants can cross the bacterial cell membrane structurally like its structure, facilitating the entry of drug molecules, therefore it can increase the solubility of the antibiotic, which results in greater antimicrobial efficiency. In addition, biosurfactants have proven antimicrobial activity, so when administered together with an inhibitory agent, it reduces the likelihood of survival of the microorganism (Joshi-Navare and Prabhune 2013).

8 Conclusion

We observed that the effect of biosurfactants on improving seed protection and seedling fertility are varied, biosurfactants can contribute to the protection of the seed by the antimicrobial activity against several phytopathogens, or by the bioremediation of contaminated soils with heavy metals and hydrocarbons to ensure the seed germinates in healthy soil.

In addition, studies show that biosurfactants contribute to seed by inducing the innate immunity of the plant, increasing resistance to diseases caused by phytopathogens, as they do not present phytotoxicity. This contributes, therefore, to better growth and development of the plants, favoring the increase of germination of seeds, growth, and elongation of roots, fresh and dry mass, and growth of seedlings, among other factors, guaranteeing the highest yield of healthy plants, without the presence of toxic compounds that can be harmful to human or animal health.

Therefore, biosurfactants are promising molecules to be used as green pesticides, which can be used alone or in synergy with other compounds, offering interesting perspectives for the future sustainability of commercial agricultural products that guarantee seed protection and fertility, and contribute to the reduction of agricultural losses. Thus, simultaneous studies of antimicrobial and biostimulant properties should continue to be investigated, for future explorations to increase seed protection and fertility in the near future.

9 Companies, Organizations, and Research Groups Working on the Topic

<https://www.allied-c-s-usa.com/>

<https://www.dispersa.ca/>

<https://www.ulster.ac.uk/staff/im-banat>

<https://www.unilever.com/news/news-search/2022/building-a-clean-green-foamproduction-machine/>

<https://scholar.google.com/citations?user=2kQzN7kAAAAJ&hl=pt-BR&oi=sra>

<https://scholar.google.com/citations?user=k1OF-vAAAAAJ&hl=pt-BR&oi=sra>

<https://www.teegene.co.uk/>

<https://corporate.evonik.com/misc/micro/biosurfactants/index.en.html>

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Role of Biosurfactants in Marine Sediment Remediation of Organic Pollutants



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1 Introduction

Petroleum crude oil and its hydrocarbons constitute a significant concern among the numerous environmental organic pollutants that adversely impact marine ecosystems and their function (Jamal 2022; Wang et al. 2022; Wei et al. 2020). Oil spills in the marine environment often lead to immediate and long-term ecological damage. In several crude oil-producing regions of the world, marine sediments have been significantly contaminated with organic pollutants with a total petroleum hydrocarbon concentration (TPH) of 44,600 mg per kg of dry soil (Feng et al. 2021). The sources of organic hydrocarbon pollutants in the marine environment include industrial zones, commercial ports, touristic cities, aquacultural/agricultural practices, oil/gas exploitation, megacities, and other anthropogenic activities (Dai et al. 2022; Kumar et al. 2021). When crude oil is spilled into the marine environment, it persists in the sediment, enters the marine food web, and exerts detrimental effects on humans and other organisms (Biswas et al. 2019; Gayathiri et al. 2022; Mgbechidinma et al. 2022a; Shuai et al. 2019). These oils consist of aliphatic and aromatic compounds that greatly hinder remediation technology while negatively impacting the surrounding environment due to their toxicity, complexity, persistence, bioaccumulation tendency, and susceptibility to long-range atmospheric transport.

Although many studies have documented different treatment processes for cleaning up organic pollutants in the environment, biological methods using microbes and plants remain a promising alternative green method (Dai et al. 2022; Kariyawasam et al. 2022; Kumar et al. 2021; Lal et al. 2018; Nayak et al. 2020;

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Sonowal et al. 2022). Biological remediation of oil-contaminated environments is highly recommended and widely practiced because of its efficiency, cost-effectiveness, and environmentally friendly nature than the mechanical or chemical methods (da Silva et al. 2021; Muneeswari et al. 2022; Pete et al. 2021). While developmental approaches to biological remediation are growing, the major strategies include phytoremediation (using plants), bioremediation (using nutrients—bio-stimulation and using microbes—bio-augmentation), and bio-electroremediation (Fdez-Sanromán et al. 2021; Laothamteep et al. 2022; Mapelli et al. 2017). Recent studies on crude oil biological remediation focus on constructing effective microbial consortiums (mono and mixed cultures), inoculating co-metabolic substrates, re-inoculating contaminated sites with indigenous microorganisms, and genetic manipulation of microbes/plants (Feng et al. 2021; Gayathiri et al. 2022; Yan et al. 2020).

Despite the advances in improving crude oil biological cleanup methods, the degradation of petroleum hydrocarbon is limited mainly by the bioavailability and toxicity of the pollutants, the spatial distribution of microbes/plants, and their metabolic capability (Huang et al. 2020; Jamal 2022; Zhou et al. 2021). In marine sediments, the bioavailability mechanism of hydrocarbon pollutants involves desorption from the soil matrix, transport, and uptake/absorption by plants or microorganisms (Feng et al. 2021). Also, hydrocarbon moieties of crude oil are highly hydrophobic, leading to their absorption unto sediments, thereby limiting the pollutant mass transfer rate. Thus, most hydrocarbon pollutants in marine sediments are not readily available to the plant or microbes as nutrient sources, hindering their degradation. The primary factor limiting crude oil bioremediation in contaminated sediments is the slow desorption of these hydrocarbons from the solid phase (sediment) to the aqueous phase, causing low bio-accessibility (Dai et al. 2022; Feng et al. 2021; Guo et al. 2022; Kumar et al. 2021). In an attempt to advert these limitations in the biological remediation of organic pollutants in the marine environment, advances have been made toward incorporating surfactants to lower the surface tension between the solid and aqueous phases (Dhanya 2021; Hentati et al. 2021).

Surfactant-enhanced bioremediation is a promising technique for improving the bio-accessibility of organic hydrocarbon pollutants (da Silva et al. 2021; Gidudu and Chirwa 2021; Liduino et al. 2018). However, as surfactants increase organic pollutants desorption and free transport into the aqueous phase to intensify remediation, petroleum-derived surfactants are not eco-friendly because of their high toxicity and low biodegradability. Hence the increasing interest in biosurfactants as a green, non-toxic alternative to their chemical counterpart. Biosurfactants are active surface secondary metabolites synthesized by microbes that can utilize substrates like simple sugars and oils as nutrient sources (Durval et al. 2020; Femina et al. 2021). They have different structures and are amphiphilic compounds with polar and nonpolar moieties. These secondary metabolites are grouped based on the type of producer, the substrate used, and their chemical composition as low molecular weight (glycolipids, phospholipids, lipopeptides, fatty acids) and high molecular weight (polysaccharide–protein complexes) biosurfactants (Sarubbo et al. 2022). Following

a systematic approach, published articles that report the application of biosurfactants in sediment remediation were reviewed and quantitatively evaluated using comprehensive databases such as Web of Science, Scopus, Google scholar, and Pub Med between 2017 and May 2022 (Fig. 1).

Figure 1 shows the rise in research incorporating biosurfactants in sediment remediation to remove and degrade pollutants. However, biosurfactants have a wide range of applications in several industries, including food, cosmetics, agricultural, and pharmaceutical (Ashitha et al. 2020; da Silva et al. 2021; Pandey et al. 2022; Xu et al. 2020). The dramatic increase in biosurfactant application in the marine environment can be attributed to its numerous environmental compatibility properties. These include reducing surface and interface tension, emulsion formation, foaming capability, oil displacement, biodegradability, nontoxic, and stability over varying environmental conditions like temperature, pH, and salinity (Dell'Anno et al. 2018; Gidudu and Chirwa 2021; Ram et al. 2019; Wei et al. 2020). These properties make biosurfactants an environmentally compatible biomolecule of the twenty-first century. Biosurfactants are produced within cells or secreted extracellularly to form thin films for cellular communications that regulate several physiological activities (Sharma et al. 2021). Many recent studies have reported successes in applying biosurfactant producer as remediation agents. However, there are advances in applying biosurfactants during pollutant remediation by employing nanotechnology, immobilization, dose-supplementation, and direct use of crude extracts (Mandal et al. 2018; Rong et al. 2021).

Following the unpredictable flow of organic pollutants such as hydrocarbons (aliphatic and aromatic), pesticides, and chlorinated solvents in the marine environment and their hazardous impact on the exposed populations, ecosystem productivity, and global economic growth, this chapter is focused on revealing the role of biosurfactants in the marine sediment remediation of organic hydrocarbon pollutants. The sediment remediation trend in biosurfactant application, as shown in Fig. 1, also emphasizes the scope of this chapter. The environmental compatibility properties of biosurfactants and the factors that influence their production are discussed. Considering the anoxic conditions of the marine environment, we reviewed biosurfactant production under aerobic/anaerobic conditions. Moreover, application strategies of biosurfactants and the interaction mechanism underlying biosurfactant–pollutants' complexation during remediation are discussed. We further revealed recent advances in biosurfactant-mediated remediations while providing future outlooks for developing efficient and eco-sustainable biosurfactant-based strategies in marine sediments remediation in view of large-scale applications. This chapter will provide relevant insight into the possible achievement of environmental sustainability of marine sediments in beaches, wetlands, marshes, offshores, and intertidal zones.

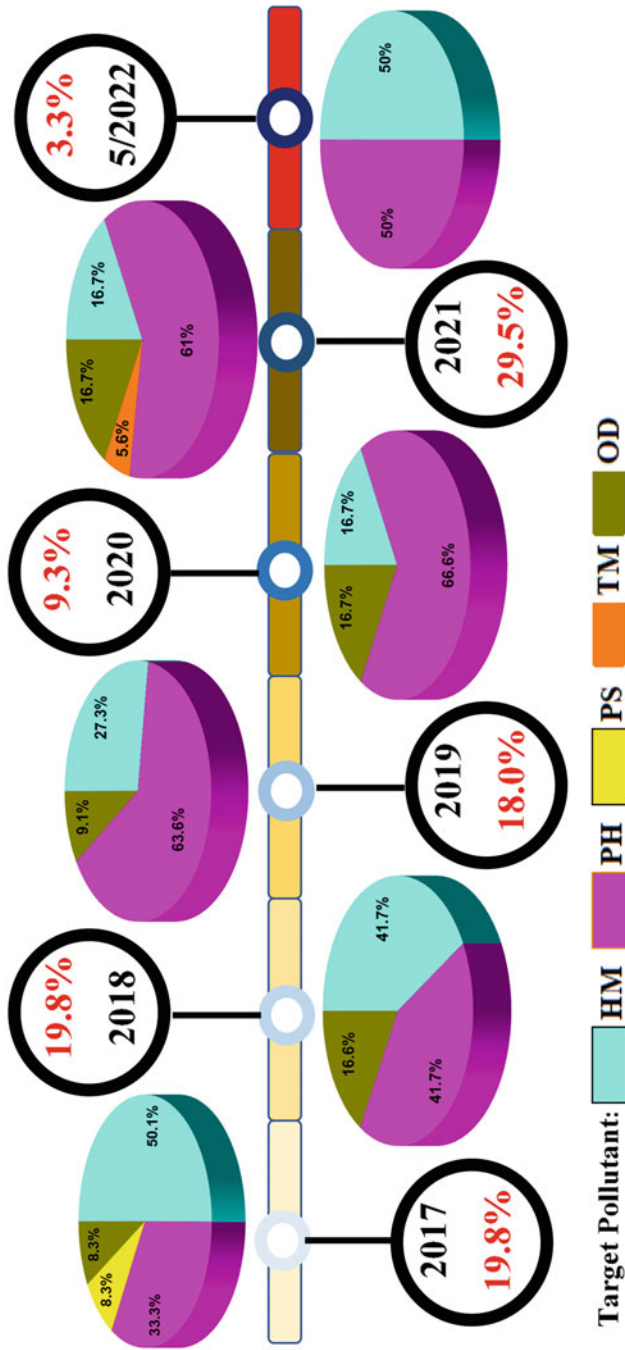


Fig. 1 The systematic trend in published articles on biosurfactant application in sediment remediation as indexed in several databases between 2017 and May 2022. The target pollutants observed were heavy metals (HM), petroleum hydrocarbons (PH), pesticides (PS), transition metals (TM), and others (OD). Others represent combined pollutants. This process was performed using limited searching terms: biosurfactant AND sediment AND remediation. The outcome measurement technique was restricted to specific criteria focusing on the title, abstract, and keywords. Duplicate search results were removed, and studies on petroleum surfactants and aqueous environmental remediation were removed

2 Biosurfactants: Production, Environmental Influence, and Remediation Properties

Intrinsic and extrinsic factors involved in biosurfactant production affect their activity. The effectiveness of these factors is commonly evaluated under conditions of different oxygen levels, temperatures, pH, salinity, and microorganism used.

2.1 Biosurfactants and Their Main Microbial Producers

Biosurfactants are amphiphilic molecules presenting hydrophobic features (consisting of saturated and unsaturated long-chain fatty acids, hydroxy-fatty acids, or α -alkyl- β -hydroxyl fatty acids or amphiphilic/hydrophobic peptides) and hydrophilic features (composed of anionic or cationic amino acids, peptides, mono-saccharides, disaccharides, polysaccharides, phosphate, carboxylic acid, or alcohol). Biosurfactants are mainly anionic or neutral, but some cationic forms have amine groups. They are classified based on their chemical composition, molecular weight, physicochemical properties, mode of action, and microbial origin into several classes like simple fatty acids, glycolipids, lipopeptides, lipopolysaccharides, phospholipids, polymeric and particulate compounds (Ashitha et al. 2020; Gayathiri et al. 2022).

Several biosurfactants produced by different microbial taxa have been isolated from marine environments and mostly reported are rhamnolipid, cellobiolipids, trehalose lipids, sophorolipids, mannosylerythriol lipids, and surfactin from microbes in genera *Pseudomonas*, *Bacillus*, *Actinobacteria*, *Ustilago*, *Rhodococcus*, *Arthrobacter*, *Candida*, and *Pseudozyma* (Gayathiri et al. 2022). Biosurfactants regulate quorum sensing and significant microbial roles like motility, antagonism, and virulence (Sharma et al. 2021). These roles form the basis of most biosurfactant interactions with microbes in terrestrial and aquatic ecosystems during pollutant degradation (Dhanya 2021; Wei et al. 2020). Table 1 shows microbial biosurfactant producers and their fermentation conditions.

2.2 Environmental Factors That Influence Biosurfactant Production

At the late exponential and stationary growth phase of microbes during fermentation, several environmental factors significantly influence metabolite production (surfactants) (Uddin et al. 2021). These factors account for the differences in biosurfactant composition, structure, and properties that affect their applicability (Filho et al. 2021). Some of the intrinsic factors include the nutrient source and the fermentation mode. Most literature now reports on the use of renewable waste as substrates for

Table 1 Biosurfactant producers and their relevant environmental conditions

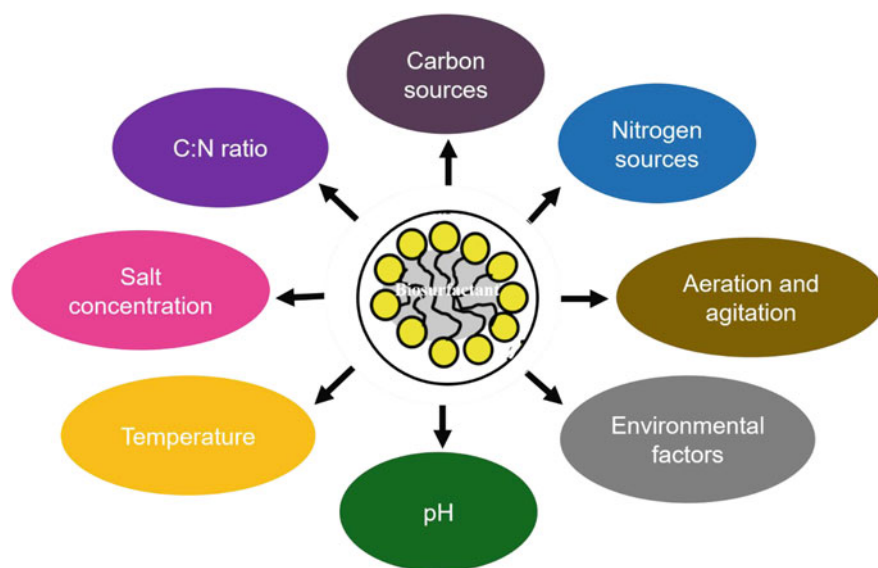
Biosurfactant producers	Isolation site	Fermentation conditions	Produced biosurfactant (Yield)	References
<i>P. aeruginosa</i> CH1	Zhoushan island, China	Anaerobic cultivation in 200 mL medium (containing 0.5 mg/L resazurin) in 250 mL serum bottle aerated with oxygen-free N ₂ gas at 30 °C, 180 rpm for 5 d	Rhamnolipid	Jiang et al. (2022)
<i>Pseudomonas mendocina</i> ADY2b	Chennai harbor	Aerobic, 28 °C, pH 7.2 at 150 rpm	Rhamnolipid	Balakrishnan et al. (2022)
<i>Enterobacter hormaechei</i>	Tamil Nadu, India	Aerobic, 35 °C, 150 rpm for 10 d	Lipopeptide	Muneeswari et al. (2022)
<i>Aeromonas hydrophila</i> RP1	Himachal Pradesh, India	Aerobic, 27 °C, pH 7 for 5 d	Glycolipopeptide	Pandey et al. (2022)
<i>B. subtilis</i> AnPL-1	Xinjiang, China	Anaerobic cultivation in 100 ml serum bottles containing 80 mL medium at 39 °C, 80 rpm for 10 d	Surfactin	Zhao et al. (2021)
<i>Vibrio sp.</i> LQ2	The South China Sea	Aerobic, 30 °C, pH 7 at 180 rpm	Phospholipid	Zhou et al. (2021)
<i>B. subtilis</i> AS2, <i>B. licheniformis</i> AS3 and <i>B. velezensis</i> AS4	Tamil Nadu, India	Aerobic, 40 °C, pH 7 at 150 rpm	Lipopeptide	Prakash et al. (2021)
<i>Staphylococcus sp.</i> CO100	Sfax, Tunisia, Mediterranean Sea	Aerobic, 37 °C, pH 7.6, 100 g/L NaCl at 180 rpm	Lipopeptide	Hentati et al. (2021)
<i>P. aeruginosa</i> ASW-4	Zhoushan island, China	Aerobic, 25 °C, pH 7 at 150 rpm	Rhamnolipid	Chen et al. (2021)
<i>P. cepacia</i> CCT 6659	São Paulo state, Brazil	Aerobic, 28 °C, pH 7, 250 rpm for 60 h	Rhamnolipid	da Silva et al. (2021)
<i>Bacillus cereus</i> UCP 1615	Pernambuco, Brazil	Aerobic, 28 °C, pH 7 at 200 rpm	Lipopeptide (4.6 g/L)	Durval et al. (2020)
<i>P. aeruginosa</i> CH1	Zhoushan island, China	Aerobic, 30 °C at 180 rpm for 8 d	Rhamnolipid	Huang et al. (2020)
<i>B. licheniformis</i> LRK1	Bhavnagar, India	Aerobic, 35 °C, pH 7, 3% salt concentration at 150 rpm	Lipopeptide	Nayak et al. (2020)
<i>Paracoccus sp.</i> MJ9	Jiaozhou Bay, China	Aerobic, 30 °C, pH 7.2 at 130 rpm	Rhamnolipid	Xu et al. (2020)
<i>Acinetobacter sp.</i> Y2	Xinjiang Uygur, China	Aerobic, 30 °C, pH 6.5–7.0 for 3 d	Lipopeptide	Zhou et al. (2020)

(continued)

Table 1 (continued)

Biosurfactant producers	Isolation site	Fermentation conditions	Produced biosurfactant (Yield)	References
<i>Bacillus</i> sp. SGD-AC-13	Chorao Island, Goa, India	Aerobic, 30 °C, pH 7.6 at 150 rpm	Novel thermostable biosurfactant with fatty alkene	Ram et al. (2019)
<i>P. aeruginosa</i> 709	Xinjiang oil reservoir, China.	Anaerobic cultivation in 250 mL serum bottles sealed with butyl rubber stoppers at 39 °C for 10-d.	ND (422.8 ± 16.23 mg/L)	Zhao et al. (2017)
<i>P. stutzeri</i> DQ1	Heilongjiang Province, China	Anaerobic fermentation medium-boiled under a stream of oxygen-free nitrogen and incubated at 40 °C, pH 7.2 for 36 h.	Lipopeptide	Liang et al. (2017)

ND not detected

**Fig. 2** Factors that influence biosurfactant production

biosurfactant production, mainly agro-industrial wastes (molasses, fruit peels, wheat straw, rice straw, cassava flour, and sugarcane bagasse), animal oil/fats (fish waste, fish peptones, and crude fish oil), dairy/distillery by-products, petroleum refining wastes (marine leachates), and food processing by-products (Das and Kumar 2018; Femina et al. 2021; Gaur et al. 2022; Mgbechidinma et al. 2022b). The major factors affecting biosurfactant production are shown in Fig. 2.

Biosurfactant production relies on the feeding methods employed in a shake flask or bioreactor fermentation. There are three modes of fermentation commonly used in microbial surfactant research. In the batch mode, the media and inoculum are added simultaneously to the bioreactor, and the product is recovered at the end of the fermentation process (Sarubbo et al. 2022). Fed-batch mode entails adding new media regularly without removing the product. In contrast, the continuous mode is run through unceasing substrate streaming and product collection once the maximum product concentration is reached (Gayathiri et al. 2022). During fermentation, biosurfactant producers grow and function in a wide range of environmental conditions such as pH, temperature, salinity, and oxygen availability (aeration and agitation speed).

According to Gayathiri et al. (2022), some ecosystem-dependent biosurfactants include trehalose lipids in cold environments, rhamnolipids in thermophilic environments, lipoproteins in acidophilic/alkaliphilic environments, and glycolipids in saline/hypersaline environments. These environmental factors form the basis for determining the fermentation parameters used during biosurfactant production. Statistical methods can optimize these parameters to investigate their variable interactions and ensure maximum biosurfactant yield at the lowest possible costs (Christopher et al. 2021; Mandal et al. 2018; Mgbekchinma et al. 2022a; Muneeswari et al. 2022; Uddin et al. 2021; Vaishnavi et al. 2021). The commonly reported fermentation conditions are pH 6–8, temperature 28–37 °C, NaCl concentration up to 10%, and aeration modulation at 150–200 rpm agitation speed (Dierickx et al. 2022; Gaur et al. 2022). Although agitation and aeration cause foam formation during biosurfactant production (Domingues et al. 2017), the presence or absence of oxygen transfer remains essential. Although previous studies on biosurfactant production have emphasized the effect of varying temperature, pH, and salinity as relevant environmental conditions, oxygen availability is addressed in this section, considering the anoxic nature of marine sediment below the surface.

2.2.1 Aerobic Biosurfactant Production

Oxygen availability in microbial cultures implies an aerobic condition, whereby molecular oxygen (>30%) is the electron acceptor and limiting factor (Domingues et al. 2017). In field experiments, bulking agents are used to save costs; however, most laboratory studies on biosurfactant production are conducted in aerated bioreactors equipped with agitators. As a result, aerobic biosurfactant production is more widely explored (Balakrishnan et al. 2022; Chen et al. 2021; Hentati et al. 2021; Muneeswari et al. 2022; Pandey et al. 2022; Prakash et al. 2021; Zhou et al. 2021). The presence or absence of oxygen affects genes that regulate biosurfactant production. Under aerobic or anaerobic conditions, the *rhl* genes expression in *P. aeruginosa* is altered, leading to rhamnolipid production with different yields, homologs, structural composition, and physicochemical properties (Jiang et al. 2022; Zhao et al. 2021).

2.2.2 Anaerobic Biosurfactant Production

The surface marine sediment might allow the aerobic production of biosurfactants by microbes; however, locations in the deep underground areas of the marine environment are anaerobic with high pressure and high salinity (Liang et al. 2017; Zhao et al. 2017). Studies show that aerobic biosurfactants-producing microbes exhibit weaker metabolic activity and lower oil displacement efficiency in anaerobic marine environments (Zhao et al. 2018). The unique biosphere of marine sediments modifies the microbial communities to adapt to diverse metabolic functions using nitrate, iron, bicarbonate, nitrous oxide, and sulfate as electron acceptors. Although some anaerobic biosurfactant syntheses are nutrient dependent, there are currently only a few microbes capable of such processes. According to Zhao et al. (2021), *Bacillus subtilis* AnPL-1 anaerobically produces surfactin (150 mg/L) with emulsification and viscosity reduction effects on crude oil at 20–50 °C, 6–9 pH, and 0–7% of NaCl. The surfactin had a mixture of C13-, C14-, and C15-surfactin congeners with 28.5 mN/m ST, 30 mg/L CMC, 70.5% emulsification index, and 40.6% viscosity reduction against crude oil. Jiang et al. (2022) also revealed that *Pseudomonas* sp. CH1 anaerobically produces rhamnolipids with lower CMC (40 mg/L) than 100 mg/L in an aerobic condition. The biosurfactant from CH1 had six homologs with 87.83% mono-rhamnolipids capable of enhancing PAHs solubilization in water from 1.29 mg/L to 193.14 mg/L with over 90% viscosity reduction.

2.3 Characteristic Remediation Properties of Biosurfactant

Although biosurfactants are ecologically safe, they can self-assemble and form micelles that define their morphological structures and specificity, like synthetic surfactants. The three main micelles forms are spherical, rod-like, and wormlike micelles (Fig. 3).

The self-assembly and micelle formation account for several favorable biosurfactant properties that can be affected by changes in the congener molecular structure (Sarubbo et al. 2022). These properties are the basis for the observable physiochemical methods for developing several rapid techniques for isolating and screening biosurfactant-producing microbes.

2.3.1 Microbial Cellular Communication

Biosurfactants mediate a myriad of cellular communication in microorganisms while allowing for physiological processes such as motility, antagonism, virulence, quorum sensing (detect and modulate cell population density), and biofilm formation/dispersion (Sharma et al. 2021). These cellular communication features can be

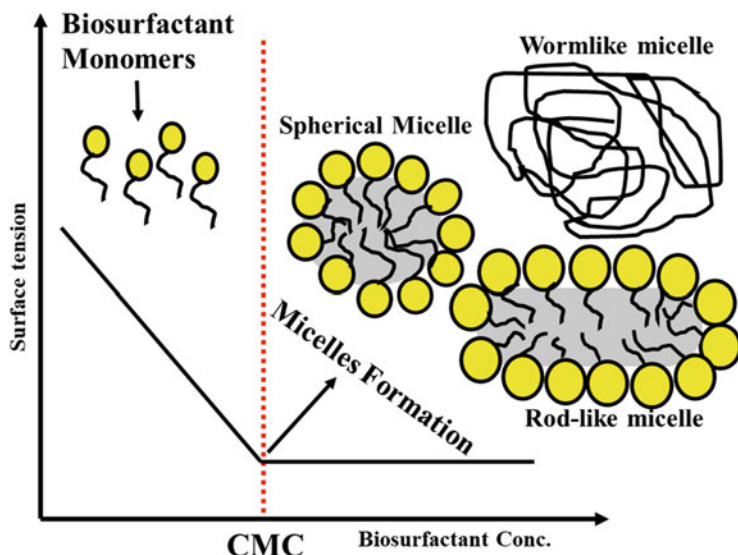


Fig. 3 Biosurfactant micelle structures

explored as an alternative approach for sustainable and economic biosurfactant production.

2.3.2 Surface and Interfacial Tension Reduction

Biosurfactants are known to reduce surface tension (ST) of water (72 mN/m) and interfacial tension (IT) between oil/water interfaces (10–40 times) more than synthetic surfactants owing to their lower CMC values (Femina et al. 2021). This implies that less biosurfactant concentration is required for maximum ST/IT reduction than synthetic surfactants. The IT measures the cohesive energy present at the interface between liquid and liquid or gas and liquid. However, ST and IT are determined similarly in mN/m units by measuring the fermentation liquids or purified biosurfactant extracts using capillary rise, Du Nouy, Wilhelmy plate, and release/drop-weight methods. The principles of these techniques include measuring (i) the counterbalance gravity force and weight of the liquid in the Capillary rise method (Das and Kumar 2018), (ii) the force required to remove a platinum-iridium ring placed on a surface or interface by the Du Nouy method (Balan et al. 2019; Gayathiri et al. 2022; Hentati et al. 2019), (iii) the direct force imposed on a platinum plate at the interface using Wilhelmy plate method (Christopher et al. 2021; Lee et al. 2018; Pandey et al. 2022; Zhao et al. 2018), (iv) the force required to remove a wire ring from a liquid surface (release method) and the droplet weight from a pipe (drop-weight method) (Gidudu and Chirwa 2021; Ram et al. 2019; Uddin et al. 2021).

Corresponding to the decrease in ST and IT, most biosurfactants have CMC values less than 2000 mg/L depending on their molecular structure.

2.3.3 Enhanced Solubilization, Mass Transfer, and Bioavailability

Biosurfactants can increase the solubility of hydrophobic organic compounds, thereby enhancing their mass transfer and bioavailability by reducing ST at the interfacial phase (Femina et al. 2021). This leads to emulsions that dispense solute inside the core of hydrophobic micelles, thus altering the CMC, size, and shape of the biosurfactant. Moreover, biosurfactant solubilization is based on ST reduction and micelle formation, which modifies the hydrophobicity of the cell surface and increases cell-substrate affinity (Zhou et al. 2021). At standard micelle formation, a most compatible phase with the hydrophobic pollutant solubilization, the biosurfactant hydrophobic ends are connected inside. In contrast, the hydrophilic ends are connected to the aqueous phase (Xu et al. 2020). However, the hydrocarbon concentration of any organic pollutant is the limiting factor for effective solubilization (Femina et al. 2021).

2.3.4 Environmental Tolerance and Ionic Strength

The surface activity of most biosurfactants is unaffected by environmental conditions like pH (3–12), temperature (up to 120 °C), and salt concentration (up to 10% w/v). Meanwhile, synthetic surfactants are inactivated at extreme pH, temperature, and salt concentrations greater than 2% (Sarubbo et al. 2022). Therefore, the biosurfactant produced by microorganisms has high foaming and emulsifying activities with stability at extreme temperatures, pH, and salt concentrations (Kumar et al. 2021).

2.3.5 Biodegradability and Low Toxicity

Biosurfactants are not persistent molecules, and their intermittent or end-products are not hazardous. They are highly eco-friendly and safe compared to synthetic surfactants, with a minimal report on their harmful effects when used without purification. Although sucrose-stearate, a synthetic surfactant, has an identical homolog to microbially produced glycolipid, the latter has faster degradability (Gayathiri et al. 2022). Assays that are commonly used to determine biosurfactant toxicity include cytotoxicity activity (using cell lines or animal samples) (Balan et al. 2019; Dierickx et al. 2022) and phytotoxicity activity (using seed or plant samples) (Wei et al. 2020). Biosurfactants are of considerable interest in the food, cosmetic, and pharmaceutical industries, emphasizing the importance of using safe producers like lactic acid bacteria due to their detoxifying and antimicrobial properties (Ashitha et al. 2020; Mgbechinma et al. 2020). In addition, biosurfactants in

hydrocarbon-contaminated environments enhance biodegradability by increasing pollutant solubilization (Feng et al. 2021; Xu et al. 2020).

2.3.6 Emulsion Forming and Breaking

The emulsifying potential of biosurfactants is independent of their ST reduction capability. Although high molecular mass biosurfactants are excellent emulsifiers, not all bio-emulsifiers can significantly reduce surface/interfacial tension. Emulsifiers (polymeric biosurfactants) are in high market demand in the food, cosmetics, and pharmaceutical industries. Also, biosurfactants are used for the demulsification of industrial waste emulsions. While a common mechanism of biosurfactant emulsion formation is polymerization, the breaking mechanism occurs through creaming, flocculation, coagulation, and coalescence (Femina et al. 2021).

3 The Fate of Organic Pollutants and Biosurfactant Mechanism of Action

Marine pollution with petroleum hydrocarbons has detrimental effects on the ecosystems and possible economic resources (Pete et al. 2021). Crude oil is one of the major globally needed natural resources, although only produced significantly by countries like Russia, Iran, Qatar, the United States, Turkmenistan, Saudi Arabia, China, United Arab Emirate, Nigeria, and Venezuela, following the recent report by the US Energy Information Administration (<https://www.eia.gov/>). As such, the global distribution of oil in line with meeting the high market demand results in crude oil spills and seepage, thus, impacting the lives of the terrestrial and aquatic communities (Pete et al. 2021). Significantly, the marine sediments are the vulnerable sink for these crude oil hydrocarbons consisting of heavy metals, volatile organic compounds (VOCs), aliphatic hydrocarbons, acidic aerosols, hydrogen sulfide, PAHs, and particulates (Gayathiri et al. 2022; Mishra et al. 2021; Wei et al. 2020).

When crude oil reaches the surface, the composition and properties of the oil change almost immediately by processes like evaporation, oxidation, emulsification, sedimentation, biodegradation, and dispersion (Fig. 4). The presence of biosurfactant increases hydrophobicity that improves the interaction of the surface-active agents with the pollutant leading to desorption (Ram et al. 2019). Pollutant solubilization is affected by the charge of the hydrophilic group and the chain lengths that determine the micelles' orientation (Dierickx et al. 2022).

In contaminated marine sediments, the hydrophobic moiety entraps the pollutant hydrocarbon, thereby increasing its adsorption by microbes. Bioremediation efficiency depends on pollutant bioavailability, microbial growth, and degradation capacity. The mechanism of action of biosurfactants in marine sediment remediation

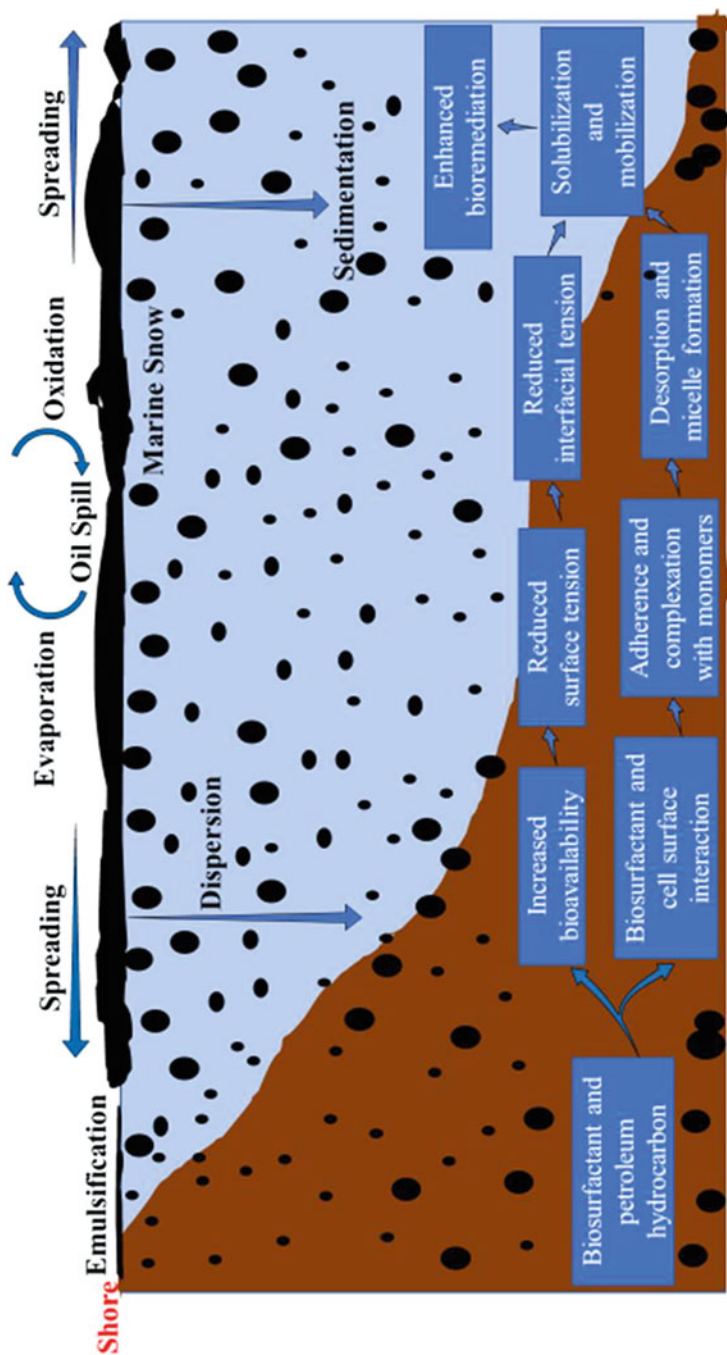


Fig. 4 Biosurfactant-mediated bioremediation. The fate of organic pollutants includes processes in the upper part of the figure, such as spreading, evaporation, oxidation, dispersion, and sedimentation. On the other hand, biosurfactant mechanisms of action are processes in the lower part of the figure, including bioavailability, surface tension, interfacial tension, hydrophobicity, micelle formation, solubilization, and mobilization

of organic pollutants is shown in Fig. 4. The mechanism of hydrocarbon removal by biosurfactant to increase substrate bioavailability for microorganisms and increases cell hydrophobicity can be described based on their molecular mass and concentration. Low molecular mass biosurfactant below CMC allows for mobilization by reducing the surface and interfacial tension between air/water and soil/water systems. Thus, the contact angle increases as the capillary force reduce. Meanwhile, solubilization is favored above the CMC for low molecular mass biosurfactant through the micelle formation with the hydrophobic ends connected inward.

In contrast, the hydrophilic ends are exposed to the exterior aqueous phase (Christopher et al. 2021). Also, a high molecular mass biosurfactant enhances emulsification, a process that forms emulsion (droplet of oil suspended in a fluid).

Interestingly, microbial surface-active agents solubilize crude petroleum oil in aqueous and solid media. Using biosurfactants from marine bacteria (*Bacillus licheniformis* MTCC 5514 producing surfactin) in comparison to synthetic surfactants, Kavitha et al. (2014) described the solubilization of crude oil in soil matrix of different types (sandy, fine sand soil, clay, and clay loam). It was observed that complete solubilization could be achieved at 2% concentration of crude oil, with the biosurfactant having >25% removal rate than the synthetic surfactants. Compared to other soil types, clay absorbs more crude oil, accounting for its least solubilization efficiency (Kavitha et al. 2014). In a dose-dependent manner, as reported by Saimmai et al. (2013), biosurfactant solubilization of PAHs such as anthracene, fluoranthene, or pyrene (15–20 times higher compared to control) is higher compared to fluorene, naphthalene, or phenanthrene (about 3–5 times compared to control). Biosurfactants increase petroleum hydrocarbon solubilization by multiple folds compared to water (Feng et al. 2021), with efficiency ranging from fivefold solubility (for cell-free supernatant containing the biosurfactant), threefold solubility (for crude biosurfactant) and between 1.6 and 2.8 fold solubility (for synthetic surfactants) (Hentati et al. 2019). This suggests the applicability of biosurfactants for microbial-enhanced oil recovery and environmental bioremediation (Mapelli et al. 2017).

Notable, non-homogenous solubilization is called “pseudo-solubilization,” the incorporation of hydrophobic pollutants into the micelle is called “solubilization,” and a high amount of hydrocarbon act as a limiting factor during solubilization in different ecosystems. The two significant solubilization mechanisms during bioremediation are the increased bioavailability of microbial substrates and the increasing surface hydrophobicity to allow hydrophobic substrates easily associate with bacterial cells (Xu et al. 2020). Although, further investigation is required to enable model predictions for ex situ sediment remediation mediated by biosurfactant solubilization.

4 Environmental Application of Biosurfactant During Pollutant Remediation

The recent advances in the environmental application of biosurfactants are shown in Table 2.

4.1 Direct Supplementation of Biosurfactant and Dose Effect

Direct addition of biosurfactant during remediation is usually conducted at a known CMC concentration. According to Sarubbo et al. (2022), the biosurfactant mechanism of action during sand washing can occur in 2 ways. Firstly, below CMC allows surfactant accumulation at the soil–pollutant interface, resulting in a change in the system's affinity for water and increasing repulsion force. Meanwhile, the second mode of action occurs above the CMC level when micelles are formed, favoring the partitioning of pollutants into the aqueous phase, increasing solubilization, recovery, demulsification, and possibly recycling to reduce remediation costs (Huang et al. 2020).

In simulated field remediation, Rong et al. (2021) demonstrated bacteria isolation and surfactant toxicity matching as a new sediment remediation technology. It was reported that adding 500 mg/kg rhamnolipid to a bacteria-enriched soil can remove 80.24% of aged total petroleum hydrocarbons (TPHs) within 30 d and significantly increase the specie richness of indigenous petroleum-degrading bacteria (such as *Massilia* and *Streptomyces*) (Rong et al. 2021). This suggests that biosurfactant addition to polluted marine sediment improves microbial community interaction, enhancing organic pollutants degradation.

Also, Huang et al. (2020) showed that glycolipid with 80 mg/L CMC has high stability over a wide range of temperatures (0–120 °C), pH (4–12), and salinity (0–16%, w/v) could be used in dose-effect to enhance organic pollutant degradation. The microbial growth and activity improved at sub-CMC (40 mg/L concentration of glycolipid) with upregulation of the expression levels of degradation-related genes and effectively promoted the biodegradation of n-alkanes (reduction from 272.21 to 56.93 mg/L) and PAHs (reduction from 61.6 to 16.36 g/L) in 7 d. The results by Huang et al. (2020) suggest that the feasibility of applying biosurfactant at known dose enhances remediation and contributes significantly to the optimization of surfactant-facilitated bioremediation strategies. Therefore, knowing the optimal dose of biosurfactant improve the remediation processes and decreases operational cost.

Table 2 Recent advances in the environmental application of biosurfactant

Biosurfactant application	Biosurfactant	Application strategy	Pollutant	Remarks	References
Direct supplementation	Rhamnolipid	500 mg/kg concentration	Petroleum hydrocarbon	80.24% TPH degradation after 30 d and increased species richness of indigenous petroleum-degrading bacteria	Rong et al. (2021)
	Rhamnolipid	5x CMC concentration	Crude oil	Hydrocarbon removal efficiency of 72.50 ± 0.11%	da Silva et al. (2021)
	Glycolipid	Sub-CMC concentration (40 mg/L)	Hydraulic fracturing flow back of petroleum hydrocarbon	Upregulation of degradation-related genes and effective biodegradation of n-alkanes from 272.21 to 56.93 mg/L and PAHs from 61.6 to 16.36 g/L in 7 d	Huang et al. (2020)
	Rhamnolipid	50 mg/L CMC concentration	PAHs	Enhanced the degradation of PAHs from 35.3% to 54.4% within 7 d	Ammami et al. (2015)
	Rhamnolipid	10 mg/L of purified rhamnolipid	Crude oil	Enhanced degradation of TPH from 63.4% to 82.1%	Antonou et al. (2015)
Supplementation of biosurfactant-producing microbes	Lipopeptide	Biosurfactant-producing <i>Enterobacter hormaechei</i> with high biocatalysts	Crude oil	85% petroleum hydrocarbons degradation within 10 d following a pseudo-second-order kinetics with rate constant k^2 0.2775 and R^2 0.9923	Muneswari et al. (2022)
	Glycolipopeptide	Biosurfactant-producing <i>Aeromonas hydrophila</i> RPI	Diesel and n-hexadecane	77.33% and 55.98% percentage degradation efficiency of diesel and n-hexadecane after 7 d	Pandey et al. (2022)
	Lipopeptide	Biosurfactant-producing halotolerant marine bacterium <i>Bacillus licheniformis</i> LRK1	Engine oil	Degrades 24.23% of engine oil after 21-d at neutral pH, 35 ± 2 °C temperature and 3% w/v NaCl concentration	Nayak et al. (2020)

	Lipopeptide	Indigenous biosurfactant-producing bacteria <i>Acinetobacter</i> sp. Y2	Hydraulic fracturing flowback of petroleum hydrocarbon	Increased microbial activity and growth. Significant decrease in chemical oxygen demand from 6646.7 mg/L to 1546.7 mg/L. Degradation of n-alkanes from 2635.4 mg/L to 159.7 mg/L and PAHs from 918.6 µg/L to 209.6 µg/L in 7 d	Zhou et al. (2020)
	Rhamnolipid	Biosurfactant-producing <i>Paracoccus</i> sp. MJ9	Diesel oil	81% degradation efficiency within 5 d	Xu et al. (2020)
	Lipopeptide	Biosurfactant-producing <i>Bacillus cereus</i> UCP 1615	Motor oil	Organic pollutant degradation with little or no detrimental effect on fish <i>Poecilia vivipara</i> (higher than 90% survival rate)	Durval et al. (2020)
	Lipopeptide	Biosurfactant-producing halotolerant marine bacterium <i>Bacillus licheniformis</i> LRK1	Engine oil	Degrades 24.23% of engine oil after 21-d at neutral pH, 35 ± 2 °C temperature and 3% w/v NaCl concentration	Nayak et al. (2020)
	Lipopeptide	Biosurfactant-producing marine bacterium, <i>Bacillus stratosphericus</i> FLU5	Petroleum hydrocarbon	Hydrocarbonoclastic effect with 50 mg/L CMC value and 28 mN/m ST	Hentati et al. (2019)
Immobilization of biosurfactant or its producers	ND	Zeolite immobilization	Crude oil	80.67% crude oil bioremediation efficiency within 21 d compared to bio-stimulation and natural attenuation	Laothamteep et al. (2022)
	ND	Immobilization on novel carrier material from coated puffed thubarb rice with calcium alginate membrane	Diesel oil	The immobilized consortium exhibited floatability, and slow nutrient release properties while degrading 86% of diesel oil	Luo et al. (2022)
	Phospholipid	Biochar immobilization prepared from corn straw biomass	Diesel oil	Upregulation of degradation-related genes (alkB and CYP450)	Zhou et al. (2021)

(continued)

Table 2 (continued)

Biosurfactant application	Biosurfactant	Application strategy	Pollutant	Remarks	References
				and 94.7% effective removal of diesel oil corresponding to 169.2 mg to 8.91 mg in 7 d compared to the 54.4% degradation by free-cell culture of LQ2	Uddin et al. (2021)
	Lipopeptide	Immobilization on functionalized nanoporous activated bio-carbon	Petroleum hydrocarbon	Bioremediation of municipal landfill leachate through lignin biosequestration mechanism	Christophers et al. (2021)
	Lipopeptide	Immobilization on activated functionalized carbon (AFC) matrix prepared from rice husk	Petroleum hydrocarbon	61.8% TPH reduction and improving seed germination of cowpea <i>Vigna unguiculata</i> after 28 d of treatment	
	ND	Size-optimized coconut fibers cellulose bio-carrier	n-hexadecane	Increased microbial biomass in contact with the pollutant and the degradation rate enhanced to 95.7% in solid phase of soil at 27 °C after 60 d	Hajjehghrari and Hejazi (2020)
Nanotechnological practices	ND	Biosurfactant and ZnO nanoparticles	Benzo[a]pyrene	Degradation kinetics was a first-order reaction with a maximum rate of 82.67 ± 0.01 (%) at 130 rpm, 30 °C, pH 7.0 after 6 d	Mandal et al. (2018)
	Rhamnolipid	Biosurfactant and nanoparticles (α -Fe ₂ O ₃ and Zn ₅ (OH) ₈ C ₁₂)	Crude oil	Improved degradation rate with 80% emulsification index and 36 mN/m ST	El-Sheshawy et al. (2014)
	Rhamnolipid	Biosurfactant layered double hydroxides	Naphthalene	Removal of hydrophobic organic pollutants with 1.3 efficiency higher than SDS, a synthetic surfactant	Chuang et al. (2010)

ND not detected

4.2 *Supplementation of Biosurfactant-Producing Microbes and Synergistic Effect*

Biosurfactant-producing microorganisms are widely used in organic pollutant remediation either as mono- or mixed culture in synergistic interactions with non-producers have been extensively detailed in previous studies (Feng et al. 2021; Shuai et al. 2019; Xu et al. 2020). The biosurfactants are secreted either intercellularly or extracellularly in the form of biofilm, which interacts with an interface and alters the surface features such as wettability and other properties.

Indigenous biosurfactant-producing bacteria *Acinetobacter* sp. Y2 was reported to increase microbial activity and growth while significantly ($P < 0.05$) removing chemical oxygen demand (from 6646.7 mg/L to 1546.7 mg/L) and increasing the degradation rate of n-alkanes (from 2635.4 mg/L to 159.7 mg/L) and PAHs (from 918.6 $\mu\text{g/L}$ to 209.6 $\mu\text{g/L}$) in 7 d (Zhou et al. 2020). Lipopeptide-producing halotolerant marine bacterium *B. licheniformis* LRK1 degrades 24.23% of engine oil after 21 d of incubation at neutral pH, 35 ± 2 °C temperature, and 3% w/v NaCl concentration (Nayak et al. 2020). The lipopeptide had a 70% Emulsification Index (EI24) and 31.43% ST reduction (Nayak et al. 2020). Similarly, Hentati et al. (2019) showed that lipopeptide-producing marine bacterium *B. stratosphericus* FLU5 is hydrocarbonoclastic and, at 50 mg/L CMC value reduces the ST of water from 72 to 28 mN/m. In Jiaozhou Bay, China, *Paracoccus* sp. MJ9 can produce rhamnolipid that enhances the bioavailability of recalcitrant hydrocarbon for easy degradation of diesel oil (81%) by microorganisms within 5 d (Xu et al. 2020).

Recently, Muneeswari et al. (2022) revealed that the successful remediation of marine crude oil spills depends on biosurfactant production and biocatalysts by the native hydrocarbon-degrading microbes. The report showed that halotolerant bacterium, *Enterobacter hormaechei*, capable of producing anionic, high molecular weight (48 kDa) lipopeptide can reduce ST of water to 35 mN/m, with an emulsification index of 46.34%, and biocatalysts of extracellular enzymes [lipase (160 U/mL) and laccase (38 U/mL)] and intracellular enzymes [alkane hydroxylase (48 U/mL), alcohol dehydrogenase (86 U/mL), and esterase (102 U/mL)]. The petroleum hydrocarbon degradation was 85% within 10 d following a pseudo-second-order kinetics with rate constant k^2 0.2775 and R^2 0.9923. This suggests that aside from biosurfactant production, the synergetic activity of the biocatalysts secreted by *E. hormaechei* enhances bioremediation.

Pandey et al. (2022) reported that biosurfactant-mediated biodegradation of 77.33% of diesel and 55.98% of n-hexadecane is greater than 26.68% of n-hexadecane and 48.36% of diesel degradation by *Aeromonas hydrophila* RP1 alone after 7 d. The biosurfactant used was glycolipopeptide consisting of pentadecanoic and octadecenoic fatty acids and having an ST reduction value of 27.4 mN/m at 123 mg/L CMC concentration. The glycolipopeptide displayed high stability over several environmental conditions (4–100 °C temperature, 2–10 pH, and 5–150 g/L NaCl concentration) with a greater than 50% emulsification index

against hydrocarbons like aviation turbine fuel, n-hexadecane, hexane, paraffin oil, and xylene.

Durval et al. (2020) reported using *B. cereus* UCP 1615 biosurfactant “lipopeptide” to stimulate the growth of autochthonous microorganisms independently of the presence of motor oil in the marine environment. The nutrient sources for the anionic lipopeptide production were 2% frying oil and 0.12% peptone. The toxicity assay revealed that aside from the lipopeptide being a good candidate for remediating polluted marine environment through enhancing pollutant solubilization and mobilization, the biosurfactant had little or no detrimental effect on fish *Poecilia vivipara* (higher than 90% survival rate).

4.3 Immobilization of Biosurfactant or Biosurfactant Producers

In nature, the violent fluctuation and complex constituents of the marine environment significantly influence the activity of free microbial cells while reducing pollutant degradability (Zhou et al. 2021). As such, immobilization of microbes using inert carriers is practiced to stabilize the interaction between the degraders and pollutants by minimizing biomass loss even under adverse environmental conditions (Hajieghrari and Hejazi 2020). Thus, ensuring biosurfactant sustainability during remediation reduces the regeneration cost, increasing cell density and reusability associated with immobilization technology (Luo et al. 2022). Immobilized biosurfactants, especially the producers, promote pollutant degradation rate with stability in varying environmental conditions. Commonly used immobilization substances include zeolites, carbonaceous materials, alginate, biosilica, and biochar, following methods like entrapment, encapsulation, adhesion/adsorption, covalent bonding, cross-linking, and combined techniques (Lapponi et al. 2022). The immobilization substances can increase cell biomass, substrate–microbe interaction, and pollutant absorption to facilitate bioremediation. Biochar is cost-effective, readily available, and has an excellent physiochemical substance with large surface area/porosity. Waste materials in immobilization matrix formation (such as biochar) enhance renewable resource recycling and reduce pollution.

Recently, Zhou et al. (2021) reported the enhanced bioremediation of diesel oil-contaminated seawater by cell immobilization technology. The biochar used was corn straw biomass, and the cell organism was a phospholipid-producing marine *Vibrio* sp. LQ2 isolated from cold-seep sediment. The biochar-immobilized phospholipid producer removed 94.7% of diesel oil, corresponding to 169.2 mg–8.91 mg in 7 d, compared to the 54.4% degradation percentage by the free-cell culture of LQ2. Besides the improvement in LQ2 biomass and activity in the immobilized matrix, the degradation-related genes (*alkB* and *CYP450*) also increased by 3.8 and 15.2 folds to the cell-free LQ2. The findings by Zhou et al. (2021) indicate that using immobilized biosurfactant-producing microbes in treating organic pollutants in the

marine environment can be feasible. Previously, Hajieghrari and Hejazi (2020) showed that immobilized biosurfactant-producing *P. aeruginosa* on size-optimized coconut fibers, a cellulosic bio-carrier, enhances biodegradation n-hexadecane (95.7%) in solid phase of soil at 27 °C after 60 d. The study revealed that optimizing the size of fibers used for immobilization influences the amount of microbial biomass in contact with the pollutant and the degradation rate.

Christopher et al. (2021) showed that lipopeptide biosurfactant (5.0 g) from *Bacillus Malacitensis* could be immobilized on Activated Functionalized Carbon (AFC) matrix (30.0 g) prepared from rice husk at 80 rpm agitation, room temperature, and pH 7 in 30 min. The immobilization process increased the amino acids (71.22% polar and 28.78% hydrophobic) content of the lipopeptide, thereby significantly enhancing the removal of recalcitrant organic pollutants through increased hydrophobicity, solubilization, micelle formation, and pollutant adsorption. The TPH (2642.5 ± 131 mg/kg) of the contaminated sediment (50 cm depth) from an industrial area used in the study was reduced by 61.8% while also improving the seed germination of cowpea *Vigna unguiculate* after 28 d of treatment. Therefore, suggesting that toxic organic pollutants affect plant metabolic rate as the soil property and microbial community are negatively impacted. Following an advanced approach, the lignin biosequestration mechanism by immobilized cationic lipoprotein biosurfactant was proposed for the bioremediation of municipal landfill leachate (Uddin et al. 2021). Optimal bioremediation was achieved using functionalized nanoporous activated bio-carbon as the immobilization material.

Studies have demonstrated that petroleum hydrocarbon consortiums containing biosurfactant producers can be immobilized to improve their functionality during bioremediation. In enhancing the bioremediation of crude oil-polluted marine sandy soil microcosms, Laothamteep et al. (2022) showed biosurfactant-producing *Mycolicibacterium* sp. PO2 can be zeolite-immobilized with other PAH degraders. It was revealed that the synergistic interactions between the bacteria strains increased the biodegradation of recalcitrant high molecular weight hydrocarbon since their genomes harbor degradative genes that allow for a meta-cleavage pathway. The zeolite-immobilized consortium has a broad activity range of pH (5.0–9.0), temperatures (30–40 °C), and salinities (20–60‰), highly increasing the indigenous hydrocarbon-degrading bacteria. The bioremediation efficiency of the crude oil (10,000 mg/kg) contaminated sandy soil was 80.67% within 21 d compared to bio-stimulation and natural attenuation. Similarly, Luo et al. (2022) reported that biosurfactant-producing *Gordonia* sp., in addition to other bacteria strains in a consortium, can be immobilized with a novel carrier material from coated puffed rhubarb rice (PRR) with calcium alginate (CA) membrane. The immobilized consortium exhibited floatability and slow nutrient release properties while degrading 86% of diesel oil.

4.4 Nanotechnological Practices

Nanomaterials are sized from 1 to 100 nm with a large surface area-to-volume ratio spatial confinement, possessing optical, magnetic, catalytic, and electronic properties (Pete et al. 2021). These properties make nanoparticles (either organic or inorganic) have good adsorbing characteristics that increase their potent preparation with biosurfactants to form biocompatible conjugates with synergistic interaction to improve organic pollutant remediation (Biswas et al. 2019). Therefore, nano-biosurfactants are a promising green technology material due to their surface activity, aggregative, stabilization, ion exchange, affinity, adsorption, and molecular sieving properties.

Biosurfactant addition to nanoparticles increases the electronegativity between the materials and their interaction, thereby acting as a dispersant, foaming agent, and flocculant to favor the removal of organic pollutants (Kumari and Singh 2016). The first report on the statistical optimization of benzo[a]pyrene degradation by response surface methodology using yeast consortium in the presence of 2 g/L ZnO nanoparticles and 3% biosurfactant was reported by Mandal et al. (2018). The pollutant degradation followed a first-order reaction with a maximum 82.67 ± 0.01 (%) rate at 130 rpm, 30 °C, pH 7.0 after 6 d. El-Sheshtawy et al. (2014) showed that maximum bioremediation of crude oil pollution at Gemsa Bay could be achieved with the use of biosurfactants from *Pseudomonas* species and nanoparticles (α -Fe₂O₃ and Zn₅(OH)₈C₁₂) after 7 d with an emulsification index of 80% and 36 mN/m ST. The application of nano-biosurfactant in pollutant remediation from the environment depends on their classification (high- or low molecular weight surface-active substance) and chemical (anionic, cationic, or neutral) nature (Kumari and Singh 2016; Plaza et al. 2014). The commonly synthesized nano-biosurfactants are metallic nanoparticles using rhamnolipids, lipopeptides, and sophorolipids through water in oil microemulsion, reverse micelle, and borohydrate reduction methods (Pete et al. 2021; Plaza et al. 2014).

Chuang et al. (2010) reported that rhamnolipid produced from *P. aeruginosa* could synthesize biosurfactant layered double hydroxides (LDHs) to remove hydrophobic organic pollutants such as naphthalene. The removal efficiency was 1.3 times higher than when SDS, a synthetic surfactant, was used, suggesting that rhamnolipid-LDH has a good adsorption capability. Biosurfactant nanotechnological practices employed for remediation have great potential, but some shortcomings like the high cost of biosurfactant production and expensive downstream processing need to be addressed.

5 Role of Biosurfactants in Marine Sediment Remediation

One of the problems with remediating contaminated marine sediments with organic hydrocarbons is their absorption into the sediment matrix, low solubility into the aqueous phase, and limited bioavailability for degradation (Dell'Anno et al. 2018). Therefore, several efforts have been taken to incorporate biosurfactants and their producers in the remediation of organic pollutants from marine sediments, as illustrated in Table 3.

5.1 *Biosurfactant-Mediated Microbial Remediation*

This concept entails enhancing the bioavailability of organic pollutants for easy accessibility by microbes. As more petroleum reservoirs are discovered and explored, oil spill into the marine environment is inevitable. These oil spills cause many severe consequences, such as heavily impacting the marine planktonic ecosystem (organisms unable to swim against water currents), bottom-dwellers, and other higher tropic organisms in the marine sediments. Bioremediation is up to date widely accepted in the remediation of contaminated marine sediments as a more sustainable approach. However, the integration of biosurfactants and bioremediation has been a significant advancement in the biological remediation section.

Feng et al. (2021) demonstrated sophorolipid-assisted bioremediation of petroleum hydrocarbon-contaminated soil with an isolated indigenous bacterial consortium. The biodegradation efficiency of TPH increased from 12.2% in the contaminated soil (control) to 44.5% and 57.7% in the isolated consortium and isolated consortium +1.5 g sophorolipid per kg dry soil, respectively. The half-life of TPH degradation also decreased from 32.5 d to 20.4 d in the treatment having sophorolipid compared with only the bacteria consortium. The sophorolipid mechanism of action includes TPH desorption from the solid matrix to the aqueous solution, increased solubilization, and improved hydrocarbon bioavailability. The stimulated microbial growth and activity observed suggest that sophorolipid also served as carbon for the bacterial community co-metabolism in the degradation system.

Although bioremediation studies on varying sediment types, including marine stones with porous spacing, are scarce, da Silva et al. (2021) recently described the removal of hydrophobic contaminant adsorbed in marine stones. The stones resulting from wave fragmentation of coral reefs had an average pore size between 230 μm and 520 μm and a porosity of 72.0%. The biosurfactant was prepared at different concentrations of $\frac{1}{2}\text{xCMC}$, CMC, 2xCMC , and 5xCMC ; however, the highest hydrocarbon removal efficiency (72.50%) was observed in treatment with 5xCMC (3000 mg/L). This suggests that hydrocarbon mobilization on porous sediment surfaces increases with increasing biosurfactant concentration. Although the changes in hydrocarbon removal efficiency from CMC to 5xCMC are not

apparent (71.30 ± 0.19 to $72.50 \pm 0.11\%$), the biosurfactant enhanced the oil viscosity reduction and formation of an oil-in-water emulsion.

Das and Kumar (2018) studied an indigenous glycolipid-producing *Pseudomonas azotoformans* AJ15 strain for remediation of petroleum-contaminated soil under hypersaline conditions. The biosurfactant substrate was agro-industrial wastes of bagasse and potato peels produced under submerged fermentation. The glycolipid class was identified as rhamnolipid following several chemical analyses, and the product had high stability against environmental stress (90 °C, 6% NaCl concentration, and varying pH). It was observed that the rhamnolipid effectively enhanced the removal of about 36.56% of trapped petroleum hydrocarbon in soil matrix under saline conditions.

In isolating indigenous microbes from Taean beach sediment, Lee et al. (2018) showed that employing biosurfactant-producing and hydrocarbon-utilizing indigenous bacteria enhances the effectiveness of crude oil bioremediation. The hydrocarbon bioavailability was increased by the biosurfactant-producing bacteria in the genus *Bacillus*, *Rhodococcus*, *Isoperitcola*, and *Pseudoalteromonas* during the degradation. The biosurfactant produced was rhamnolipid with a reduced ST of 33.9–41.3 mN/m, high oil spreading (1.2–2.4 cm), and hydrocarbon emulsification (up to 65%), justifying the hydrocarbon degradation performance observed in the marine sediment tested.

5.2 Biosurfactant-Mediated Phytoremediation

Phytoremediation is an eco-friendly approach for repairing and restoring contaminated lands, and with recent advances in the biotechnology field, its application potential is widening and has opened up new possibilities in the reclamation of the degraded sediments in marine ecosystems (Sonowal et al. 2022). This green technological approach uses site adaptive or endemic plants (Lal et al. 2018) to stabilize, extract, accumulate, degrade, or transform organic pollutants in marine sediments into less toxic molecules (Sonowal et al. 2022). While several promising assisted phytoremediation methods such as genetic engineering, nanoparticle-assisted, microbial-assisted, and electrokinetic-assisted approaches are gaining increasing attention (Yan et al. 2020), studies on the incorporation of biosurfactants are limited. As such, we focus on revealing biosurfactant-assisted phytoremediation considering the limitations highlighted by Moradi et al. (2021).

Moradi et al. (2021) reported the physiological responses and phytoremediation capability of *Avicennia marina* to PAHs contamination sediment in the vulnerable coastal ecosystems of the Persian Gulf area. The *A. marina* phytoremediation mechanism involved allocating more biomass to the root than shoot regions and activating the antioxidative enzymatic/non-enzymatic reactions (activities of peroxidase, ascorbate peroxidase, and polyphenol oxidase). The decreasing pattern of PAHs in the polluted sediments with *A. marina* rhizosphere was 37 ± 0.4 , 21.84 ± 0.27 , 12.78 ± 0.11 , and $14.74 \pm 0.03\%$, corresponding to 2.5, 5.0, 7.5,

Table 3 Role of biosurfactants in marine sediment remediation

Biosurfactant-assisted remediation	Organic pollutants	Soil/sediment	Biosurfactant used	Biosurfactant mechanism of action	Observed effects	References
Bioremediation	Petroleum hydrocarbon	Contaminated soils (5–15 cm depth) were obtained from an abandoned plant located at the Yangtze River Delta, China	Sophorolipid	TPH desorption from solid matrix to the aqueous solution, increased solubilization, and improved hydrocarbon bioavailability. Stimulated microbial growth, activity, and co-metabolism of the bacterial community	Decreased TPH biodegradation half-life and increased removal efficiency from 12.2% in the contaminated soil (control) to 57.7% in isolated consortium + 1.5 g sophorolipid per kg dry soil	Feng et al. (2021)
	Crude oil	Marine stones were collected at Suape beach, Ipojuca city—Pernambuco, close to the coral reefs	Rhamnolipid	Enhanced oil viscosity reduction and formation of an oil-in-water emulsion	Hydrocarbon removal efficiency of 71.30 ± 0.19 to $72.50 \pm 0.11\%$ from CMC to 5xCMC	da Silva et al. (2021)
	Crude oil and PAHs	Heavily contaminated Taean beach sediment on coast of Taean, Korea	Rhamnolipid	ST reduction ($33.9\text{--}41.3$ mN/m), high oil spreading, and hydrocarbon emulsification (up to 65%)	Hydrocarbon degradation in the marine sediment	Lee et al. (2018)
Phytoremediation	Petroleum hydrocarbons	Soil samples contaminated with oily	Rhamnolipid	No significant effect ($p < 0.05$)	Over a 90-d, 4 mg/kg rhamnolipid	Liduíno et al. (2018)

(continued)

Table 3 (continued)

Biosurfactant-assisted remediation	Organic pollutants	Soil/sediment	Biosurfactant used	Biosurfactant mechanism of action	Observed effects	References
		residues were collected from petrochemical facility in Sao Paulo State, Brazil		on the structure of the dominant bacterial community in the contaminated industrial soil sample analyzed	results in 58% TPH and 48% PAH concentrations reduction. The metal removal percentage was Ni (41%), Cr (30%), Pb (29%), and Zn (20%)	
	Dichlorodiphenyltrichloroethane (DDT)	The DDT-contaminated soil was collected from a long-term cotton field in Henan province, China	Direct use of biosurfactant-producing <i>Pseudomonas</i> sp. SB	Increased pollutant bioavailability and microbial community	The removal efficiency of tall fescue in treatment containing biosurfactant-producing <i>Pseudomonas</i> sp. SB increased from 40.3 to 59.4%	Wang et al. (2017)
	Crude oil	Spiked soil (5000 mg/kg, TPH) collected from the upper layer (0–20 cm) of a farm in Guangzhou higher education mega center	Rhamnolipid and soybean lecithin	Enhanced the soil microbial populations, which correlated with the crude oil degradation efficiency	The saturated hydrocarbons were greatly reduced while the recalcitrant PAH degradation was enhanced in treatment having rhamnolipid in the	Liao et al. (2016)

	Gasoline	Spiked soil	Biosurfactant from <i>Serratia marcescens</i>	Increased bacterial richness at the root surface and stimulated bio-decomposition of pollutants through enhanced rhizodegradation. The biosurfactant also acts as biocatalysts to increase the performance of phytoremediation by <i>Ludwigia octovalvis</i>	The biosurfactant removed a significant amount (up to 93.5%) of TPH compared to other additives that removed only 85.4% (<i>Serratia marcescens</i>), 70.3% (<i>Serratia marcescens</i> culture supernatant) and 86.3% (sodium dodecyl sulfate)	Almansoori et al. (2015)
Bio-electrokinetic remediation	Diesel oil	Spiked soil located in Serkkadu (Tamil Nadu, India)	Rhamnolipid from <i>Staphylococcus epidermidis</i> EVR4	Synergistic role of biosurfactant and catabolic enzymes	With a maximum degradation efficiency of 96% within 4 d	Vaishnavi et al. (2021)
	Petroleum hydrocarbon	Spiked soil from Pretoria (South Africa)	Rhamnolipid (7 mono-rhamnolipid and 4 di-rhamnolipid homologues) from <i>P. aeruginosa</i>	Biosurfactant and electrode with different currents.	Microbial survival and biosurfactant yield decreased 36.25 ± 3.75 mg/mL, 22.5 ± 5 mg/mL, 6.25 ± 1.25 mg/mL with increasing currents of 0.5 A, 1 A, and 1.5 A	Gidudu and Chirwa (2021)

(continued)

Table 3 (continued)

Biosurfactant-assisted remediation	Organic pollutants	Soil/sediment	Biosurfactant used	Biosurfactant mechanism of action	Observed effects	References
Crude oil	Spiked soil collected from Thiruvalluvar university campus, Serkadu, Vellore district (India)	Lipopeptide	Improved solubilization. Increased pollutant bioavailability and microbial accessibility of hydrocarbons as carbon and energy source. The microbes were <i>B. subtilis</i> AS2, <i>B. licheniformis</i> AS3, and <i>B. velezensis</i> AS4	Degraded higher molecular weight hydrocarbons (C8–C28) with about 92% crude oil which is more than the conventional electrokinetic method (60%) in 2 d operation	Prakash et al. (2021)	
Engine oil	Spiked soil from Pretoria (South Africa)	Rhamnolipid from <i>P. aeruginosa</i> PAI	Biosurfactant and lower electrode spacings enhanced desorption and demulsification	Oil recovery was improved to 75.15% in 240 h	Gidudu and Chirwa (2020)	
PAHs	Polluted dredged sediment was collected from the disposal site of a French harbor in Normandy	Rhamnolipid viscosin-like biosurfactant from <i>P. fluorescens</i> PfA7B	Biosurfactants and periodic voltage gradient	Removal of 16 PAHs	Ammami et al. (2015)	

and 10% crude oil contamination, respectively. The highest percentage rate of PAH fraction removal was observed for fluoranthene (71.18 ± 0.56) in 2.5% crude oil contamination sediment and anthracene (69.45 ± 6.33 , 55.66 ± 4.38 , and 35.97 ± 0.22) in 5.0, 7.5 and 10% crude oil-contaminated sediments. The findings by Moradi et al. (2021) indicate that *A. marina* is an excellent phytoremediation candidate for small-scale oil pollution and can only remove some PAH fractions from contaminated marine sediments. Therefore, incorporating biosurfactant treatment into the studies could have enhanced the activity of *A. marina* in removing more PAHs fractions easily.

The previous studies on phytoremediation are more focused on addressing the removal of heavy metals. However, Liduino et al. (2018) reported the multi-decontamination of organic hydrocarbon pollutants and heavy metals through biosurfactant-assisted phytoremediation. The study revealed the mixed functionality of commercial biosurfactant (rhamnolipid) and sunflower (*Helianthus annuus* L.) cultivation. Over a 90-d examination, the best phytoremediation efficiency occurred in the treatment with 4 mg/kg rhamnolipid, reducing 58% TPH and 48% PAH concentrations. The metal removal percentage was Ni (41%), Cr (30%), Pb (29%), and Zn (20%). Nevertheless, rhamnolipid addition and phytoremediation activity of *Helianthus annuus* L. had no significant effect ($p < 0.05$) on the structure of the dominant bacterial community. Still, increased pollutant degradation in the soil samples analyzed.

Wang et al. (2017) revealed that biosurfactant-producing microorganisms could promote the phytoremediation of organic pollutants in soil media. The biosurfactants produced by *Pseudomonas* sp. SB increased the bioavailability of organic pollutants and enhanced their microbial degradation. In synergetic response, the plants (tall fescue and perennial ryegrass) improved the rhizosphere environment for *Pseudomonas* sp. SB proliferation, thus promoting an integrated phyto- and bioremediation system. The removal efficiency of the different treatments for the pot experiment conducted: T0 = fertilizer (control), T1 = fertilizer + tall fescue, T2 = fertilizer + tall fescue + *Pseudomonas* sp. SB, T3 = fertilizer + perennial ryegrass, and T4 = fertilizer + perennial ryegrass + *Pseudomonas* sp. SB were 40.3, 59.4, 65.6, 69.0, and 65.9%. The result observed suggests that while biosurfactant addition has a significant effect on pollutant removal, the plant type influenced the rate of remediation and soil bacterial community, with Proteobacteria, Acidobacteria, and Actinobacteria being the three most dominant phyla in all groups.

Liao et al. (2016) demonstrated that biosurfactant-enhanced phytoremediation of organic pollutants as a green technology for treating contaminated soil. Microbially synthesized (rhamnolipid and soybean lecithin) and chemically synthesized (Tween 80) surfactants were employed in the phytoremediation of crude oil-contaminated soil using maize (*Zea mays* L.). It was observed that the addition of surfactants inhibited the chlorophyll fluorescence of the maize leaf but had no significant effect on the maize biomass production at $p < 0.05$. Compared to Tween 80, rhamnolipid and soybean lecithin enhanced the soil microbial population, which correlates with the crude oil degradation efficiency observed. Among the crude oil constituents, the

saturated hydrocarbons were greatly reduced (100%), while the recalcitrant PAH degradation occurred more at the plant root region than the leaf.

Almansoori et al. (2015) showed the potential application of a biosurfactant extracted from hydrocarbon-degrading bacteria *Serratia marcescens* in improving phytoremediation technology using *Ludwigia octovalvis*. Biosurfactant addition (10%) resulted in up to 93.5% TPH removal compared to the other treatments that removed only 85.4% (*S. marcescens*), 70.3% (*S. marcescens* culture supernatant), and 86.3% (synthetic surfactant). The biosurfactant-assisted phytoremediation of the gasoline-contaminated soil was pseudo-second-order kinetics with 0.9318 coefficient of determination (R^2) and 0.0032 second-order rate constant (k^2) (g TPH/kg plant d). It was observed that the biosurfactant increased the bacterial richness at the root surface and stimulated the bio-decomposition of pollutants through enhanced rhizodegradation. Hence, *S. marcescens* secreted biosurfactant is an effective biocatalyst for the phytoremediation of polluted sediment.

5.3 Biosurfactant-Mediated Bio-electrokinetic Remediation

Electrokinetics (EK) is used to remove organic pollutants in solid sediments based on charges using limited direct current (DC) applied to electrodes. The outcome effectively separates the hydrocarbon molecules based on electroosmosis, electromigration, and electrophoresis (Prakash et al. 2021). Electrochemical factors such as the slow bio-oxidation process, low microbial biomass, and pH fluctuations greatly impact the rate of pollutant degradation. However, coupling bioremediation and electrokinetic remediation methods known as the bio-electrokinetic technique are highly advantageous for simultaneously increasing the degradation rates and cell yield in the polluted marine environment (Fdez-Sanromán et al. 2021).

The major reason for integrating bioremediation and electrokinetic remediation is the long degradation period required to clean up contaminated marine sediments compared to the aqueous media (Ammami et al. 2015). This reason can be attributed to several factors such as microbial types, nutrient availability, pollutant composition, and level of environmental parameters. Using electrochemical techniques can increase the temperature inside the soil system, thereby improving nutrient supply and possible biosurfactant production by inherent microbes (Ammami et al. 2015). These processes enhance pollutant solubilization and mobilization (electromigration) through the soil matrix toward the electrode chambers, thus improving biodegradation. Besides temperature increase, the electrokinetic technique using anode and cathode in the same soil compartment can modulate pH (polarity reversal approach to maintain soil acidity or alkalinity) and pollutant concentration (nutrients distribution across the soil), which correlates with the remediation rate (Fdez-Sanromán et al. 2021). These mechanisms can increase marine sediment permeability and functionality of biosurfactant producers.

According to Prakash et al. (2021), bacterial biosurfactant “lipopeptide” is applied in a system containing *B. subtilis* AS2, *B. licheniformis* AS3, and

B. velezensis A enhances the remediation efficiency of crude oil in contaminated soil from 60% to 90% after 2 d. The mechanism of bio-electrokinetic involves increasing the solubility of the organic pollutant, which leads to faster electromigration of hydrocarbon constituents to the anodic compartment, which then confirms the decrease in the total organic content. Similarly, Vaishnavi et al. (2021) show that biosurfactant secretion by *Staphylococcus epidermidis* EVR4 can improve diesel degradation in a bio-electrokinetic remediation system. The remediation efficiency observed was 100% degradation of nonane (C9) to tricosane (C23) hydrocarbons, while pentacosane and octacosane were degraded at 85%, and 47%, respectively. The improvement in the degradation of TPHs 96% (liquid system) and 84% (soil system) was recorded within 4 d. This can be attributed to the synergistic role of biosurfactant and catabolic enzymes (dehydrogenase, catalase, and cytochrome C). Thus, making biosurfactant-assisted bio-electrokinetic remediation a potential method for the in situ removal of organic pollutants in marine sediment.

Gidudu and Chirwa (2020) reported that integrating high voltage, low electrode spacing, and biosurfactants enhances bio-electrokinetic remediation. The experiment was conducted at a varying voltage (30 V and 10 V), electrode spacing (335 mm and 185 mm), and rhamnolipid from *P. aeruginosa* PA1 with ST 30.35 mN/m at 156 mg/L CMC concentration. The result revealed that the bio-electrokinetic remediation run at 30 V and 185 mm with biosurfactant had the highest petroleum hydrocarbon recovery efficiency in the soil system. The high voltage allowed higher electroosmosis and electrophoresis in favor of electron transfer within the soil. Meanwhile, the biosurfactant and 185 mm electrode spacing enhanced the hydrocarbon remediation rate by decreasing energy expenditure and increasing desorption and demulsification. Although the change in voltage had no significant detrimental effect ($p < 0.05$) on the cells except close to the electrode (pH extremes), higher microbial proliferation was recorded in the compartment with 185 mm electrode spacing. When field scale (in situ) environmental conditions were further evaluated, it was observed that microbial survival and biosurfactant yield decreased 36.25 ± 3.75 mg/mL, 22.5 ± 5 mg/mL, 6.25 ± 1.25 of the organic pollutants with increasing currents of 0.5 A, 1 A and 1.5 A (Gidudu and Chirwa 2021).

6 Remediation Evaluation Techniques of Contaminated Marine Sediment

While developing appropriate remediation methods is required to mitigate the possible risk of organic pollutants in the marine environment, determining the suitable extraction and qualification assay is important. According to Zhang et al. (2021), the major criteria for evaluating a sediment remediation technology include the organic pollutant type, duration, residue, costs, safety, technological readiness level, efficacy/monitoring, reliability/maintenance, and preliminary investigations, auto-sustainability, acceptability. The analytical flow for evaluating

biosurfactant-mediated remediation of organic pollutants in marine sediments is extensively discussed and illustrated in Fig. 5.

6.1 Organic Pollutant Extraction

Due to the low aqueous solubility of hydrophobic pollutants, they tend to adsorb tightly to the organic matter in soils and sediments, thereby making the pollutants less extractable and difficult to recover if the sample size is small or the contaminants are at trace levels (Ammami et al. 2015; Chen et al. 2021; Dell'Anno et al. 2018; Lee et al. 2018). The regularly used techniques to extract organic pollutants from sediments are soxhlet, ultrasonic, liquid–liquid, and solid-phase extractions, although the need for large solvent volumes, longer period, more purification steps, limited efficiency, and analyte loss limit their application. However, selecting the most efficient and sustainable technique plays a major role in minimizing organic solvent wastage and human exposure. More rapid, simplified, safe, eco-friendly, and cost-effective techniques are employed in modern studies, including supercritical and subcritical fluid extraction, microwave-assisted solvent extraction, plant oil-assisted extraction, and microextraction methods (Kariyawasam et al. 2022).

6.2 Organic Pollutant Quantification

Quantification of the organic hydrocarbons extracted from contaminated marine sediment matrices is essential to understanding the extent of remediation. The most common analytic techniques with high sensitivity and low detection limit are gas chromatography (GC) and high-performance liquid chromatography (HPLC). The coupled detection systems include mass detectors (MS), flame ionization detectors (FID), fluorescence detectors (FLD), diode array detectors (DAD), and ultraviolet detectors (UV). Fourier transforms mass spectrometry (FT-IR) has been reported to reveal non-target hydrocarbons after multidimensional ionization (Kariyawasam et al. 2022).

7 Some Relevant Enterprises Working on the Massive Biosurfactant Production

Some companies, organizations, and research groups working on the massive production of biosurfactants for possible application in sediment remediation of organic pollutants in the environment are listed below:

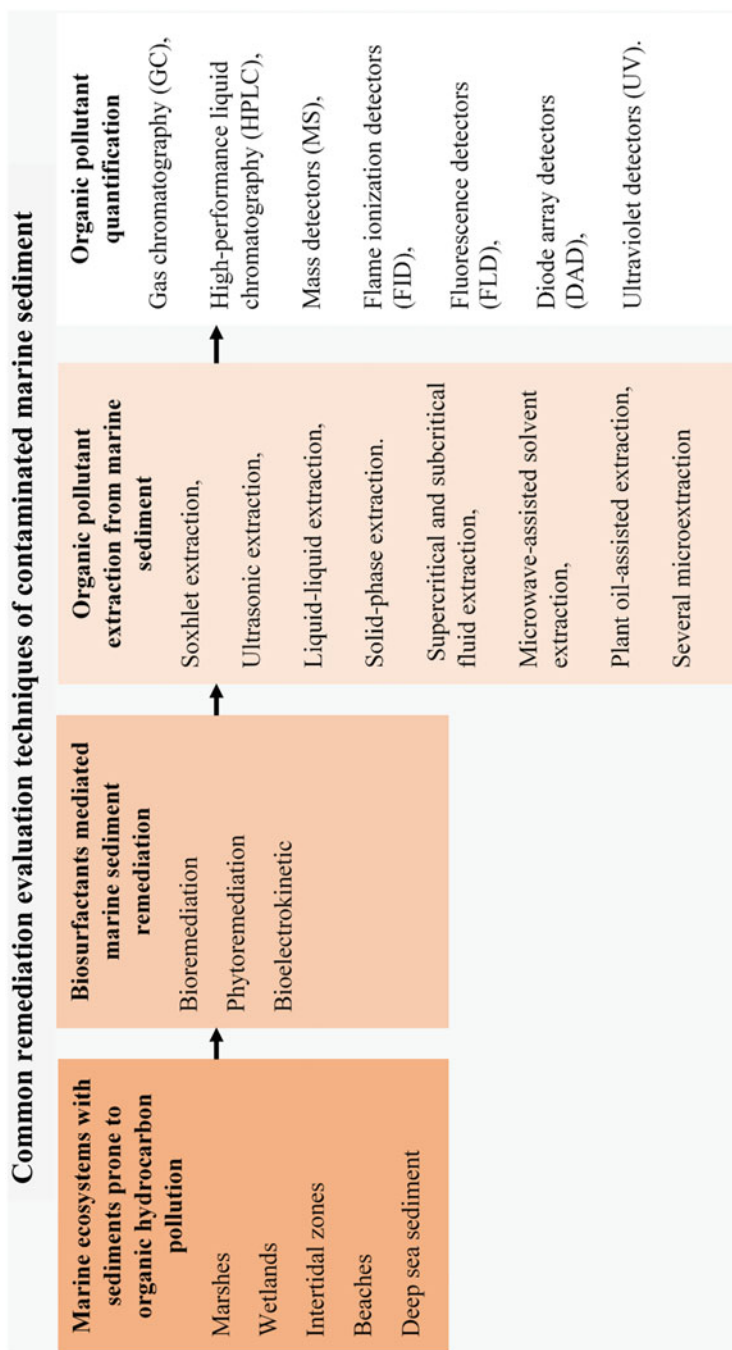


Fig. 5 The analytical flow for evaluating biosurfactant-mediated organic pollutant remediation in marine sediments

- (i). Holiform, a private company located in the United Kingdom and founded in 2018—<https://holiform.com/>
- (ii). TeeGene Biotech, a private company located in the United Kingdom and founded in 2014—<https://www.teegene.co.uk/>
- (iii). Rhamnolipid, Inc., a company located in the USA, uses artificial intelligence and machine learning to determine rhamnolipid application—<https://www.rhamnolipids.com/>
- (iv). AGAE Technologies, a private company located in the USA and founded in 2010—<http://www.agaetech.com>
- (v). Logos Technologies LLC, a company located in the USA and known for the production of sulfate and phosphate-free biosurfactants—<https://www.logostech.net/?s=Biosurfactant>
- (vi). National Science Foundation, sponsored Columbia University research on biosurfactant production by anaerobes and their cleansing/environmental remediation performance—https://www.nsf.gov/awardsearch/showAward?AWD_ID=0942962&HistoricalAwards=false
- (vii). KAM Biotechnology Ltd. is a leading international company located in British Columbia, Canada, and established in 1981. <http://www.kambiotechnology.com/>
- (viii). G&C Ambientpetrol V, Inc. is a site remediation company located in Florida, USA, and is known for producing biodegradable and ecological products. <https://gcambientpetrol.com/>
- (ix). Micro-Bac International, Inc., an environmental biotechnology research company based in the USA. <https://www.micro-bac.com/>
- (x). MCF Environmental Services, Inc., founded in 1989, USA. <https://mcfenvironmental.com/>
- (xi). Hull's Environmental Services, Inc. was founded in 1983. <https://www.hullsenvironmental.com/services/environmental-remediation-services/>
- (xii). Professor Zhang Chunfang research group, Microbiology laboratory, Institute of Marine Biology and Pharmacology, Ocean College, Zhejiang University, Zhejiang Province, China. https://person.zju.edu.cn/en/zhang_cf/#673570

8 Future Prospects

Future research should be devoted to:

- Understand the trophic transfer and impacts on human health of different petroleum hydrocarbons accumulated in marine sediments.
- Investigate the mechanism of pollutants solubilization by biosurfactants is required to enable model predictions as information regarding biosurfactant-pollutant interactions based on their structure, texture, complexity, and geochemical characteristics is scarce.

- Optimize factors that influence biosurfactant-mediated remediation in marine sediments focusing on site characteristics, surrounding environment parameters (oxygen availability, temperature, pH, and salinity), indigenous microorganisms, hydrocarbon components, and remediation cost. This will allow for a comparative analysis of different biosurfactants for assisting pollutant remediation and the scaling-up of this practice for in situ applications.
- Incorporate new micro-nano methods for applying biosurfactant in a biosurfactant-mediated remediation system while developing on the existing methods like immobilization and use of bubbles. Also, culture-independent techniques need to be developed to reduce the cost of isolating and screening biosurfactant producers.
- Develop monitoring tools and technologies to study biosurfactant-mediated remediation's efficacy in marine sediments and understand the persistence of petroleum hydrocarbon. These techniques need to be advanced, smart, sustainable, cost-effective, and energy efficient.
- Determine the effect of different sediment texture types on biosurfactant-assisted remediation. Sediment properties are often not considered during experimental design. However, the knowledge can create a link and cross-talk between remediation method, sediment texture, biosurfactant application, and microbial community structure/diversity.

It is known that intense human activities around and within marine ecosystems lead to serious pollutant accumulation in sediments; therefore, in addition to biosurfactant-assisted remediation as a sustainable approach, governmental interventions, and policy implementation is required.

9 Conclusion

Several technologies have emerged over the years for petroleum hydrocarbon remediation in marine sediments to transform these pollutants into less and non-toxic forms at a minimum environmental cost. As cost-effective remediation technologies are being developed at a slow pace, biosurfactant-assisted systems are being employed to enhance remediation performance in marine sediment reclamation. Thus, this chapter reveals the sustainable strategies for applying biosurfactants in organic pollutant remediation of marine environments and the possibility for improvement.

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The Role of Biosurfactants in Biofuel Production



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1 Introduction

Energy is one of man's most basic needs apart from air, food, and water. The importance of it to human activity cannot be overstated. Humans have needed energy since the beginning of time to power some of their activities in order to live better lives. Energy is demonstrated by experts as the act to accomplish a task and can be converted from one form to another. Humans have known means of shifting from one form of energy to another to achieve their goals, thus making modernization a reality. Humans use energy to exercise and move various machines for means of transportation, to brighten the environment, to ignite fire for cooking meals on stoves, to generate ice in freezers, to lighten our houses and factories, to make products, and to operate various machines and equipment. Gravitational pull, chemical, electrical, heat, light, and motion are all examples of diverse types of energy.

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1.1 Significance of Energy

Most actions in modern civilization require energy (Mustafa and Zehra 2017). Its use or consumption is commonly used as a barometer of one's living standard. To make living more comfortable and convenient, we employ energy sources such as firewood, fossil fuels, and electricity. At home, electricity is utilized for lighting, fans, air conditioning, water heaters, room warmers, ovens, microwaves, washing machines, and dryers, among other things. Automobiles, such as cars, buses, and lorries, run on gasoline, diesel, or compressed natural gas. Agriculture and manufacturing utilize a significant amount of energy. In offices, energy is utilized to power office equipment among other things. Fuel from fossil is utilized to power aircraft, vehicles, trains, among other things, and transportation accounts for a significant portion of total energy use. The significance of energy was brought to limelight in civilization.

1.2 Types of Energy Sources

Energy sources can be categorized as renewable or nonrenewable. Because energy is so vital to our survival, it is critical that we understand the many sources of energy. Energy can be classified as renewable (which can be used for a long period and reuse) and nonrenewable sources (when the energy source cannot be reused) are the two basic kinds of energy sources. Wind, solar, biomass, wave energy, geothermal, and hydrogen are all examples of renewable energy. The modern world's economy and technology are heavily reliant on petroleum-derived energy sources known as fossil fuels, yet these resources are both detrimental to the environment and limited. As a result, developing renewable energy technology has become a necessity (Mustafa and Zehra 2017). Primary energy, such as heat, and secondary energy, such as electricity and hydrogen, can both be produced using renewable and nonrenewable energy sources.

1.2.1 Sources of Nonrenewable Energy

Nonrenewable energy resources have a finite number of stocks. When compared to the rate of consumption, the regeneration rate of nonrenewable energy supplies is minimal. That is, nonrenewable energy that we consume cannot be replenished in a reasonable amount of time, if not in our lifetime. The main sources of energy are from fossil. Coal, lignite, and peat are fossil fuels found in the liquid and gaseous form beneath the ground and below the sea floor (petroleum, for example). Fossil fuels are the relics of prehistoric plant and animal life that have been discovered on the planet. Heat is produced when fossil fuel energy is released.

Nonrenewable energy sources are energy supplies that have been expended by living creatures for many years after they have been consumed. This form of energy now encompasses the most widely used fossil-based energy types in the world, including coal, oil, and natural gas. This form of energy shapes international relations, domestic politics, economics, environmental politics, and many other social areas in the modern world. This includes coal, oil, and natural gas.

1.2.2 Sources of Renewable Energy

Renewable sources of energy are those sources of energy that can be replenished after it has been expended and they include biomass and biodiesel. The trend in the production of renewable energy is gaining momentum globally as illustrated in Fig. 1.

1.2.2.1 Biomass

Biomass energy, also known as bioenergy, is derived from organic materials like firewood, branches, plant parts that have died, bovine dung, manure from livestock, and animal tissue that have died. Sunlight is transformed into chemical energy by plant leaves, which is then stored in the plants. Crop residues, animal waste, kitchen waste, and municipal solid waste are all sources of biomass fuels. Biomass, which

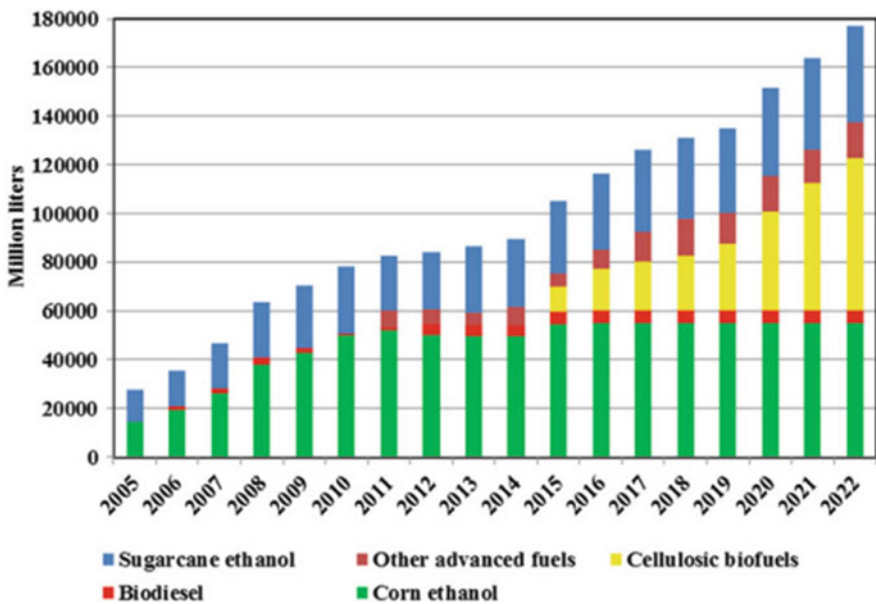


Fig. 1 Global biofuel production. Source: Jerald et al. (2016)

would typically be a challenge to dispose of, is now transformed into electricity (e.g., crop residue and stubbles) as many wastes of biological components can now be used for energy production.

1.2.2.2 Biodiesel

Trans-etherification of vegetable oils yields biodiesel. Biodiesel could be made from wild plant seeds that are rich in nonedible oils. Pongamia, Jatropha, and Neem seeds are popular for making biodiesel. Energy from biomass has the ability to moderate greenhouse gas emissions significantly. Biomass produces almost equivalent volume of carbon iv oxide to fossil, but as photosynthetic plants grow and develop, they remove carbon dioxide from the atmosphere.

2 Biofuel Production

Fuel is any fluid that produces heat or energy when it reacts with other substances, allowing a mechanism or machine to perform work in a precise proportion. Biofuels are fuels made from live organisms or their derivatives that are used to power machines. Biofuels production refers to the process of creating energy-rich chemicals using biological means or obtained in the biological components. As the world's population grows, a greater deal of energy is needed to improve people's wellbeing.

According to Allakhverdiev et al. (2009), biofuels could be one of the options for meeting the world's energy mandate (Bhat et al. 2022). "Many years ago, fossil fuels have been used as a primary source of energy; nevertheless, their use is unsustainable and causes environmental problems due to fossil fuel burning" (Voloshin et al. 2015; Razzak et al. 2013). As a result of this problem, fossil fuels may be substituted with ecologically benign and environmentally stable energy sources in form of biofuels.

Unlike some renewable energy sources, biomass may be changed straight into liquid fuels, identified as "biofuels," to take care of vehicular fuel demand. Ethanol and biodiesel are the two most often exploited biofuels currently, and they are both representatives of the first generation of biofuel technology (Voloshin et al. 2015).

Recent methodologies for microbial biofuel generation have been thoroughly investigated and recognized (Demirbas 2009; Heiman 2016), and microalgal culture stratagems for direct energy translation in making biofuels have been suggested. Biofilm culture of cyanobacteria or microalgae, for example, would provide a fresh contrivance for biomass making paths which could eventually be studied for biofuel processing. "Plant biomass has been the most well-known source of biofuels for decades. Increasing research suggests that algal biomass is a promising source for biofuel production" (Bhat et al. 2022; Dragone et al. 2010). The capacity to photosynthesize is a key property that differentiates plants from other sources.

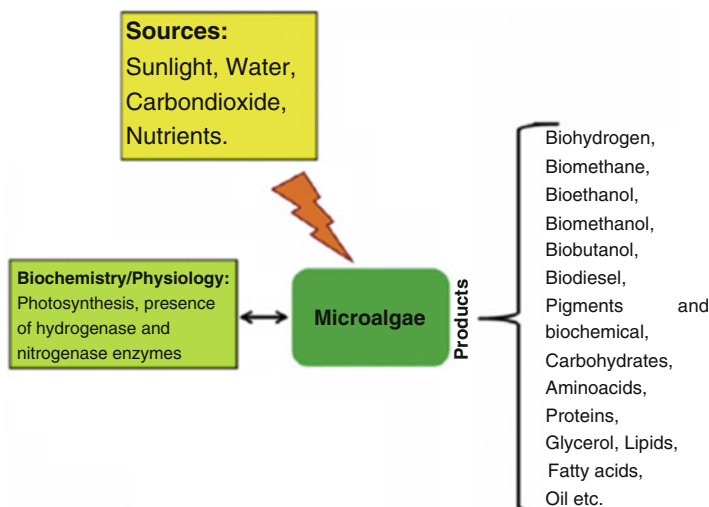


Fig. 2 Functioning Machinery of Microalgae for Energy Production

“Photosynthesis is the process of forming sugars from carbon dioxide in the atmosphere using sun energy” (Voloshin et al. 2015). It is the unique manner that plants and green algae fix carbon in nature, demonstrating that photosynthesis is the only way for plants and algae to generate biomass, which is used as raw items for biofuel generation.

The most fundamental molecular substrate for bioethanol and biomethanol generation is sugar (Bhat et al. 2022; Dias et al. 2009). Utilization of green plants or green culture as a source of biofuel is both inexpensive and practicable, as CO₂ from the atmosphere serves as a carbon supply and sunlight as an energy source during the light-dependent and light-independent photosynthesis (Voloshin et al. 2015).

The most common biofuels are biodiesel, ethanol, carbohydrates, alcohols, triglycerides, fatty acids, and organism biomass, which can be formed by a variety of organisms (Poudyal et al. 2015). Reports showed that “microalgae is being explored as an attractive feedstock for biofuels production based on existing information” (Slade and Bauen 2013). Hydrogen can be produced from microalgae (Poudyal et al. 2015). In the same manner, biomethanol, bioethanol, and biodiesel are produced (Chisti 2007), or some biomolecules, or added substances that are employed in pharmaceutical businesses, based on the species and growing method (Carlsson et al. 2007). Generation of biofuels derivative from algal involves only sunlight, CO₂, H₂O, and produces a variety of renewable energy products Fig. 2.

Output of biofuels from algae is a highly greater than biofuel from seed plants. During fermentation by microorganisms, its biomass can constantly be treated to make biofuels. Several bacteria have been discovered that can efficiently create biofuels.

Bioalcohol, isoprenoids, and fatty acid derivatives were also generated in greater quantities by *Escherichia coli* and *Bacillus subtilis*. Furthermore, some bacteria species have special features that make them suitable for use as a biofuel source. *Clostridium acetobutylicum* and *Clostridium beijerinckii* have been utilized in acetone–butanol–ethanol fermentation to produce biofuels (Gronenberg et al. 2013). Lactic acid and glutamic acids are produced by some bacteria, such as *Bacillus* and *E. coli*, as a source of various compounds (Hasunuma et al. 2013). Several bacteria species have been found as having the ability to generate ethanol. *Caldicellulosiruptor*, *Thermococcus*, *Pyrococcus*, and *Thermotoga* species had higher hydrogen generation and lower ethanol production, according to genetic studies. “The popular microbe *Saccharomyces cerevisiae* has been regarded as a model organism for the effective generation of ethanol and lipid via the fermentative process” (Tai and Stephanopoulos 2013).

2.1 Biofuels’ Types

Biofuels are divided into primary and secondary biofuels. Ordinary biofuels produced unswervingly from plants, fauna droppings, and crop residue are known as primary biofuels while secondary biofuels are produced right from Photosynthetic floras and microbes and this could be categorized into three groups (generations) as shown in Fig. 3. The manufacture of ethanol from starch-containing arable crops including some cereals and potatoes, or biodiesel from sunflower, soybean, and visceral fat, is the first generation of biofuels. The second generation of biofuels are bioethanol and biodiesel made from plants like jatropha, cassava, miscanthus, straw, grass, and wood. “Biodiesel made from microalgae and microorganisms is the third generation of biofuels” (Abdelaziz et al. 2013).

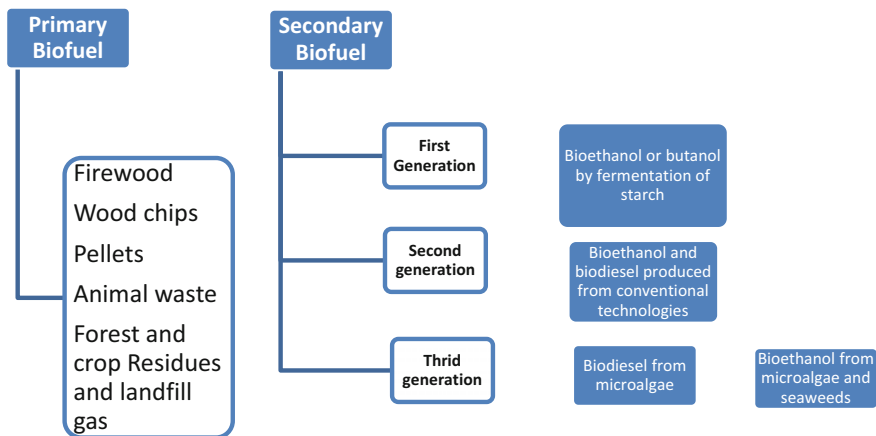


Fig. 3 Biofuel classification

2.2 Biohydrogen Production

The synthesis of hydrogen molecules is among the utmost auspicious renewable energy producing options through the cultivation of photosynthetic bacteria (Ghirardi et al. 2009). The development occurs at room temperature and requires only sunlight, water, and a few macro- and micronutrients. Because the hydrogen formation from photosynthesis produces no greenhouse gases or other pollutants, further efforts are expected to lead to the development of an environmentally favorable droppings mechanism for the industrial synthesis of ecofriendly energy (Seibert 2009). Biohydrogen could be used openly for inner burning of engines or in fuel cells to produce electricity; however, the only byproduct in both cases is water.

Multiple [NiFe]- and [FeFe]-hydrogenases have been discovered in fermentative bacteria. H₂ generation in *E. coli* is related to [NiFe]-hydrogenase through the pyruvate–formate hydrogen lyase reaction, while H₂ production in *Clostridium* sp. is linked to [FeFe]-hydrogenases through the pyruvate ferredoxin oxidoreductase reaction. “Fermentative H₂ generation at this site has been reported to reach up to 15 LH₂/(Lh) utilizing sucrose as a substrate” (Hay et al. 2013). Dark fermentation is less energy-intensive than fermentation initiated by sunlight when numerous factors are considered (Fig. 4) (Roy and Das 2015a, b; Pinto et al. 2002). Bacteria that absorb organic matter, either facultatively or obligately, go through this process (Oey et al. 2015). Multiple environmental parameters, such as medium pH and metal cofactor availability, are discovered to be relevant in dark fermentation (Chong et al. 2009). Not only does dark fermentation produce hydrogen, but it also produces a variety of other biofuels (Guwy et al. 2011).

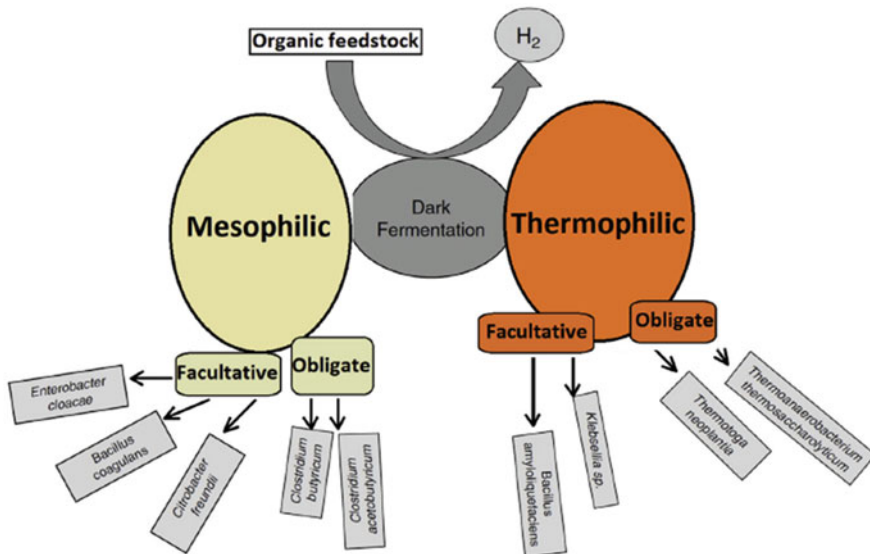


Fig. 4 Fermentative level for hydrogen generation (Roy and Das 2015a, b)

2.3 Production of Other Biofuels

Traditional methods on the use of bioalcohol as the basis for fuel formation have been practiced for many eras. Bioalcohol is now known as a non-relic unconventional source for vehicular use. “Plant material having significant sugars and starch, such as grain crops and sugarcanes, has been the primary source of bioalcohols to date, but focus has recently switched to perennial grasses such as switchgrass and *Miscanthus*” (Shah and Sen 2011). Despite the fact that those crops do not thrive with diet, the fermentation and purification processes require cellulosic biomass to be converted into biomolecules (sugars) first. The utmost frequent bioalcohol is ethanol, followed by biopropanol and biobutanol. Microorganisms ferment starch-rich crops to create these alcohols (Shah and Sen 2011). The quantity of biomass generated by plants is dependent on the effectiveness of their photosynthetic activity as well as the environment under which they are grown. Figure 5 shows the map showing the locations of biorefineries globally.

Low-temperature combustion is feasible as a result of existence of oxygen in its molecular form, which reduces the discharge of various hazardous compounds including carbon II oxide (CO), nitric oxide (NO), and explosive natural substances. “Agricultural wastes, lignocellulosic biomass, rice straw, and sugarcane are some of the sources for bioethanol production” (Dias et al. 2009). As illustrated in Table 1, “feedstocks for bioethanol production include sucrose from sugarcane, sugar from beets, starch from corn, wheat, and lignocellulosic materials including straw, wood, and bagasse (the dry pulpy residue of sugarcane stems remaining after juice extraction)” (Dias et al. 2009). When the crop residues from sugarcane, bagasse, are used, it creates a large amount of bioethanol.

Algae as another cause of bioethanol can also be used to make biohydrogen. Algae have been acknowledged lately as a possible feedstock for biofuel production, as they contain roughly 50% lipids for biodiesel synthesis and the balance constituent sugar and polymer of amino acid for bioethanol formation. Methanol is presently derived primarily from fossil fuels. Methanol is made via a chemical procedure that involves three steps: steam reforming, synthesis, and distillation.

Among fuel sources used for many decades is bioalcohol using traditional methods. It is now known as non-fossil mode of transportation. Until recently, the primary source of bioalcohols was plant constituents with significant sugars with starch, such as cereal crops and sugarcanes, but the focus has recently moved to perennial grasses like switchgrass and *Miscanthus*. Although these plants do not contend with food, the fermentation and purification procedures required the transition of cellulosic biomass, first into sugars. “Ethanol, biopropanol, and biobutanol are the three most common bioalcohols to make the alcohols, bacteria ferment carbohydrate-rich feedstocks” (Shah and Sen 2011). Gasification can also be used to produce biomethanol from microalgae like *Spirulina* sp. Because of its corrosive and toxic properties, bioethanol has been more recognized than biomethanol (Yeole et al. 2009).

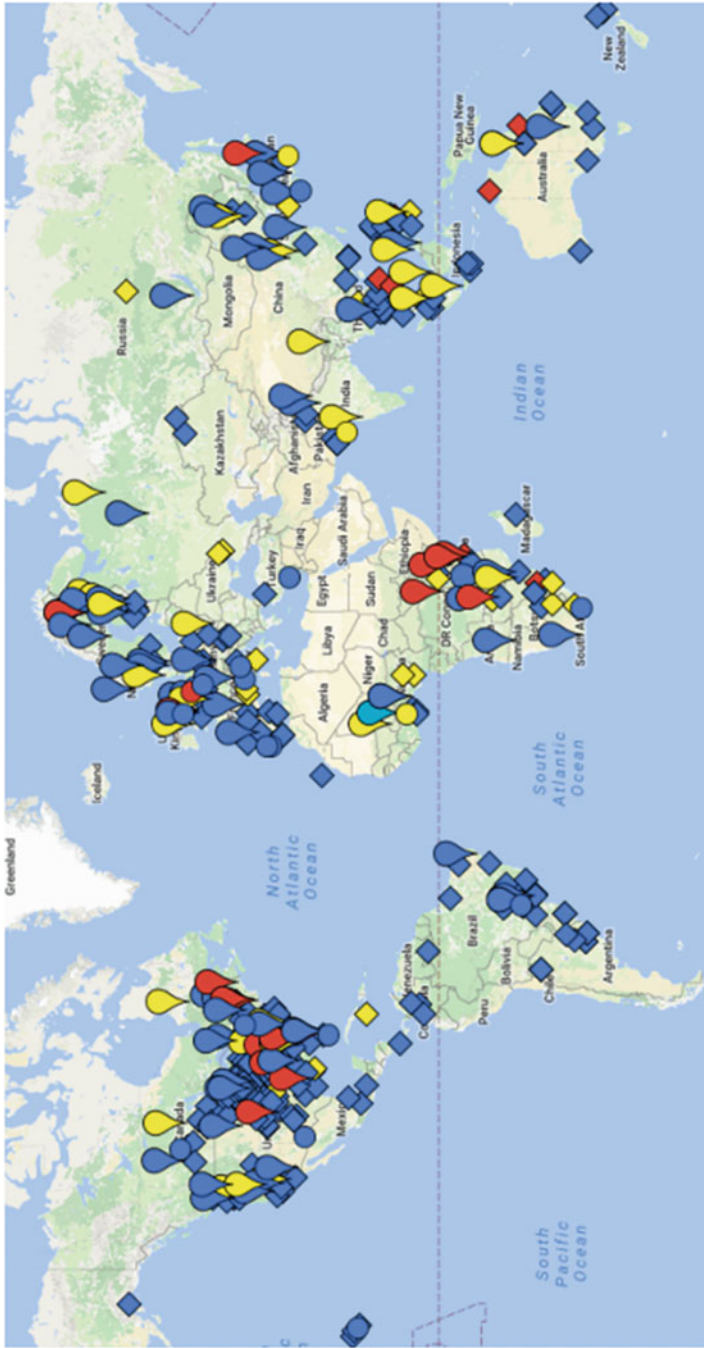


Fig. 5 Global biorefineries. **Blue** markers display operational biorefineries. **Yellow** markers display biorefineries in development. **Red** markers display biorefinery developments that have been suspended. Map produced by Dovetail Partners. For the full scale, interactive map, Source:http://www.dovetailinc.org/programs/responsible_materials/maps/global_biorefineries

Table 1 Ethanol generated from plant constituents

S/N	Sources	Ethanol yield (gal/acre)	Ethanol yield (L/ha)
1	<i>Panicum virgatum</i>	1100–1200	93,525
2	<i>Chlorella</i> species	10,000	5845
3	<i>Beta vulgaris</i> subsp.	625	3740
4	<i>Zea mays</i> L	400	2500–2680
5	<i>Triticum</i> sp.	270–284	6845
6	<i>Saccharum officinarum</i>	732	3560
7	<i>Sorghum bicolor</i>	376	3300–3320
8	<i>Manihot esculenta</i>	350–358	1225
9	Maize residues	131	93,525

Source: Modified from Dias et al. (2009)

Biodiesel is significant for several reasons: (a) it can provide a low-cost, locally produced fuel for local parsimonies; (b) the fuel is maintainable and ecofriendly; and (c) it produces slight toxic waste due to its huge biodegradable inputs and outputs (Cadenas and Cabezudo 1998). Furthermore, biodiesel is environmentally stable (Khan et al. 2009) and can be used to enhanced engine performance (Gerpen 2005). It is made of non-noxious compounds and unlike petrochemicals, and without emission of gas containing sulfur and nitrogen during burning.

Biodiesel production is a two-step process as shown in Fig. 6. Fatty acid extraction from faunal or floral tissues is the starting stage. The second stage is catalytic trans-esterification of lipid fraction with ethanol to produce fuel (biodiesel) (Rodionova et al. 2017).

Biodiesel is comparable to diesel generated from crude oil in chain length, viscidness, and energy strength and could be a “drop in” fuel demanding slight alteration of current inner combustion engines (Rodionova et al. 2017). Vegetable oil esters have 10–11% oxygen, which allows them to burn faster than hydrogen-based diesel. Furthermore, the vicissitudes of triglycerides into methyl or ethyl esters through the trans-esterification activity reduce the molecular weight to one-third of the triglycerides, reduce the viscosity by a factor of about eight, and marginally increase the volatility of biodiesel, resulting in a marginally lower volumetric heat capacity than mineral diesel (Rodionova et al. 2017). Another appealing feature of biodiesel is that it reutilizes carbon iv oxide. *Jatropha curcas*, an economically significant plant, has been discovered as a possible cause of biodiesel formation. It may be cultivated in both hot and subtropical climates (Rodionova et al. 2017).

2.4 Advantage of Biofuel

- It can easily be used independently of any other.
- It enhances the stability of an economy.
- The cost is relatively low.

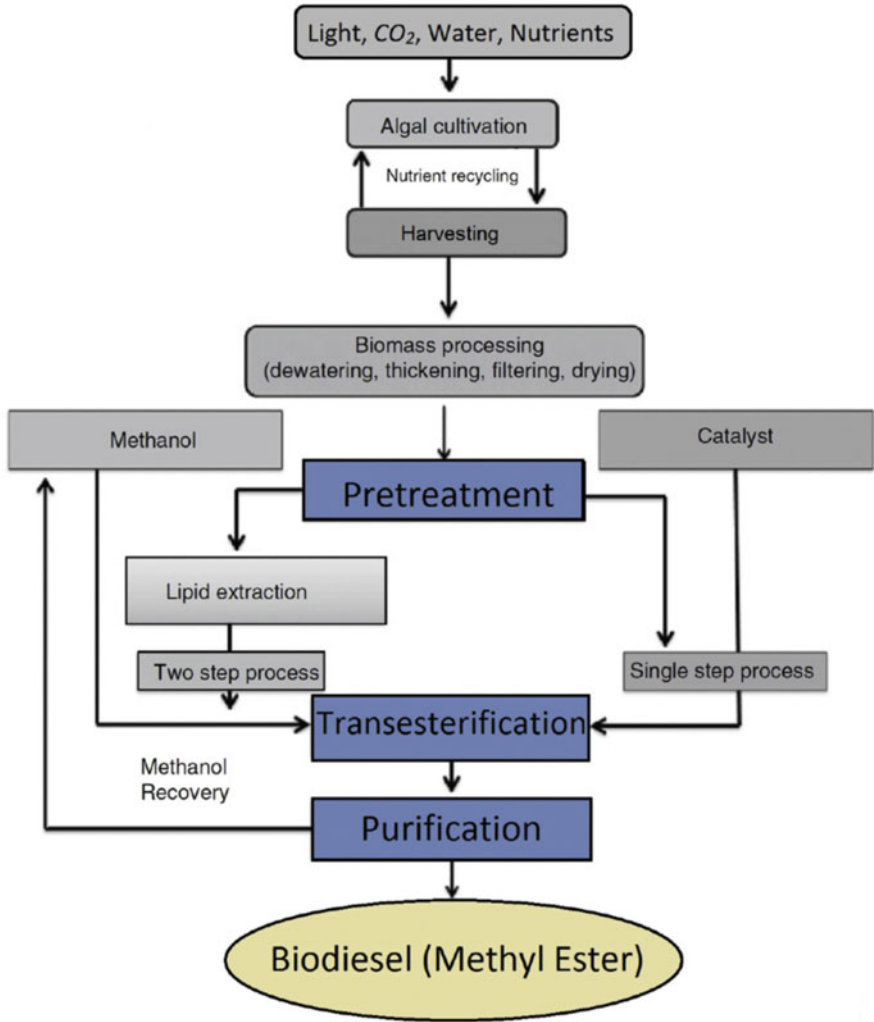


Fig. 6 Algal cultivation for biodiesel production (modified from Roy and Das 2015a, b)

- The fuel is cleanest type.
- It produces less smoke.
- They help to reduce monopoly in energy sector.
- Lower toxicity in the atmosphere.
- They generate revenue and employment for the locals.
- Biofuel does not produce sulfur.
- It promotes agricultural sector.

(Source: www.conserve-energy-future.com)

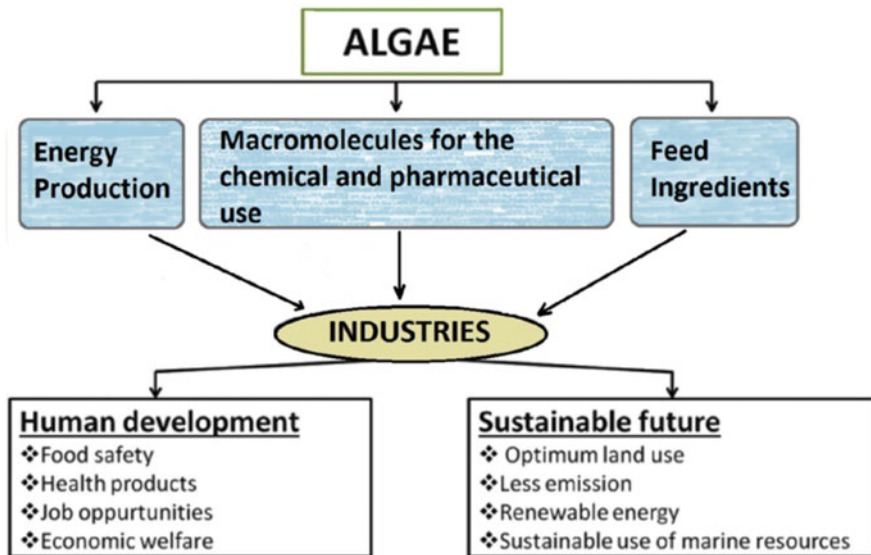


Fig. 7 Biorefinery concept using algae as potential applications (modified from Das 2015)

The plant has been regarded a good option for biodiesel because of its quick growth and high seed output. *J. curcas* seeds could be principal source of oil because they include 6.2% moisture, 18% protein, 38% fat, 17.5% fiber, and 5.3% ash (Raja et al. 2011). “Microalgae is the third generation of biofuels, which overcomes the drawbacks of the first and second generations” (Nigam and Singh 2011). Photosynthetic microalgae have recently emerged as the strongest prospect for meeting world energy demand (Fig. 7). “Microalgae can produce biodiesel 200 times more efficiently than traditional crops, according to estimates, because they can harvest after only a few hours to ten days of cultivation” (Schenk et al. 2008). Microalgae transform carbon iv oxide into biological molecules more effectively than seed plants because microalgae employ light energy (Schenk et al. 2008).

While global energy consumption is growing, fossil fuels continue to have flaws and pose a serious environmental concern. The quantity of globally consumed fuel, with its demand, is predicted to increase quickly, and the usage of fossil fuels poses substantial problems and has a negative influence on the earth’s ecology. The current global energy issue has sparked widespread concern throughout the world. Renewable energy sources are essential for addressing the worldwide energy crisis. Biofuels are a great sample of sustainable energy which could be made from biological organisms and can help to lessen reliance on fossil fuels. Using atmospheric carbon dioxide, photosynthesis may enhance the quantity of plant and algal biomass on a huge measure (Das 2015).

“Biofuels, or biomass-derived fuels, are based on photosynthesis and may be the answer to meeting energy demands while being environmentally friendly and cost-effective” (Voloshin et al. 2016).

3 Biosurfactant in Biofuel Production

Surfactants are chemical groups that have both hydrophilic and hydrophilic pur-views and groups in an amphiphilic molecule. Of most sectors of modern industry, this has been a required component in manufacturing lines. “The large volumes employed and wide variety of applications they are used in, spanning from food and beverage, agriculture, public health, healthcare/medicine, textiles, and bioremediation, demonstrate their relevance” (Nikolova and Gutierrez 2021). However, in recent decades, a major push was made on the identification of surfactants from natural/organic sources—specifically, biosurfactants—as supreme surfactants used in commercial applications currently are synthesized by organo-chemical formation utilizing petrochemicals as antecedents.

“Not only because they are made from nonrenewable resources, but also because of their environmental incompatibility and potential toxicological impacts on humans and other organisms,” says the report (Nikolova and Gutierrez 2021). In recent years, the challenge has been to shrink dependence on fossil and shift to more environmentally friendly energy that is environmentally benign and has no toxicological implications. “Given microorganisms’ huge genetic diversity, they hold great promise in developing innovative forms of biosurfactants to replace those created through organo-chemical synthesis, and the sea environment has tremendous promise in this regard” (Nikolova and Gutierrez 2021).

3.1 Types of Biosurfactants

Biosurfactants are amphiphilic surface-potent chemicals produced from living organisms such as bacteria, faunae, and plants. “Due to their great genetic variety, bacteria, yeast, and archaea are the most prevalent commercially suitable and viable sources of surface-active chemicals which could be pieces of or parts of the micro-organism’s cell wall, or they could be released by extracellular mechanisms out of the cell”. Biosurfactants can be soluble in aqueous and non-aqueous solution due to their amphiphilic nature. The ability of a surfactant to reduce the surface tension (ST) and interfacial tension (IFT) between two immiscible phases of air/fluid and polar/non-polar fluids phases, correspondingly, determines its effectiveness (www.ncbi.nlm.nih.gov).

Surface tension could be defined as degree of the energy (per unit area) necessary to raise the surface area of a fluid because of intermolecular forces, exposing more surface area for reaction and energy production. It takes less effort to bring a molecule to the surface when a surfactant is available, thereby resulting in a decrease in surface tension. Water’s surface tension can be reduced from 72 to 35 mN/m, and the interfacial tension between water and n-hexadecane can be reduced from 40 to 1 mN/m with a viable biosurfactant.

“A biosurfactant with a low critical micelle concentration (CMC), which is the lowest concentration value required to trigger micelle formation and often connects surface tension with interfacial tension, is an efficient biosurfactant”. With a low CMC, less biosurfactant is required to minimize the ST or IFT.

Average quantity of bacterial colonies is reliant on the high biosurfactant production rate, according to Sari et al. (2019). “Biosurfactant-producing bacteria can boost metabolism because biosurfactants on the cell surface aid in the transportation of nutrients across the cell membrane, resulting in a faster rate of growth” Sari et al. (2019).

3.2 Chemical Composition of Biosurfactants

Biosurfactants’ chemical composition differs widely amongst microbe species and can be generically categorized on the value of their molecular strength or chemical reaction. “Surface-active compounds are classified as either low-molecular-weight (LMW) surfactants, which reduce surface tension between two immiscible liquids, or high-molecular-weight (HMW) emulsifiers, which enable the formation of oil-in-water or water-in-oil emulsions and are commonly composed of exo polysaccharides (EPS)”.

Glycolipids, phospholipids and fatty acids, lipopeptides, and lipoproteins are some of the major chemical structures of LMW biosurfactants. “As single macromolecules, polymers, and/or particulate structures, these structures can create biosurfactant”. Biosurfactants’ chemical composition varies widely between microbe species and can be generically categorized based on their molecular weight or chemical charge. “Surface-active compounds are categorised as either low-molecular-weight (LMW) surfactants, which reduce surface tension between two immiscible liquids, or high-molecular-weight (HMW) emulsifiers, which enable the formation of oil-in-water or water-in-oil emulsions and are commonly composed of exo polysaccharides (EPS)”. Because they are LMW, their primary purpose as surface-dynamic compounds is to reduce surface and/or interfacial pressure between the non-mercurial liquid segments, such as liquid and solid or liquid and gas. The most researched biosurfactants are glycolipids, which are made up of diverse biomolecules linked to β -hydroxy fatty acids (carbohydrate head and lipid tail), whereas lipopeptides are made up of cycloheptapeptides with amino acids coupled to fatty acids of various chain lengths (Uzoigwe et al. 2015; pure.hw.ac.uk).

Heteropolysaccharides, lipopolysaccharides, lipoproteins, and proteins make up high-molecular-weight bioemulsifiers, which are more complex than biosurfactants. EPS is another name for them. Extracellular Polymeric substance (EPS) molecules, like low-molecular-weight biosurfactants, can effectively emulsify two immiscible liquids (e.g., oil and water), but are less successful for reducing ST” (www.ncbi.nlm.nih.gov). EPS molecules attach securely to scattered hydrocarbons in oil-polluted environments, preventing oil droplets from aggregating to “bursting” open. The enormous quantity of mercurial constituents shown in their arrangements has been

attributed to this procedure, which is known as emulsion equilibrium (Uzoigwe et al. 2015). Emulsan, alasan, liposan, sphingan, and xanthan gum are the most studied microbial EPS (Table 2).

3.3 *Biosurfactant-Producing Bacteria in Aquatic Environment*

To date, a controlled diversity of bacterial species capable of producing biosurfactants with commercial usefulness or have potential in this regard have been isolated from aquatic settings. Although several species, such as *Pseudomonas* and *Rhodococcus*, were originally isolated from terrestrial habitats, some members of these genera with biosurfactant-producing characteristics were obtained from nautical locations. The features of biosurfactants generated by these species are detailed underneath (Uzoigwe et al. 2015).

3.3.1 *Bacillus*

Bacillus members were isolated mostly from oil pools or oil-polluted earths and proved and known as highly effective biosurfactant makers in MEOR operations. For less than two weeks of incubation, *Bacillus methylotrophicus* USTBa has the ability and capacity to extract over 90% of the crude oil. “A strong glycolipid type biosurfactant was created by the bacteria, as evaluated by the ST of the culture media, which was 28 mN/m”. When cultivated on crude oil as the only carbon source, *B. subtilis* strain A1 could make 78% emulsification process by producing lipopeptide biosurfactant. After a week of incubation at 40 °C, this strain effectively and completely destroyed a range of low-molecular-weight alkanes (C₁₀–C₁₄) and up to 97% of high-molecular-weight alkanes (C₁₅–C₁₉). “A low-yield lipopeptide biosurfactant (1 g/L) generated by a non-pathogenic *B. licheniformis* R2 reduced the ST to 28 mN/m and the IFT between heavy crude oil and formation water-brine utilized in core flooding to 0.53 mN/m” (Joshi et al. 2015).

3.3.2 *Antarctobacter*

Antarctobacter is a Gram negative bacteria genus that belongs to the Rhodobacterales order and is exclusively aerobic. Only *Antarctobacter heliothermus*, a species isolated from Antarctica, has been validly taxonomically defined. *Antiarctobacter* sp. strain TG22 was able to create an extracellular aqueous glycoprotein polymer (named AE22) that produced stable suspensions with various floral oils at concentrations as low as 0.02%. The species generated an average dry-weight yield of 21 mg/L when cultivated in maritime broth complemented with

Table 2 Biosurfactant sources with chemical composition and application

S/N	Biosurfactant type	Source	Chemical composition	Application	References
1	Glycolipids Rhamnolipid	<i>Pseudomonas aeruginosa</i> <i>Burkholderia thailandensis</i> <i>Marinobacter</i> sp. strain MCTG107b	Rhamnose monosaccharide/s linked to 3-hydroxyl fatty acid unit via β -glycosidic bond	Bioremediation Agriculture Cosmetics Pharmaceuticals Marine oil spills	Funston et al. (2016), Chong and Li (2017), Twigg et al. (2018), and Tripathi et al. (2019)
2	Sophorolipid	<i>Candida</i> spp.	Dimeric sugar sophorose head linked to a long-chain hydroxy fatty acid tail	Cosmetics Personal care products	Van Bogaert et al. (2007), Kurtzman et al. (2010), and Santos et al. (2017)
3	Trehalose lipids	<i>Rhodococcus erythropolis</i>	Trehalose sugar linked to long-chain fatty acids (C20–C90)		Peng et al. (2007)
4	Mannosylerythrol lipids (MELs)	<i>Candida antarctica</i> <i>Pseudozyma</i> spp.	Long-chain fatty acids linked to a mannopyranosyl-meso-erythritol hydrophilic head group	Food	Adameczak and Bednarski (2000), Morita et al. (2013), and Niu et al. (2019)
5	Lipopeptides Surfactin, iturin, and fengycin	<i>Bacillus subtilis</i>	Cyclic lipopeptide consisting of long hydroxyl fatty acid chain and hydrophobic amino acid ring	Soil bioremediation MEOR	Vanittanakom et al. (1986), Al-Wahaibi et al. (2014), Inès and Dhouha (2015), and Liu et al. (2015)
6	Lichenysin	<i>Bacillus licheniformis</i>	Cyclic lipopeptide similar to surfactin	MEOR	Yakimov et al. (1995), Joshi et al. (2015), and Coronel-León et al. (2016)
7	Viscosin	<i>Pseudomonas fluorescens</i>	Cyclic lipopeptide similar to surfactin	Agriculture	Laycock et al. (1991) and De Bruijn and Raaijmakers (2009)
8	Emulsan	<i>Acinetobacter calcoaceticus</i> RAG-1	A complex of a lipoheteropolysaccharide (apoemulsan) and a protein	MEOR	Rosenberg and Ron (1999) and Uzoigwe et al. (2015)
9	Alasan	<i>Acinetobacter radiorensistens</i> KA53	A complex of anionic polysaccharides rich in alamine and proteins with high molecular mass	Bioremediation MEOR	Navon-Venezia et al. (1995) and Toren et al. (2001)

10	Liposan	<i>Candida lipolytica</i>	A complex of heteropolysaccharides and protein	Pharmaceuticals Food Cosmetics	Cirigliano and Carman (1985) and Campos et al. (2013)
11	Sphingan	<i>Sphingomonas</i> spp.	Linear tetrasaccharide backbone (glucose-glucuronic acid-glucose-rhamnose/mannose) to which glucosyl, rhamnosyl, mannosyl or acetyl side chains are attached	Food Textile Pharmaceuticals Oil and gas	Schultheis et al. (2008), Prajapati et al. (2013), Kaur et al. (2014), and Li et al. (2016)
12	Xanthan	Gum <i>Xanthomonas campestris</i>	A backbone of repeating sub-units of 3–8 monosaccharides	Food Oil and gas	Kuppuswami (2014), De Mello Luvielmo et al. (2016), and Kang et al. (2019)

Source: Nikolova and Gutierrez (2021)

1% glucose. Glucosamine, glucuronic acid, fucose, and mannose dominated carbohydrate constituents of AE22 (total of 15%).

3.3.3 Rhodococcus

Rhodococcus is a genus with metabolically diverse species that can flourish in a variety of environments. The ability of members of the genus to digest hydrocarbons and contaminants from various settings has been the focus of research. *Rhodococcus aurantiacus*, *Rhodococcus ruber*, and *Rhodococcus erythropolis* are some of the most recognized Rhodococcus biosurfactant manufacturers (Peng et al. 2007). It is only n-alkanes as the only source of carbon allowed *R. erythropolis* 3C-9 to thrive and generate biosurfactant (CMC of 50 mg/L), whereas glucose had no effect on its productivity (Peng et al. 2007). The 3C-9 biosurfactant was made up of fatty acids ranging in length from C₁₀ to C₂₂ (docosenoic acid was the most common, followed by hexadecenoic acid) and two glycolipids (individually controlled by glucose and trehalose monosaccharides).

3.3.4 Pseudomonas

Pseudomonas, a genus of Gammaproteo bacteria, can colonize a wide range of environments and create a variety of biosurfactant compounds, including glycolipids (rhamnolipids) and lipopeptides (e.g., amphisin, syringomycin, tolaasin, and viscosin). “While the bulk of isolated *Pseudomonas* spp come from terrestrial environments, representatives of this genus are widespread in aquatic environments”. *P. aeruginosa* produces biosurfactants that are commonly investigated, and it thrives well on a variety of non-hydrocarbon and hydrocarbon mechanisms, producing rhamnolipids, which create stable emulsions with unrefined oil. Extra work discovered that *P. aeruginosa* has a huge attraction for crude oil (93% cell adherence to unrefined oil), indicating that it produces biosurfactants. The surface tension of culture broth was lowered by the *P. aeruginosa* DQ8 strain from 63 to 38 mN/m when various component of crude oil is available, including PAHs (Zhang et al. 2014). “It has been shown that *P. aeruginosa* can use organic or non-hydrocarbon substrates like soybean oil, fish oil, mannitol, and glycerol to generated non-poisonous biosurfactants that could be useful in oil spill bioremediation as an alternative to chemical dispersants or as a substitute for synthetic surfactants in commercial dispersant formulations”. *P. aeruginosa* strains cultured on glycerol generated rhamnolipids (3.8 g/L; CMC 50 mg/L) that lowered the surface tension to 29 mN/m and blended petrol (EI24 70%) and diesel (EI24 80%), indicating its possible use in oil recovery and bioremediation. *Pseudomonas putida* strain BD2 could thrive on glucose while concurrently producing rhamnolipid and sophorolipid; the rhamnolipid lowered ST to 31 mN/m, and blended vegetable oil at 70% effectiveness. It has an ability to act on various organic substrates to release biosurfactant, which is used to produce biofuel from biomass constituents.

3.4 Activities of Biosurfactants in Biofuel Production

“Microbial enhanced oil recovery (MEOR) is a technique that involves injecting microorganisms and/or their metabolic by-products into mature oil reservoirs in order to recover residual crude oil that was not removed during the primary and secondary extraction procedures” (www.ncbi.nlm.nih.gov). MEOR is based on the notion that when ideal conditions exist in the reservoir, the injected microorganisms will proliferate geometrically, and their biochemical products will utilize the remaining oil. MEOR has both advantages and disadvantages, have extensively explored the many stages of its deployment.

MEOR is founded on some basic principles. The first concept entails microbial action demeaning but also removing sulfur and heavy metals from heavy oils by varying the interfacial properties of the oil–water–minerals disarticulation effectiveness (i.e., reduce in IFT to intensify media permeability), propelling pressure (pool force), volatility (miscible flooding; viscosity reduction), and sweep proficiency (selective plugging; mobility control) and the second concept entails microbial action demeaning but also eliminating sulfur and heavy metals from weighty oils. In the majority of MEOR field trials, pre-cultured bacteria or a consortium of bacteria were injected with nutrients along with indigenous (or other MEOR compatible) bacteria (e.g., oxygen and nitrogen). Because bacteria can manufacture biosurfactant in situ, this approach has been the preferred option because it lowers operational expenses. “The capillary forces that prohibit oil from flowing through rock pores can be reduced by biosurfactants, but the decrease in IFT must be at least two orders of magnitude to achieve oil mobilization. IFT between hydrocarbons and water is typically between 30 and 40 mN/m”. Biosurfactants must drop the IFT to 103 mN/m in order to have any effect in MEOR. Furthermore, the kind of oil pool (sandstone, carbonates, etc.), remaining oil saturation, and incremental oil recovery should all be considered. The amount of biosurfactant required to achieve a 30–60% oil recovery rate should be quite large, making it neither feasible nor cost-effective. Biosurfactants do change the damping of rock formations, intermingling crude oil, and contribute to the microbial metabolism of viscous oil, in addition to lowering IFT.

Nonetheless, numerous research groups investigating biosurfactants for MEOR applications have reported some encouraging results. Lipopeptides were the most commonly employed biosurfactants in laboratory-based MEOR research, because to their capacity to lower the IFT to <0.1 mN/m. “Both bench scale and in-situ lipopeptide synthesis by *Bacillus spp.* stains have been successful in increasing oil recovery, even from wells nearing their output limits” (Al-Wahaibi et al. 2014). Surfactins have been found to retain activity over an extensive temperature, pH, and salinity range while recovering sand-bound oil. *B. subtilis*, for example, developed surfactin at high temperatures that could emulsify diesel with 90% proficiency and convalesce more than 60% of the oil contained in sand cores. Surfactin has lately been proven to change the wettability of CO₂ injected into a subterranean rock formation, indicating that it could be useful in carbon apprehension and stowage.

Lichenysin has been shown to lower the IFT to 102 mN/m (reduced concentrations of 10–60 mg/L) and to have outstanding steadiness at temperatures as high as 140 °C, 6–10 pH series, 10%, salinities up to NaCl, and calcium (as CaCl₂) of 340 mg/L concentrations.

In main swamping trials, partly pure lichenysin recovered up to 40% of residual oil from sandstone cores, vs just 10% when artificial surfactants were used. The adding of biosurfactants to chemical surfactant flooding can help to increase overall flooding performance. The surfactant alkylbenzene sulfonate's adsorption to sandstone was reduced by 25–30%, and the grade of oil recovery improved by 7% in the presence of rhamnolipids. By adsorbing firstly to oil sands, rhamnolipids operate as sacrificial agents, enhancing the surfactant's availability for displacement activity and altering the wettability of porous media. At small concentrations of 0.5 mg/mL, macromolecular biopolymers like emulsan have been proven to eliminate up to 98% of pre-adsorbed crude oil from limestone core samples. "A biopolymer generated by *Rhizobium viscosum* CECT908 was recently found to be more effective than xanthan gum in the recovery of heavy oil" (www.frontiersin.org).

3.5 Significance of Biosurfactants in Biofuel Production

Crude oil is very hydrophobic, containing huge amount of hydrocarbon and non-hydrocarbon spp., as well as metals, all of which have different water solubilities. As a result of its lower density than water, an oil will glide on the surface of a liquid phase when injected. Surface tension, along with viscosity, indicates how quickly and to the extent an oil could flow across the surface and, once dispersed, into the subsurface with the larger the extent of spreading, the lower the interfacial tension with water. Chemicals are introduced to an oil slimy to improve the solubility of oil in water (i.e., to reduce the friction between oil and water's surface).

"Surfactants (biogenic or synthetically created) are used to disperse/emulsify the oil, speeding up the biodegradation process. Biosurfactants have been proven to help disperse crude oil and speed up the biodegradation process. It reduces the solubility and increases the stability of the alkanes (nC₁₃-C₁₅) chain. Within 5 days, the rhamnolipid encouraged the growth of hydrocarbon degraders, which were able to use 50% of the crude oil saturated portion" (Nikolova and Gutierrez 2021; www.ncbi.nlm.nih.gov).

In the presence of rhamnolipids, LMW PAHs, as well as the biomarkers pristine and phytane, were dramatically reduced. The type of element used in the treatments (organic lipophilic or water-soluble) in combination with rhamnolipids, could enhance crude oil dissolution in seawater and sediment settings. The cultivation of biofuel-producing microbes necessitates a variety of favorable environmental conditions, including adequate light, temperature, nutrients, salinity, and pH (Cheng and He 2014). Bioemulsifiers could be utilized to respond to oil spills with positive results. The bioemulsifier exopolysaccharide EPS2003, generated by the bacteria *Acinetobacter calcoaceticus*, has been shown to improve crude oil biodegradation in

natural saltwater microcosms. “In *E. coli*, targeted modifications in central carbon metabolism, such as overexpression of isocitrate dehydrogenase and deletion of glutamate synthase, have been shown to efficiently increase ethylene synthesis” (Lynch et al. 2016). *Synechococcus* sp. due to heterologous generation of an acyleacyl carrier protein reductase and an aldehyde decarbonylase, *Synechococcus* sp. PCC 7002 generates alkanes (Zhang et al. 2015).

However, the full-scale commercial production of biosurfactants for biofuel production is still limited by a number of factors going by the global population trend and demand for the energy need by man. Efforts are needed to make this a worthwhile endeavor and operational to meet the human demand.

4 Conclusion

Finally, it is evidently clear if biofuel production takes its full course, pollutions, greenhouse effect, and associated problems will be reduced with the use of biofuel. In biofuel production, the indispensability of biosurfactant-producing microbes cannot be gnarled. Those microorganisms including *P. aeruginosa* produce biosurfactants that perform essential functions in enhancing the full-scale production of biofuel from biomass by reducing the interfacial tension and the surface tension in the biomass through unique and inimitable enzymatic hydrolysis potential by the biosurfactants, especially the Rhamnolipids as it enhances full oil recovery. The use of biosurfactant is indispensable as it is ecofriendly and biodegradable without production of any toxic substance to the environment. It is therefore highly recommended for use in biofuel production for full oil recovery to reduce overdependence on fossil fuel with associated the pollution and greenhouse effect in the globe.

5 Important Websites

1. International Renewable Energy Association (http://www.se4all.org/sites/default/files/IRENA_RE_Jobs_Annual_Review_2016.pdf).
2. (<https://energy.gov/eere/bioenergy/2016-billion-ton-report>)
3. <http://www.Unilever.com>
4. U.S. Energy Mapping System. (<http://www.eia.gov/state/maps.cfm>).
5. <http://www.holiferm.com>
6. <http://www.allied-c-s.co.jp>
7. <http://www.lehigh.edu>
8. <http://www.agaetech.com>
9. <http://www.logostech.net>
10. <http://www.dispersa.ca>

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Part III
Biosurfactants: Current Biomedical
Applications

Role of Biosurfactants in Biocidal Activity and Wound Healing



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1 Introduction

Biosurfactant (BS) can be described as a surface-active molecule formed by microbes (bacteria, fungi, or yeast) with a large range of applications. In current years, due to their distinct characteristics such as high specificity, lower toxicity, highly biodegradable, high specificity, and relatively easy preparation from various renewable origins, functionality under harsh conditions; biosurfactants (BSs) have carved a niche for themselves (Sen et al. 2017). They are amphiphilic molecules with both hydrophobic and hydrophilic moiety which interact differently at the interface between fluid phases which are made up of several levels of hydrogen and polarity bonds, including air or water and oil or water and air interface (Banat et al. 2010; Rodrigues et al. 2006a, b). BSs have been found to enhance nutrient transport within membranes and influence different host–micro interactions. The majority of these microbial surfactants are complex compounds that are made up of different structures such as phospholipids, fatty acids, glycopeptides (GPs), and glycolipids (GLs) (Rahman and Gakpe 2008; Sen et al. 2017). The main famously and widely isolated and studied biosurfactant is glycolipids (Guatam and Tyagi 2006).

The most recognized glycolipid BSs includes trehalolipids, rhamnolipids (RLs), mannosylerythritol lipids (MELs), and sophorolipids (SLs); they consist of disaccharides or monosaccharides with long-chain hydroxylaliphatic acids (Van Bogaert et al. 2007). Sopholipids (SLs) are majorly produced by fungi such as *Candida bombicola*, *Rhodotorula bogoriensis*, and *Candida apicola*, while mannosylerythritol lipids are mainly formed by *Pseudozyma rugulosa*, *Pseudozyma*

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antartica, and *Pseudozyma aphidis* (Konishi et al. 2007; Sen et al. 2017). SLs are synthesized by noninfectious fungi (yeasts) different from other well-researched rhamnolipids where the major effective producers are opportunistic enterobacteria *P. aeruginosa*. More so, SLs have been revealed to have higher production than RLs causing its larger commercialization. SLs have shown a wide range of antimicrobial action against different organisms and the possible mode of their actions could be through improved membrane permeability and destabilization (Bluth et al. 2006). Some studies have reported the antimicrobial activity of some BSs against some yeasts and bacteria (Dengle-Pulate et al. 2014; Haque et al. 2016; Sen et al. 2017).

The wound healing process can be a regulated, dynamic, and coordinated cascade of cellular activities which starts after wound formation. This biological activity of wound healing can be classified into inflammation, cell proliferation, extracellular matrix, and wound remodelling (Beldon 2010; Sonam et al. 2017). In some medical conditions such as smoking, diabetes, aging, surgery, and starvation; healing in these wounds can be delayed, leading to severe illness and even death (Lu et al. 2020). Surfactin has been found as another amphipathic cyclic lipopeptide (LP) produced by a heptapeptide sequence joined with a β -hydroxyl fatty acid-forming lactone ring structure. They are commonly developed by different strains of *Bacillus subtilis* which was largely utilized as soil remediation agent, bacteria fertilizer, and biotic pesticide in agricultural fields. There has been an increasing trend in the application of BSs among developing nations with a lack of microbial and nutrient resistance that has been of great concern. Furthermore, several studies had revealed the potential roles of glycolipids produced by *B. licheniformis* SV1 in wound healing (Giri and Park 2022; Gupta et al. 2021; Gupta et al. 2017a). Some studies had reported the effect of a novel function of surfactin A produced from *Bacillus subtilis* at various levels of wound healing such as scar tissues, inflammatory response, cell migration, and angiogenesis (Yan et al. 2020). BSs have shown several advantages over chemical surfactants and there has been a higher demand worldwide. Thus, this chapter reviews the roles of biosurfactants in biocidal activity and wound healing.

2 Genetics of BS Production

Different functional and structural diversity can be exhibited in BSs formed by microbes. BS-producing strains including *Candida*, *Acinetobacter*, *Bacillus*, and *Pseudomonas* sp. had been reported from several origins including industrial effluents, soil, and water (Kumar and Das 2018). There are different genes required in producing various types of BSs. The genomic regulation of BS development had been investigated on the rhamnolipid produced from the *P. aeruginosa*, the report showed that the BS production was induced by quorum sensing signalling compounds (Dusane et al. 2010; Soberón-Chávez et al. 2021). Three consecutively enzymatic reactions occur during the production of biosurfactants. In the first reaction, there is a synthesis of 3-hydroxyalkanoyloxy alkanic acid (HAA) by RhIA through the esterification of Acyl carrier protein-bound two 3-hydroxyacyl

compounds. For the second reaction, dTDP-L-rhamnose is transferred to hydroxyalkanoyloxy alkanolic acid to form mono-rhamnolipid. In addition, dTDP-L-rhamnose had been seen to be originated from glucose-6-phosphate formed by the central carbon metabolic pathways. Lastly, di-rhamnolipid can be produced by adding another compound of dTDP-L-rhamnose to the mono-rhamnolipid catalyzed through the enzyme rhlC. The rhlC and rhlAB gene expressions can be regulated by Quorum sensing signalling molecules including C₄-HSL (N-butyryl homoserine lactone). The joining of the rhlA promoter to the RhlR-C₄-HSL can be activated by rhlC and rhlAB genes which can lead to the transcription of the rhlAB gene (Dusane et al. 2010).

3 Classification of Biosurfactant and Their Microbial Origin

Based on the recent literature, BSs can be generally classified into higher and lower molecular mass molecules. Biosurfactants with higher molar mass are good emulsifiers while others with lower molecular mass molecules can be more efficient to decrease interfacial and surface tensions (Banat et al. 2010). The two groups are further classified into various types according to their chemical constituents (Ndlovu et al. 2017; Sandeep and Rajasree 2017) (Table 1).

3.1 High Molecular Weight Biosurfactants Produced by Bacteria Around the Environment

Several studies have revealed that there are relationships between communities located in various sites of the body such as the vagina and oral cavity, between the oral cavity and stool specimens. Several studies have reported that bacteria can migrate via the gastrointestinal tracts and can share an ecological environment (De Giani et al. 2021; Ding and Schloss 2014). Those stable microbiotas are found in the vaginal and stool, while most of the unstable microbiotas are from the oral cavity. *Lactobacillus* genus with a biosurfactant activity are few in the vaginal site, and many biosurfactant compounds can change the surface tension and disseminate in the milieu to prevent the organisms (Banat et al. 2010). Strains of the *Lactobacillus* genus can be utilized to produce biosurfactant which is majorly composed of phosphate, proteins, and polysaccharides in diverse ratios (Brzozowski et al. 2011); mostly, they can be categorized as glycolipoproteins (Banat et al. 2010) (Table 1). Likewise, BS compounds have antimicrobial activity against different common potential infectious bacteria such as *K. pneumoniae*, *S. aureus*, *E. aerogenes*, *S. saprophyticus*, *E. coli*, *N. gonorrhoeae*, and antifungal activity in fighting *C. albicans* (De Giani et al. 2021).

Table 1 Different classes, types, and sources of biosurfactants

Category of biosurfactant	Origins of biosurfactant	Type of biosurfactant	Class of pathogens	References	Category of biosurfactant	Origins of biosurfactant	Type of biosurfactant	Class of pathogens	References
Neutral, phospholipids and fatty acids	Low molecular weight <i>Corynebacterium diptheria</i> and <i>C. lepus</i>	Corynomycoic acid	Actinobacteria	Al-Hazmi (1990) and Cooper et al. (1979)	High molecular weight Lipopeptides	<i>Bacillus polymyxa</i>	Polymyxin	Bacilli	Duttagupta et al. (2016) and Yu et al. (2018)
		Phosphatidylethanolamine	Gammaaproteobacteria	Jain and Raghav (2021), Karlapudi et al. (2018), and Santos et al. (2016)			Aneurinofactin	Actinobacter	Balan et al. (2017) and Morikawa et al. (1993)
		Spiculisporic acid	Eurotiomycetes	Fengold (2021) and Rod-in et al. (2020)			Surfactin	Bacilli	Ghazala et al. (2017) and Mnif et al. (2021)
Polymeric biosurfactants	<i>Talaromyces trachyspermus</i> and <i>Penicillium spiculsporium</i> <i>Acinetobacter radiorisistens</i> KA53	Alasan	Gammaaproteobacteria	Toren et al. (2001, 2002)	Serratia marcescens	<i>Serratia marcescens</i>	Serrawetitin	Gammaaproteobacteria	Clements et al. (2019a), Matsuyama et al. (1992), Matsuyama et al. (2010), and Roldan- and Carrillo et al. (2021)

<i>Saccharomyces cerevisiae</i> and <i>Acinetobacter</i> sp.	Mannoproteins	Gamma proteobacteria	Almenteros et al. (2010), Katemai (2011), and Nikolova and Gutierrez (2021)	<i>B. amyliquesfaciens</i> and <i>B. subtilis</i>	Iturin	Bacilli	Kim et al. (2010) and Wang et al. (2020)
	Lipomanan	Saccharomycetes	Ribeaux et al. (2017)				
<i>Candida tropicalis</i>				<i>Pseudomonas fluorescens</i>	Viscosin	Gamma proteobacteria	Alshimm et al. (2014) and Laycock et al. (1991)
<i>Acinetobacter calcoaceticus</i> RAG-1	Biodispersan	Gamma proteobacteria	Mujumdar et al. (2019), Nikolova and Gutierrez (2021), and Rosenberg and Ron (1999)	<i>B. licheniformis</i>	Lichenysin	Bacilli	Coronel-León et al. (2015) and Yeak et al. (2022)
<i>Candida lipolytica</i>	Liposan	Saccharomycetes	Rufino et al. (2014)	<i>Ustilago scitaminea</i> , <i>Pseudomonas</i> spp., <i>Candida antarctica</i> , and <i>Arthrobaacter</i> sp.	Mannosylerythritol lipids	Ustilaginomycetes, Saccharomycetes and Actinobacteria	Morita et al. (2009, 2011), Nikolova and Gutierrez (2021), Santos et al. (2016), and Yu et al. (2015)

(continued)

Table 1 (continued)

Category of biosurfactant	Origins of biosurfactant	Type of biosurfactant	Class of pathogens	References	Category of biosurfactant	Origins of biosurfactant	Type of biosurfactant	Class of pathogens	References
Particulate biosurfactants	Cyanobacteria and many other bacteria	Whole cells	Cyanophyceae	Gudina et al. (2016), Karanth et al. (1999), and Sharma et al. (2021)		<i>Melothermus ruber</i> , <i>T. aquaticus</i> , <i>Burkholderia</i> sp., <i>P. aeruginosa</i> , and other <i>pseudomonas</i> sp.	Rhamnolipids	Deinococci, Betaproteobacteria, and Gammaproteobacteria	Bernier et al. (2017) and Gayathri et al. (2022)
	<i>Serratia marcescens</i> , <i>P. marginills</i> and <i>Acinetobacter</i> sp.	Vesicles	Gammaproteobacteria	Alves et al. (2014), Clements et al. (2019b), and Hu et al. (2018)		<i>Gordonia</i> sp., <i>Corynebacterium</i> sp., <i>Mycobacterium</i> sp., <i>Rhodococcus erythropolis</i> , and <i>Norcardia</i> sp.	Trehalolipids	Actinobacteria	Franzetti et al. (2010), Kigler et al. (2015), Kuyukina et al. (2015), and Vijayakumar and Saravanan (2015)
						<i>Wickerhamiella domercqiae</i> , <i>Rhodotorula bogoriensis</i> , <i>Candida kuoi</i> , <i>T. apicola</i> , and <i>Torulopsis bombicola</i>	Sphorolipids	Microbotryomycetes and ascomycetes	Leyton et al. (2021), Sen et al. (2017), and Zhang et al. (2011)

Table 2 Available genes for the biological production of BS with their biocidal action formed from bacterial-related human health

Strains	Products	Genes	References
<i>P. aeruginosa</i>	Chain of A, B, and C of the enzymes <i>rhlA</i> , <i>rhlB</i> , and <i>rhlC</i> , respectively	<i>rhlA</i> , <i>rhlB</i> , <i>rhlC</i>	De Giani et al. (2021) and Sood et al. (2020)
<i>L. plantarum</i> RI-515	Uncertain compounds that have 5289 amino acid length	<i>npsA</i>	De Giani et al. (2021)
<i>B. subtilis</i> , <i>B. amyloliquefaciens</i> , and <i>B. thuringiensis</i>	It produces a putative surfactin transcriptional terminator	<i>sfp</i>	De Giani et al. (2021) and Perez et al. (2017)
<i>S. marcescens</i>	It can produce <i>swrW</i> (non-ribosomal serrawettin W1), <i>sphA</i> (stephensiolides), and serrawettin W2 synthetase	<i>swrA</i> , <i>sphA</i> , and <i>swrW</i>	Clements et al. (2019a, b), De Giani et al. (2021), Li et al. (2005), and Maglangit et al. (2020)
<i>L. reuteri</i>	Ketansynthase domain which includes Ab hydrolase, β -ketoacyl-synthase II, and PKS condensation	Type 2 PKS genes	Chen et al. (2011), De Giani et al. (2021), and Selvin et al. (2016)
<i>B. pumilus</i> SF214	SrfAC, srfAB, and srfAA	srfAD, srfAC, srfAB, and srfAA	De Giani et al. (2021), Saggese et al. (2018), Théâtre et al. (2021), and Zhi and Xu (2017)

The capacity to produce antimicrobial biosurfactants is evident not for the strain of *Lactobacillus* bacteria only but also for other inhabitant organisms of various human body sites. El-Sheshtawy and Doheim 2014 revealed that *P. aeruginosa* can be the most produced biosurfactant; for instance, *Pseudomonas aeruginosa* ATCC10145 can create a biosurfactant cellular-free rhamnolipid with antifungal and antimicrobial actions (Table 2). The most category of antimicrobial biosurfactants from bacteria related to human health include glycolipoproteins, glycopeptides, glycolipids, and lipopeptides (Abdalsadiq et al. 2018; Abdalsadiq and Zaiton 2018; De Giani et al. 2021). Hence, the cell-rated antimicrobial biosurfactants are more complex and characterized by different components. In addition, they can be known as cell-related biosurfactants; the strains of the *Lactobacillus* genus are commonly cell-related BSs due to their intrinsic properties. The chemical characterization analysis of cell-related biosurfactants has shown that BSs are compounds of high molecular weight that majorly consist of sugars, fatty acids, and proteins with various percentages. A study had revealed the isolation of a glycolipid type of biosurfactant from *L. acidophilus* NCIM 2903 which has the capacity of reducing surface tension from 45 to 26 mNm⁻¹ and CMC to 23.60 mgmL⁻¹ (critical micelle concentration).

3.1.1 Glycopeptides Biosurfactants

Glycopeptides BSs are glycoproteins that contain biosurfactant and antimicrobial properties; they are produced only by the *Lactobacillus* sp. Some studies had confirmed the potential capacity of three *Lactobacillus* strains (*L. cellobiosus* TM1, *L. delbrueckii* N2, and *L. plantarum* G88) in promoting the growth of biosurfactants on sugar cane and glycerol molasses. Their results showed concentrations ranging from 2.43 to 3.03 gL⁻¹ with E24 (49.89–81%) on sugar molasses and glycerol of 2.32 and 2.82 gL⁻¹ with E24 (41.81–61.81%). The compounds formed on glycerol medium mainly consist of lipids compared with the sugar cane molasses. This has revealed that lactobacilli can direct the glycerol in the gluconeogenesis and lipolytic pathway, hence producing more lipids (De Giani et al. 2021; Mouafo et al. 2018). The growth of *L. delbrueckii* N2 and *L. cellobiosus* TM1 on sugar cane molasses resulted in the production of glycolipids with no lipid fractions. The sugar and protein constituents' analyses showed about 27.10 g/100 gMS and 52.93 g/100 gMS for *L. cellobiosus* TM1-BS; 51.13 g/100 g MS; 63.64 g/100 g MS for *L. delbrueckii* N2-BS, respectively. The antimicrobial properties showed that GPBs were more sensitive than GNB. For instance, the growth of *Bacillus* sp. BC1 was found to be majorly influenced by the activity of *L. delbrueckii* N2 GLP BS, indicating an inhibition zone of 57.50 mm (De Giani et al. 2021).

3.1.2 Phospholipids (Glycolipoprotein) Biosurfactants

According to De Giani et al. (2021); Hippolyte et al. (2018), and Mouafo et al. (2018), glycolipoprotein biosurfactants can only be produced from two *Lactobacillus* sp. such as *L. paracasei* subsp. *tolerans* N2 and *L. plantarum* G88, nevertheless these complex compounds are cell-bound due to their large dimensions. Mouafo et al. (2018) confirmed that the *Lactobacillus plantarum* G88 growth on the sugar cane molasses could yield some compounds characterized by 39.60 g/100 g MS lipids, 8.96 g/100 g MS proteins, and 51.13 g/100 g MS sugars. Differentiating the antimicrobial action of *P. putida* PSV1 and PSJ1, *E. coli* E6, and *Salmonella* sp. SL2 had proved their inhibition haloes of 41, 32.00, 32.00, 32.00, and 32.00 mm, respectively. In addition, a study utilized *L. paracasei* subsp. *tolerans* N2's capability in producing biologically active molecules on sugar cane molasses using mathematical modelling to determine the optimization production of an antimicrobial biosurfactant. The results forecasted the production yield and two values showing biosurfactant characteristics such as surface tension, a measure of growth inhibition diameter and antimicrobial potential associated with the surfactant activity. After the fermentation at 33 and 34 °C optimal conditions and at 5.49–6.35% of sugar cane molasses concentration, they got an active biosurfactant containing an experimental surface tension (37.04 mNm⁻¹) compared to the predicted value (36.65 mNm⁻¹) (Hippolyte et al. 2018).

One major effective phospholipid development area of the strongest antimicrobial effect involved the least molasses of 5.49% at a temperature of 33 °C. The inhibition halo measured against *P. putida* PSJ1 was 63.89 mm in diameter compared to the forecasted diameter of 62.07 mm. Besides, the assessment of antimicrobial effect against other bacteria such as *Salmonella* sp. SL2, *E. coli* MTCC 118, and *Pseudomonas aeruginosa* PSB2 were reported. Nevertheless, *S. aureus* STP1 and *Bacillus* sp. BC1 was reported as the major susceptible pathogens to the glycolipids at a minimum inhibition concentration of 3.20 mgmL⁻¹, but *E. coli* and *Salmonella* sp. produced the lowest susceptibility at a minimum inhibition concentration of 12.81 mgmL⁻¹. The limited chemical characterization showed the major compositions as lipids, proteins, and sugars (1.10 g/100 g DM, 63.64 g/100 g DM, and 35.26 g/100 g DM, respectively) to confirm the nature of the glycolipoproteins.

3.1.3 Particulate Biosurfactants

These are referred to as extracellular vesicles and full microbial cells. According to Sharma et al. (2021), these vesicles are made up of lipopolysaccharides, phospholipids, and proteins. For example, *Acinetobacter* sp. grows on hexadecane to produce vesicles that are about 1.15 g³/cm buoyant in density and 20–50 nm in diameter on the cell surface. While the full microbial cells possessed non-hydrocarbon and degrading hydrocarbon characteristics. An example of an emulsifier is *Acinetobacter calcoaceticus* 2CA2 (Sharma et al. 2021).

3.1.4 Polymeric Biosurfactants

The most famous polymeric BSs include polysaccharide–protein complexes, emulsan, lipomannan, liposan, and alasan (Saravanan and Vijayakuma 2015). Liposan type of polymeric BS is usually a water-soluble compound made up of 17% protein and 83% carbohydrate; it is formed by *C. lipolytica* extracellularly (Cirigliano and Carman 1985). Alasan is produced by *Acinetobacter calcoaceticus* and it is a strong emulsifier with complex proteins, polysaccharides, and alanine (Navon-Venezia et al. 1995). Emulsan is made up of three unbranched dideoxydiaminohexose, galactosanminouronic acid, D-galactosamine, and amino sugars in equal ratios with about 10–22 carbon long-fatty acid chains. It has an average molecular mass of around 1000 kDa produced from *A. calcoaceticus* (Zosim et al. 1982). According to Alcantara et al. (2013) and Jagtap et al. (2010), mannoproteins are known as glycoproteins which consist of carbohydrates and proteins, formed by *Saccharomyces cerevisiae*, *Acinetobacter* sp., and so on. Similarly, they can produce strong and stable emulsions with several kinds of hydrocarbons, oils, and antimicrobial activities.

3.2 Lower Molecular Weight Biosurfactants

3.2.1 Glycolipids Biosurfactants

Glycolipids are the most studied BSs of lower molecular weight, they can be synthesized from olive oil and frying wastes, industrial wastes, and hydrocarbons (Inès and Dhouha 2015; Thakur et al. 2021). Glycolipids BSs are complex compounds with a structural component of a hydrophilic moiety of carbohydrates such as trehalose, galactose, mannose, rhamnose, glucose, sophorose, and hydrophobic of a lipid fraction (Thakur et al. 2021). Inès and Dhouha (2015) reported that glycolipids could be very effective and efficient in fighting different fungi, mycoplasma, viruses, and bacteria due to their effective function to destabilize the biological membrane through the production of ion pores and channels. Among the glycolipid BS producers, *Pseudomonas* strains formed the major important species. Some GPBs can give similar kinds of molecules expelled into the environment, this includes the *Lactobacillus* genus. Some important examples of glycolipids are trehalose lipids of *Rhodococcus* and *Mycobacterium* sp.; MELs A-C of *Candida* sp.; and rhamnolipids of *Pseudomonas* sp. (Santos et al. 2016; Vecino et al. 2017; Adu et al. 2020).

The antimicrobial activities of glycolipid BSs had revealed more prevalence in fighting GPB than GNB. It inhibited *S. aureus* at a concentration between 15 and 31 nm and *P. aeruginosa* showed concentrations at 20.00–100.02 mgmL⁻¹. Thakur et al. (2021) reported that glycolipid can hinder the peptidoglycan layer of the GPB which may lead to the weakening of the cell wall. In addition, glycolipid can serve as an anti-adhesive agent at 50 mgmL⁻¹ and inhibit *P. aeruginosa* at 75% and *S. aureus* at 78%, respectively. *E. cloacae* B14 is among the commensal bacteria in the gastrointestinal tracts that can produce a glycolipid-like substance of about 39.80 mg of biosurfactant when using fungi extracts as a substrate. The biocidal effects were more prevalent against GPB such as *S. aureus*, *B. subtilis*, and *B. cereus* with inhibition haloes ranging from 20.70 to 26.70 nm than the GNB with inhibition haloes between 9.7 and 17 nm against *S. marcescens*, *P. aeruginosa*, and *E. coli*. Ekprasert et al. (2020) reported that biosurfactant was more successful than the generally utilized tetracycline in combating *B. subtilis*; it can reduce the growth of the tetracycline-resistant strain of *S. marcescens*. As it had been reported that *P. aeruginosa* is the major researched Gram-negative bacteria used in the production of rhamnolipid. They are usually produced by a sugar known as rhamnose moiety that binds to a biosurfactant with an aliphatic variable chain feature. Some studies had revealed that several rhamnolipids can exhibit antimicrobial properties like the one produced by *P. aeruginosa* CR1 (Sood et al. 2020; Wahib et al. 2020).

Sood et al. (2020) suggested that *P. aeruginosa* CR1 biosurfactant can also demonstrate obvious emulsification and antimicrobial activities; the E24 was about 53% and the surface tension was reduced to 35 mNm⁻¹. Its recuperation occurred after the bacteria grew on both the basal media supplemented with rice bran oil and Luria Bertani broth enriched with glycerol to show the highest formation of 10 gL⁻¹. It had been detected that *P. aeruginosa* strain CR1 cannot produce di-rhamnolipids

except mono-rhamnolipids only. The genomic analysis had also established the fact that the absence of *rhlC* gene coding could play a role in the synthesis of di-rhamnolipid. A study had investigated the strains of *P. aeruginosa* isolated from a clinical sample; the results showed its capacity to produce 20 gL^{-1} of antimicrobial biosurfactant after it had been grown on glycerol media. This biosurfactant was chemically analyzed as di-rhamnolipids and mono-rhamnolipids with an E24 of 88.18%. Obviously, at 1 gmL^{-1} concentration, rhamnolipid BS could reduce the growth of *S. aureus*, *K. pneumoniae*, and *E. coli*, and it demonstrated the highest antimicrobial activity against *S. aureus* (Ekprasert et al. 2020).

3.2.2 Lipopeptide Biosurfactants

Lipopeptides (LPs) generally belong to the group of surface-active compounds that are made up of peptide moieties and fatty acids of different lengths. Structurally, LPs are classified into different isoforms including syringomycin, pseudofactin, athtrotactin, tensin, viscosin, bacillomycin, surfactin, plipastatin, fengycin, iturin, and so on; although, one microorganism may not be able to produce more than a single isoform (Salek and Euston 2019). LPs are formed by different kinds of aerobic bacteria, actinomycetes, moulds, and yeast. According to Chen et al. (2015), surfactin is one of the isoforms that is of interest because of its anti-mycoplasma, antifungal, antiviral, and antibacterial properties. It is one of the well-recognized lipopeptides produced by GPB *B. subtilis*. It can act as a cell lysate promoter and inhibit fibrin clotting. Also, Lu et al. discovered surfactin as an antioxidant, anti-wrinkle, and anti-photo aging repairing activity along with improved skin penetration function and increased the production of skin's collagen.

Among the class of *Lactobacillus* genus, Abdalsadiq et al. (2018) and Emmanuel et al. (2019) revealed some strains which are capable of producing antimicrobial LP biosurfactants. Abdalsadiq et al. (2018) reported the antimicrobial effect of the LP fraction which was compared to the GL fraction isolated from the *L. pentosus* and *L. acidophilus* cell cultures in fighting different antagonists including *S. aureus*, *P. mirabilis*, *K. pneumoniae*, *S. pneumoniae*, and *Candida albicans*. The AWDA was used to calculate the inhibition of the extracted biosurfactants; the results showed haloes between 14 and 44 mm against *K. pneumoniae* and *S. aureus*, respectively. The minimum inhibition concentration assessment showed that the LP biosurfactant can achieve better biocidal activity at the lowest minimum inhibition concentration between 7.80 and $62.50 \text{ }\mu\text{gmL}^{-1}$ against *P. mirabilis* and *K. pneumoniae*, respectively, while GL fraction ranging between 15.60 and $62.50 \text{ }\mu\text{gmL}^{-1}$. In addition, most of the organisms showed about 65 and 93% anti-adhesive effect against *P. mirabilis* and *K. pneumoniae* at $250 \text{ }\mu\text{gmL}^{-1}$ concentration based on the concentration of the LPs fraction, while the glycolipid fraction anti-adhesive activity can produce a lower percentage of inhibition from 45 to 72.70%. Furthermore, the anti-biofilm activity of LPs had been revealed at $250 \text{ }\mu\text{gmL}^{-1}$ with 100% success. The LP fraction had shown a 100% anti-biofilm effect against *P. mirabilis* and *K. pneumoniae*, and 85% against *S. aureus*.

Emmanuel et al. (2019) reported another LP biosurfactant produced by the strains of *Lactobacillus* sp. isolated from homemade curd, the results showed the concentration at 3.20 gL^{-1} , E24 of 58% and characterization due to the presence of alkene, alkyne, and a conjugated diene. Their anti-biofilm and antimicrobial effects of LP biosurfactants had been examined only against those of *E. coli*. It produced a similar antimicrobial effect to sodium dodecyl sulfate, and the biosurfactant showed inhibition against the biofilm of *E. coli*. After 6 hours that the concentration of lipopeptides was increased, the biofilm of *E. coli* cells become smaller. Many strains can be used as probiotics due to the production of spores that can withstand harsh conditions, these include lower gastric pH. As soon as it entered the intestinal tract, spores will begin to grow; hence, strains of *Bacillus* sp. grow and can be re-sporulated by using their antimicrobial property and other important activities. Some studies had established the surfactin-forming capacity of the species by recognizing sfp gene marker and their genus affiliation. Another study revealed the strain of *Pseudomonas* sp. UCMA 17988 isolated from fresh cow milk and their capacity of producing LP biosurfactant; *Pseudomonas* strains are popular in the production of rhamnolipid (Pornsunthorntawe et al. 2010).

4 The Omic Method in Detecting Biosurfactants With Antimicrobial Property

Different studies are ongoing to look for new biosurfactants and antimicrobial molecules that may be utilized for medical and biotechnological uses in fighting against resistant pathogens. Due to the primary methods that required different planting situations and experimental methods before understanding which type of secondary metabolite is released by a pathogen, these efforts had shown a lack of understanding in the area of the molecular mechanisms which are behind the BS production (De Giani et al. 2021; Emmanuel et al. 2019; Hippolyte et al. 2018; Omar et al. 2017). Zampolli et al. (2018) reported that in the last 20 years, the emergence of the “omic era,” has changed the perspective since most studies were undertaken on a genomic-wide scale. In addition to the introduction of modern bioinformatics tools, the number of bacterial genetic sequences available for the rebuilding of BGCs to encode pathways has increased the capacity to produce specialized metabolites (Ceniceros et al. 2017). Thus, finding a new antimicrobial agent and biosurfactant compounds can start through the exploration of a strain genome.

The *S. marcescens* is the commonest strain of the *Serratia* genus, their members are usually known to be opportunistic organisms that can cause nosocomial diseases such as bloodstreams, respiratory tracts, surgical wounds, and urinary tract infections (De Giani et al. 2021). Besides, strains of the environmental *Serratia* sp. are nonpathogenic food-related bacteria strains (De Giani et al. 2021; Sandner-Miranda et al. 2018). Because of this condition, it can be categorized and considered as one of the antimicrobial biosurfactant producers related to humans. BSs developed by the

Serratia genus are nonionic, and lower molecular weight lipopeptides with an antimicrobial property made up of stephensiolides A to K and serrawettin W1, W2, and W3 (Clements et al. 2019a). Clements et al. (2019b) exploited the findings to screen about 22 bacteria forming biosurfactants isolated from urban effluent treatment plants to produce serrawettin A. A primer set outline was used to identify the *swrW* and *swrA* genes that can encode for the non-ribosomal serrawettin W2 and W1 synthetase enzymes.

Moreover, a similar method was used to discover new antimicrobial biosurfactants produce from the *Bacillus* strains. The *B. subtilis* releases an LP biosurfactant surfactin that can be classified into a cyclic heptapeptide which binds a β -hydroxy fatty acid (Perez et al. 2017). The *sfp* gene encoded the surfactin transcriptional terminator that is used in form of a marker sequence (Isa et al. 2020). In addition, Kanmani et al. (2013) stated that *srfA* operons could be utilized to predict surfactin production from *Bacillus* strains. A study had reported the *srfAD*, *srfAC*, *srfAB*, and *srfAA* genes encoding surfactin synthase thioesterase and surfactin synthase subunits 1, 2, 3, respectively (Saggese et al. 2018). The *srfA* genes of *Bacillus* and *srw* genes of *Serratia* can be regarded as a part of the NRPS family (Clements et al. 2019a, b; De Giani et al. 2021; Saggese et al. 2018), which are made up of multiple modular enzyme complexes that are important in the synthesizing of antibiotics (Singh et al. 2017).

The *rhl* operon is another essential gene discovered from the strains of the *Pseudomonas* genus which can be used as a marker sequence to look for biosurfactants containing an antimicrobial property encoding for RL biosurfactants development. These genes cluster includes *rhIA* gene-encoding for the chain A of a rhamnosyl transferase to produce fatty acid dimer through the help of ACP- β -hydroxy acids; the *rhIB* gene-encoding for the chain B of a rhamnosyl transferase which catalyzed the production of mono-rhamnolipids by the help of the TDP-L-rhamnose and fatty acid dimer, and *rhIC* gene-encoding for chain C of a rhamnosyl transferase 2 to produce di-rhamnolipids from rhamnose moiety and mono-rhamnolipids (Sood et al. 2020). In addition, Sood et al. (2020) utilized the *rhlAB* operon in silicon analysis to predict RL biosynthetic pathways before the production of biosurfactants from the strains of *Pseudomonas* sp. CR1. Moreover, the Type II PKS (polyketide synthase genes) had been found as a better-characterized gene family which can be used to produce antimicrobial biosurfactant compounds. The enzymes were formed as gene products for the biosynthesis of polyketides which are made up of different domains; and they can be generally classified as non-ribosomal peptide synthetases (NRPSs) because of their complicated production of secondary metabolites with antimicrobial characteristics and biosynthetic pathways (De Giani et al. 2021; Singh et al. 2017). According to Selvin et al. (2016), a large number of KS domains in the polyketide synthase genes were reported in Actinobacteria-producing biosurfactants with antimicrobial activity. Although, another study had shown a strain of *L. reuteri* harboring a polyketide synthase gene cluster along with gene products such as Ab hydrolase (ah), β -ketoacyl-[ACP] synthase II, and polyketide synthase (PKS) condensation.

5 Biocidal Activity of Microbial-Derived Biosurfactant

BSs can demonstrate a highly effective killing effect on several classes of cells causing red blood cell lysis or forming fungal zoospores which can be employed in a bioassay. The most common and interesting question is whether bacteria with cell walls can be killed by BSs. For instance, BSs from sophorolipids can increase sepsis in animal model methods (Bluth et al. 2006; Hardin 2007). Nevertheless, the study by Sleiman (2009) showed that SLs had no antibacterial effect. Live organisms that have health benefits are called probiotics; they are majorly seen inside the intestine of animals and humans. This contributed positively by preventing the colonization of infectious pathogens in the bowels. The mechanism of antimicrobial actions in the probiotic microorganisms can lead to the formation of hydrogen peroxide, organic acids, anti-adhesion factors, bacteriocins, BS compounds, and others (Fig. 1) (Fijan 2014; Gasbarrini et al. 2016; Satpute et al. 2016). Currently, there have not been many studies carried out on the potential wound healing activities of biosurfactants. Other studies by Piljac (2008) and Stipcevic (2006) had used a lower concentration of 0.10% rhamnolipids, the results reflected encouraging results against burns and ulcers. This is another new field of research that certainly needs further study on other biosurfactant compounds that can provide a huge market for a cheap and safe wound healing activity of over-the-counter products. The microbial-derived BSs are largely utilized in agricultural, oil, cosmetic, food, and textile industries (Fig. 2). They can also be used as antibacterial, anti-biofilm, and antifungal agents (Naughton et al. 2019). These microorganisms formed BS have been examined in different areas of research such as anticancer treatments, antimicrobial activity, dermatological care, wound healing, drug delivery systems, and anti-biofilm activity (Table 3).

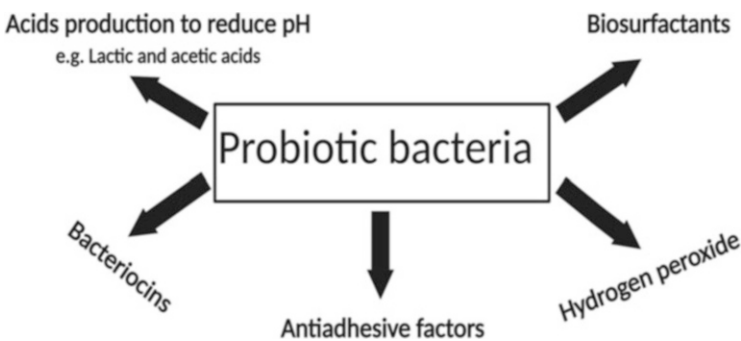


Fig. 1 Different kinds of antimicrobial actions of probiotic bacteria (Satpute et al. 2016; Adu et al. 2020)

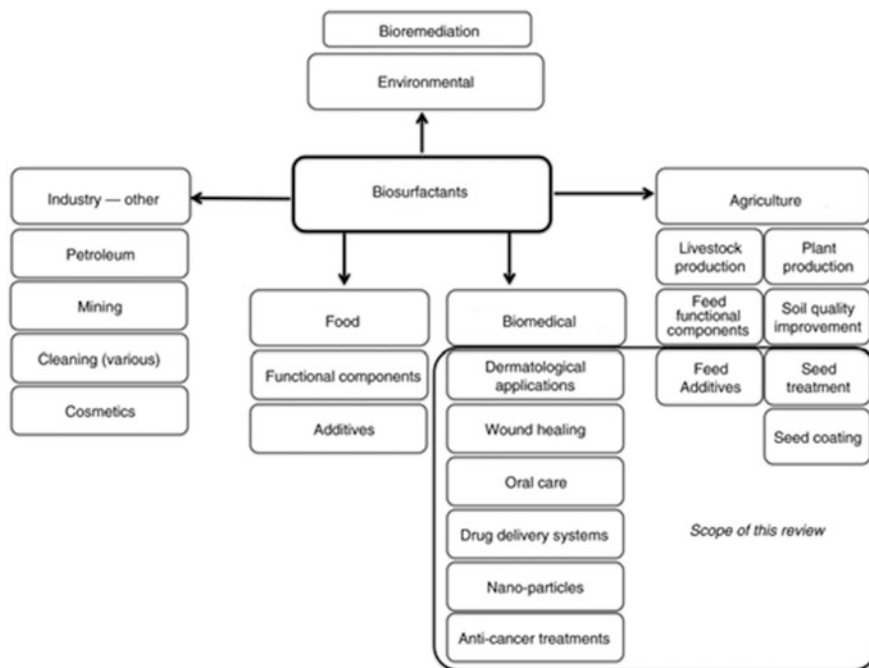


Fig. 2 Different applications of biosurfactants (Naughton et al. 2019)

5.1 Antibacterial Activity of Biosurfactants

Antibacterial property has been revealed among different microbes isolated from the aquatic areas of the Brazilian Amazon basin (Motta et al. 2004). Among the bacteria is a strain from *Bacillus* forming an antimicrobial peptide isolated from the gastrointestinal tract of a fish called Piau-com-pinta (Motta et al. 2007). A study had revealed the antimicrobial property of BS against six human infectious bacteria such as *E. coli*, *S. aureus*, *S. enterica Typhi*, and *P. aeruginosa*; the outcome showed that BS formed by *Bacillus subtilis* C19 could inhibit the growth of GPB and GNB (Yuliani et al. 2018). Mani et al. (2016) suggested that BS formed by *S. saprophyticus* SBPS has demonstrated antibacterial activity against *S. aureus*, *B. subtilis*, *V. cholera*, *E. coli*, and *K. pneumonia*. The BS could inhibit the growth of some GPBs such as *M. flavus*, *B. pumulis*, and *Listera monocytogenes* in food (Naughton et al. 2019). Loiseau et al. (2018) highlighted the activity of BSs produced by *Pseudomonas sp.* as an anti-legionella agent. The results showed that a high number of tested strains were seen to be active, and this antibacterial property was correlated to the presence of tension-active agents in the culture supernatants. Anti-legionella agents had been reported used in the water industry for treatment.

Another study by Ekprasert et al. (2021) reported the use of agricultural wastes to produce, characterize, and investigate the antimicrobial properties of BSs produced

Table 3 Different roles played by various types of biosurfactants in biocidal activity and wound healing

Classes	Type of BS produced	Producing microbes	Biological actions	References
Lipoprotein and lipopeptides	Lipopptide Surfactin	<i>Bacillus licheniformis</i> VS16, <i>B. subtilis</i> VSG4 <i>Acinetobacter junii</i> B6 <i>B. circulans</i> <i>Bacillus natto</i> TK-1 <i>Bacillus subtilis</i> G2III, MI and C4	Adhesive, antibacterial, and antioxidant properties Antiproliferative, anti-biofilm, and antimicrobial activities Ant-adhesive effect Antimicrobial anticancer, and anti-adhesive Antimicrobial action	Cao et al. (2009), da Silva and Sarubbo (2022), Das et al. (2009), Giri et al. (2019), Ohadi et al. (2020), and Sabaté and Audisio (2013)
Glycolipids	Sophorolipids Rhamnolipids Rhamnolipids and surfactin Trehalolipids Glycolipid Heteropolysaccharide	<i>Candida bombicola</i> ATCC22214 <i>Burkholderia thailandensis</i> <i>Halomonas</i> sp. (BOB-3) <i>P. aeruginosa</i> (PAO1) <i>P. licheniformis</i> and <i>P. aeruginosa</i> <i>B. subtilis</i> and <i>P. aeruginosa</i> <i>Mycobacterium tuberculosis</i> and <i>Rhodococcus erythropolis</i> and <i>Acinetobacter</i> sp. <i>Serratia marcescens</i> <i>Trichosporon montevidense</i>	Disorder of bactericidal and biofilm agent for biotechnology use Inhibitor of biofilms formation and antimicrobial function Anti-biofilm effects It affects the architectural process of the biofilm Anti-adhesive property Antimicrobial, anti-biofilm, and anti-adhesive properties Immuno-modulating activities Antimicrobial activities Biofilm disturbance, antimicrobial, and anti-adhesive effects Inhibition of biofilm formation	de Araujo et al. (2016), Dusane et al. (2010), Gomes and Nitschke (2012), Kayanadath et al. (2019), Nickzad and Déziel (2014), Elshikh et al. (2017), Vieira De Araujo et al. (2011), de Araujo et al. (2016), Dusane et al. (2010), Gomes and Nitschke (2012), Kayanadath et al. (2019), Nickzad and Déziel (2014), Elshikh et al. (2017), Shao (2011), and Vieira De Araujo et al. (2011)

Neutral lipids	Phosphatidylethanolamine	<i>Rhodococcus erythropolis</i> and <i>Acinetobacter</i> sp.	Antimicrobial and anti-biofilm properties	da Silva and Sarubbo (2022) and Alvarez et al. (2021)
Phospholipids	Spiculisporic acid	<i>Acinetobacter</i> sp.	Biofilm adhesion	da Silva and Sarubbo (2022), Ishigami et al. (2000), and Sharma et al. (2021)
Fatty acids	Corynomycolic acid	<i>Penicillium spiculisporum</i> and <i>Corynebacterium lepus</i>	Antimicrobial effects	da Silva and Sarubbo (2022) and Kügler et al. (2015)
Polymeric BS	Emulsan Lunasan Mannoprotein Rufisan	<i>Acinetobacter</i> sp. <i>Candida lipolytica</i> UCP 0995 and <i>Yarrowia lipolytica</i> <i>Saccharomyces cerevisiae</i> <i>Candida lipolytica</i> UCP0988	Anti-adhesion and antimicrobial effects Antimicrobial and anti-adhesive properties Anti-adhesive activities Antimicrobial and anti-adhesive effects	da Silva and Sarubbo (2022), Kügler et al. (2015), Luna et al. (2011), and Rufino et al. (2011)
Animal BSs	Calfactant, beractant, and poractant	Oxen Pigs	Antimicrobial activities	Jeon et al. (2015), Qiu et al. (2020), and Zayek et al. (2018)
Vegetable BSs	Saponins	<i>Asparagus officinalis</i> , <i>Glycine max</i> , <i>Quilaja saponarin</i> , <i>Panax ginseng</i> and <i>Cicer arietinum</i>	Anticancer, antifungal, antiviral, antibacterial, and insecticidal activity	Bissinge et al. (2014), Elekofehinti (2015), Güçlü-Ustündag and Mazza (2007), and Rai et al. (2021)

by soil bacteria. Chioma et al. (2017) investigated the production and antibacterial effects of BS formed by some bacteria such as *Corynebacterium* sp., *Proteus* sp., *P. aeruginosa*, *Bacillus* sp., and *S. aureus*. The results showed that *Corynebacterium* sp. produced the least (0.10 g) while *S. aureus* produced the greatest (0.50 g). The BS showed antibacterial properties against *P. aeruginosa* and *S. aureus*. The BS produced from *P. aeruginosa* provided the largest zone of inhibition of 25 mm against *Bacillus* sp., *Corynebacterium* sp., and *S. aureus*, respectively. The BS formed by *S. aureus* gave the largest zone of inhibition against *P. aeruginosa* (39 mm) and *Bacillus* sp. with a minimum zone of inhibition of 25 mm. Moreover, the biosurfactant extracted by *P. synxantha* NAK1 had been reported in the field of medicine (Mukherjee et al. 2014). The antimicrobial effects of the soya bean biosurfactants were not effective to combat *Serratia marcescens*, *Streptococcus pneumonia*, *S. typhimurium*, and *S. aureus*; while *tapai* BSs could inhibit GNB and GNP tested bacteria (Isa et al. 2020). Satpute et al. (2016) reported a few strains of *Lactobacillus* that produce glycolipid BSs; this showed effective antibacterial properties against Gram-negative and Gram-positive MDR bacteria in biofilm and planktonic at in vitro states. Based on the study by Sharma and Singh et al. (2014) on GL biosurfactants produced from *L. casei* MRTL3 utilizing FTIR, it was revealed that the compounds formed were made of lipid and carbohydrate moieties that were established employing 1H-Nuclear magnetic resonance. The results revealed that these biosurfactants could show antibacterial activities against *S. aureus* and *P. aeruginosa*. Furthermore, a study investigated the capability of BS produced by *Georgenia daeguensis* isolated from hydrocarbon-contaminated soil. Their results showed that the BS was able to produce an effective exhibiting antibacterial effect against *K. pneumoniae* (Yağmur et al. 2016).

5.2 Antifungal Activity of Biosurfactants

Recently, a novel *R. babjevae* YS3 had been isolated in Assam's northeastern part of India from an agricultural field. This study showed a major emphasis on the characterization and evaluation of the antifungal effect of the sophorolipid BSs formed from the yeast strain of *R. babjevae* YS3. The results showed that the sophorolipid BSs exhibited potential antifungal effects against some broad groups of infectious yeast such as *F. oxysporum*, *T. rubrum*, *F. verticillioides*, and *C. cassiicola* (Sen et al. 2017). Moreover, a study investigated the antifungal actions of a BS produced by lactic acid bacteria against *Aspergillus* and *Penicillium* isolated from food products utilized in human nutrition. The result of the in vivo study suggested that the BS formed by the new LCM2 (lactic acid bacteria strain) used in biotechnology could act as a substitute antifungal agent in food companies (Matei et al. 2017). Pradnya and Unnati (2015) carried out in vitro study of the antifungal effect of RL biosurfactant formed by *P. aeruginosa* utilizing olive oil as substrates against *C. albicans*. The study revealed that the RL biosurfactant demonstrated antifungal action against *C. albicans*; after purification, it can be utilized as an antimicrobial agent for pharmaceutical and biomedical uses (Pradnya and Unnati

2015). Fang et al. (2014) investigated the antifungal effect and extraction of LPP biosurfactant. The results showed a halo of 31 mm diameter against the *S. sclerotiorum*; it also produced antibacterial activity against *Pseudomonas aeruginosa*, *Rosenbach*, and *S. aureus* (Fang et al. 2014).

Another study investigated the antifungal activity of bio-protective isolates against *Fusarium moniliforme*, *Fusarium pallidoroseum*, and *Botrytis cinerea*. About 22 bacterial isolates were investigated for inhibitory action against fungal phytopathogens. The results revealed that 9 out of 22 bacterial isolates inhibited all the 3 yeasts: such isolates ranging from 40 to 61%, 60 to 68%, and 51 to 62% for *Fusarium moniliforme*, *Fusarium pallidoroseum*, and *Botrytis cinerea*, respectively (de Senna and Lathrop 2017). In addition, a study investigated the quantitative and qualitative antifungal activity of several water-soluble carbon sources on the BS formed by *B. amyloliquefaciens* strain AR2. The results showed that strain AR2 formed an LP type of BS while growing on water-soluble carbon sources. The strain AR2 demonstrated exhibition of carbon-source dependent ST (surface tension) which decrease between 30 and 37 mN/m, CMC range from 80 to 110 mg/L, and emulsification index between 32 and 66% (Singh et al. 2014). Gharaghani et al. (2019) investigated the evaluation of biosurfactants produced by the strains of *Rhodotorula* and its antifungal effect in laboratory conditions. The antifungal effect was evaluated against different saprophytic yeast. Their results showed that all the tested fungi were inhibited at 40 μ L of the BS and about 7.50% of *Rhodotorula* species had the strongest (+5) BS effect while 20.40%, 25.80%, 29.50%, and 16.70% had +1, +2, +3, and +4, respectively. However, biosurfactants produced by fungi had been restricted to some strains of *Aspergillus*, *Penicillium*, *Yarrowia*, *Pseudomonas*, and *Candida* species (Amaral et al. 2010; Gautam 2014; Gharaghani et al. 2019; Kiran et al. 2009). Currently, researchers are investigating the production of BS from *Rhodotorula paludigena*, *R. mucilaginosa*, and *R. glutinis* (Foaad 2007; Halvaezadeh and Mahmoudabadi 2017; Kawahara et al. 2013). The outcomes revealed that strains of *Rhodotorula* are the major producers of BS; thus, they have shown new potential for industrial uses (Gharaghani et al. 2019).

5.3 Antiviral Activity of Biosurfactants

The antiviral action of surfactin, a cyclic LP biosurfactant and antibiotic formed from *B. subtilis* were investigated for a broad spectrum against several viruses such as Semliki Forest virus (SFV), Murine encephalomyocarditis virus (EMCV), Herpes simplex virus, Suid herpes virus, Feline calicivirus, Vesicular stomatitis virus, and Simian immunodeficiency virus (SIV) (Yuan et al. 2018). The in vitro studies demonstrated both herpes and retroviruses inactivation kinetics during therapy against the enveloped viruses. This in vitro study was reported to be more efficient in the enveloped viruses than viruses that are not enveloped. For instance, in a 5% fetal calf serum medium, surfactin had been reported to be very active at 25 mm (Metcalf 2020). These results proposed that the antiviral activity can be caused by physical and chemical reactions of the active-membrane surfactant and the lipid membrane of the virus. This

surfactin had been reported to help improve virus safety applications for pharmaceutical and biotechnological products (Yuan et al. 2018). Besides, biosurfactants have proven to be important in different ways which are vital to managing the pandemic by attacking the signs and symptoms generated by the virus itself.

Currently, BSs are utilized across a wide range of medical and industrial applications; their natural capacity has allowed their usage for a wide range of coronavirus-associated uses (Randhawa and Rahman 2014). However, biosurfactants are used in hand washes and cleaning agents to avoid the virus from targeting and relieving the signs after infection; they can be used to produce reliable antiviral facemasks and drug delivery strategies (Fig. 3). The amphiphilic property

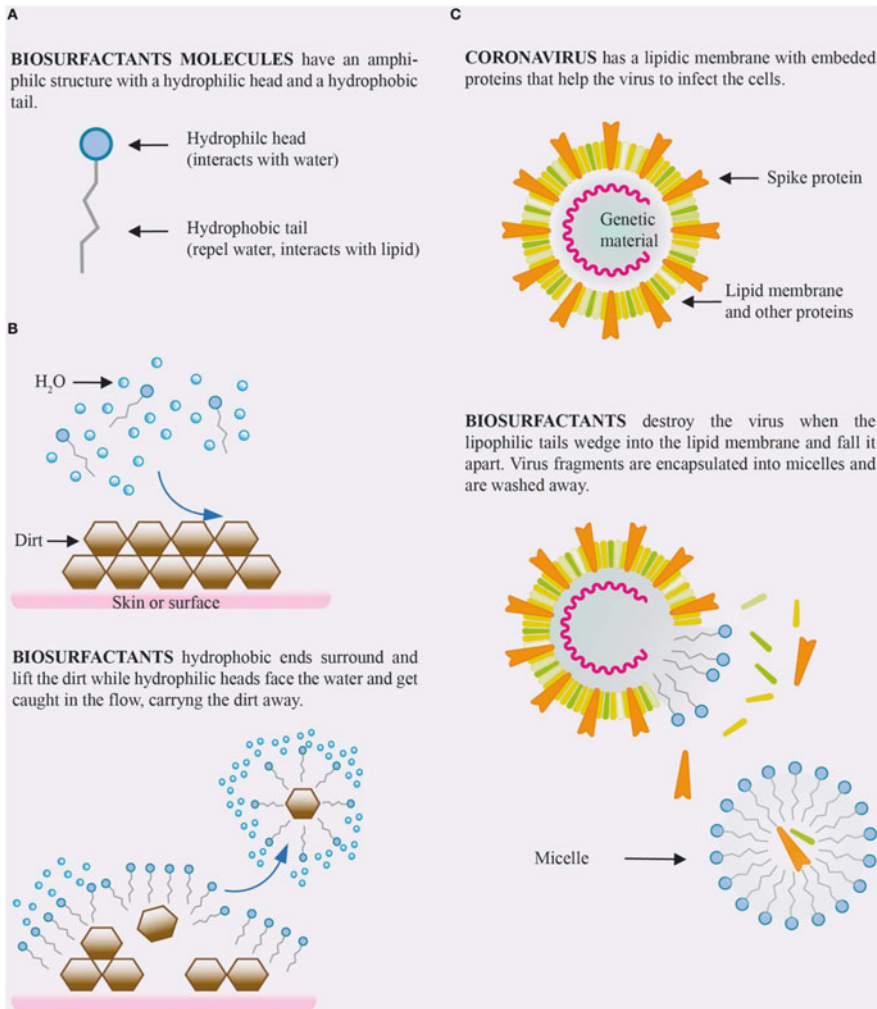


Fig. 3 Schematic representation of antiviral property of biosurfactant against coronavirus

of BS allowed the interaction of the hydrophobic domain, the virus's lipid membrane, and other hydrophilic compounds. These characteristics can help in disrupting the structure of the virus and thus, lead to the deactivation of the virus (Sandeep and Rajasree 2017). The advantage of using biosurfactants alongside their usefulness in the food and pharmacological industries proves them to be an important way to get novel solutions in eradicating the COVID-19 pandemic; hence, this has justified their extensive research to be moving forward (Fracchia et al. 2018; Nitschke and Silva 2018; Ribeiro et al. 2020; Smith et al. 2020).

BS development has often been seen to happen wherever strains have encountered a reduction in resources, including the periods where they can gain from their antimicrobial property. Khan (2017) employed the guarding property of biosurfactants to expand the usage of bioactive peptides to activate enveloped viruses. A similar study had investigated cyclosporine A which is a biological peptide formed from *T. inflatum*; it had been found in reducing the growth of the influenza virus by attacking the viral cycle (Khan 2017). Cyclosporine A cannot influence RNA replication but, it can inhibit steps in protein synthesis including budding and assembly. Smith et al. (2020) carried out in vivo study on B-cell and T-cell responses to human immunodeficiency virus Type 1 against foot and mouth infection (Smith et al. 2020). According to Borsanyiiova et al. (2016), SL one of the classes of microbial glycolipids formed from fungi had been revealed to have shown characteristics of an immunomodulatory, anti-inflammatory, and improved sepsis survival in experimental animal models. Gross and Shah (2007) and Shah et al. (2005) reported that sophorolipids showed inhibition against herpes virus and human immunodeficiency virus through the acetylation of the sophorose head groups. This modification was found to have increased the hydrophilicity of sophorolipid; hence, facilitating its cytokine stimulating and antiviral properties.

5.4 Anti-adhesive Activity of Biosurfactants

BSs were reported useful in preventing the adhesion of infectious pathogens to disease or solid sites and surfaces. Hence, before the adhesion of BS to solid sites, it consists of an effective and modern way to combat the growth of infectious pathogens (Rodrigues et al. 2006a, b; Singh and Cameotra 2004). The function played by the strains of *Lactobacillus* as probiotics in preventing urogenital diseases in the female urinary and genital is to create a disturbance to disease. These pathogens are responsible for the control of vaginal microbiological organisms by competing with other pathogens for adherence to epithelial cells to produce BS. Some studies had revealed the inhibition roles played by BS produced from *L. acidophilus* against yeasts and uropathogenic biofilm formation on silicon rubber (Reid 2000; Rodrigues et al. 2006a, b). A study investigated *L. fermentum* RC-14 producing surface-active constituents that can prevent the adhesive property of *E. faecalis* (Heinemann et al. 2000). Rodrigues et al. (2006a, b) put great effort to develop methods in preventing the microbial growth of silicon rubber voice

prostheses. They evaluated the BS action produced by the probiotic strains of *S. thermophilus* A and *Lactobacillus lactis* 53 against the adhesive property of four bacterial and two fungi species isolated from explanted voice prostheses to pre-coated silicone rubber.

These results have illustrated that BSs were active in reducing the first deposited rate and the number of microbial cells adhering after 4 hours. The BS formed by *S. thermophilus* A was reported to be positive in combating *Rothia dentocariosa* GBJ 52/2B which causes valve prosthesis failure. Rodrigues et al. (2006a, b) further showed that when rinsing the flow chambers designed in monitoring pathogens adhesion with a rhamnolipid BS solution containing deposition and the adhesion was revealed to significantly decrease different strains of yeast and bacterial isolated from explanted voice prosthesis to silicon rubber. This rhamnolipid can also be utilized as a biological detergent solution for prosthesis cleaning to directly help the laryngectomized patients and prolong their lifetime. The function of biosurfactants in defense against inflammation and infection in the human body is a famous phenomenon. A synthesized lipoprotein compound can be known as a pulmonary surfactant secreted by the epithelial lung cells into the outer surface to reduce ST at the air–liquid phase of the lungs. It can also be the main factor in fighting against inflammatory lung infections and other diseases (Wright 2003).

6 Role of Biosurfactants in Wound Healing

Wound healing has been a significant biological process that involves the regeneration and repairing of tissues. A wound can be described as a detriment or disturbance of skin function and structure causing damage to the intrinsic skin barriers (Boateng et al. 2015; Boateng and Catanzano 2015; Zouaria et al. 2016). Boateng et al. (2008) reported healing as an intricate and complex process established in response to an injury that can reinstall the integrity and function of damaged tissues. Wound healing may expose steady cell-to-cell as well as cell-to-matrix interactions to concede the process in three overlying phases; 0–3 days of inflammation, 3–12 days of cellular proliferation, and 3–6 months of remodelling (Schmidt et al. 2009). Besides, platelet aggregation during hemostasis can set free different soluble mediators preceding the healing process and hemostasis may result from a small last inflammatory condition caused by increased complement activation, capillary permeability, vasodilatation and macrophage, and polymorphonuclear movement into the wound site within 3 days (Jones et al. 2007; Tsala et al. 2012). Archana et al. (2013), Boateng and Catanzano (2015), and Kim et al. (2015) revealed that moisturized wounds could be less exposed to infection than dry wounds, this could be because the moisture around the wounds may help in stimulating tissue remodelling, cosmesis, and preventing the infectious attack. The use of available drugs to stimulate the wound healing process had been found limited. Besides the drugs, about 1–3% indexed of pharmacopeias in Western countries had been reported to be utilized as topical administration on wounds (Kumar et al. 2007).

Bouaziz et al. (2014) revealed that the application of polymers and natural molecules had been a leading advancement in regenerating and reconstructing tissues. According to Mandal et al. (2013), large arrays of bioactive metabolites had been demonstrated as a potential for dermatological uses. LP BSs have been applied safely to some dermatological products since they showed lower cytotoxicity against human cells (Mandal et al. 2013). Different LPs have been well documented, this includes surfactin formed from the *Bacillus* genus has gained a lot of interest due to its multipurpose applications in the industry (Nitschke and Costa 2007; Zouaria et al. 2016). Surfactin has been the major researched BS with significant surface features which reduce the surface tension of water from 72 to 27.90 mNm⁻¹ (Zouaria et al. 2016). Regarding continuous study on surfactin, Kanlayavattanakul and Lourith (2010) had revealed that surfactin reduced surface tension to about 26.70 mNm⁻¹ from 54.4 mNm⁻¹ with an interfacial tension between 0.36 and 34 mNm⁻¹ at the CMC of 1–240 mM. Surfactins have also been reported to be exceptionally biocompatible because of their reduced cytotoxicity to mammalian cells, lower irritation to human skin and support for certain applications (Patel et al. 2015).

A study reported different industries that have utilized some surfactin derivatives in dermatological formulations and as cleansing cosmetics agents with washing ability (Mandal et al. 2013). In addition to their anti-wrinkle and cleansing agents, Sun et al. (2006) had explained that surfactin can be used to stimulate elastin and collagen production, because of their moisturizing and free radical scavenging properties; LP BSs can show potential antimicrobial action. Mandal et al. (2013) also reported that lipopeptide BSs could demonstrate potential against multidrug-resistant fungi and bacteria. A similar study by Ghribi et al. (2012) investigated the mixture of crude lipopeptide BS released by *B. subtilis* SPB1; its inhibitory activity was found against 8 and 11 strains of fungi and bacteria, respectively. The results showed significant antimicrobial activity against bacteria and fungi with multidrug-resistant properties. Mnif et al. (2016) used mass spectrum assessment to investigate *B. subtilis* SPB1. This was detected to release surfactin isoforms with molecular weights of 1035, 1021, and 1007 Da; iturin isoforms with the molecular mass of 1060, 1040, and 1028 Da; and fengycins isoforms with the molecular mass of 1446 and 1432 Da. Two new clusters of lipopeptide isoforms with molecular weights of 1411 and 1423 Da and 974 and 988 Da, respectively.

6.1 *In Vitro* Study of Biosurfactants in Dermal Wound Healing

The microbial lipopeptide biosurfactant is made of distinct surface characteristics such as moisturizing, antimicrobial, anti-wrinkle, and antioxidant effects. They can be safely utilized for dermatological products, because they show low cytotoxic effects against human cells (Kanlayavattanakul and Lourith 2010). Zouaria et al.

(2016) investigated the evaluation of in vitro antioxidant and wound healing activities of *B. subtilis* SPB1 LP BS on excision wounds induced in the experimental rats. The result showed that the free radical scavenging effect of *B. subtilis* SPB1 biosurfactant on DPPH radical at 1 mg/mL was around 0.55 mg/mL (70.40%) at IC₅₀. The BS formed from the strains of *B. subtilis* SPB1 can produce an effective inhibition capacity and important activities in the b-carotene test (IC₅₀ = 2.26 mg/mL) when compared to BHA as a reference standard. Most importantly, SPB1 BS showed about 80.32% ferrous ion chelating effect at 1 mg/mL. Besides, Ayed et al. (2015) had reported the topical use of *B. subtilis* SPB1 BS made of gel on the wound site in a rat model every 2 days, the results showed a significant increase in the percentage of wound healing after 13 days when compared to the CICAFLORATM-treated and untreated groups. The BS-based gel SPB1 role in wound healing action was confirmed by a histological study (Zouaria et al. 2016).

6.2 *In Vivo Study of Biosurfactant in the Evaluation of Wound Healing*

A study investigated by Sonam et al. (2017) revealed the wound healing power of a GL formed by the strains of *B. licheniformis* SV1. In confirming the microbial GL wound healing activity, biosurfactant ointment as a transdermal alternative was formulated by mixing the ointment base with the microbial GL and applying the mixture to the wounded skin of Wistar albino rats using an excision wound model. Moreover, collagen and H & E histological stain; hydroxyproline contents and tensile strength were examined to confirm the potential of this biosurfactant-based ointment if it can be utilized as a transdermal alternative. *B. licheniformis* can be regarded as a noninfectious Gram-positive bacteria, which contains the potential to form a GL type of biosurfactant (Gupta et al. 2017b). The Wistar albino rats were divided into four groups and each group had six rats weighed between 200 and 220 g using the in vivo wound model. These rats were anesthetized using ketamine hydrochloride (1%) in an induction chamber. The rats' backs were shaved and cleaned; the biopsy punch was used to create about a 6-mm size excision wound on the rats. The Group I untreated animals were used as a control, Group II animals were administered with ointment base only, and Group III animals were administered with 5% w/w betadine that serves a standard. Furthermore, Group IV animals were administered with 300 mg/kg of BS ointment that serves as the test group animals. The ointments were topically administered to the wounds once per day and assessed for the percentage of closure, time of epithelization, and wound healing effects. The wounded areas were examined until healing was complete.

Chronic wounds can be classified as a decubitus ulcers, venous ulcers, and diabetes-induced traumatic wounds ulcer. The general system required in pathological and physiological chronic wounds includes a cellular response, polymicrobial colonization, and tissue hypoxia, modified systemic and ischemia-reperfusion injury

(Mustoe et al. 2006). A chronic wound is generally colonized by polymicrobial communities which are responsible for continuous inflammatory action and impeded healing processes; thus, decreasing the standard of living (Kalan et al. 2016; Percival et al. 2015). Nevertheless, the therapy required for this kind of wound microbial-derived drug formulations can be greatly sought after as a remedy to treat several chronic wounds and tissue injuries produced outside the epidermal layer of the skin. Microbial-derived surface-active amphipathic compounds can decrease surface tension, bioemulsifier, biodegradable, and non-toxic to produce effective wound healing activity against tissue injuries. A study had reported that *B. licheniformis* SV1 can produce hemolytic activity but, the purified extracellularly formed one shows a better potential for dermal wound healing that was established to be glycolipid in nature after NMR, GC-MS, FTIR, and TLC analyses. Furthermore, phase-contrast microscopic images demonstrated the fibroblast cell proliferation effect of GL which was later confirmed using DAPI fluorescent staining. This dye is known as DAPI fluorochrome, a fluorescent dye that can bind greatly to the A-T-rich region of intact DNA (Varshney et al. 2017). Sophorolipids and rhamnolipids had been reported to increase the bacterial cell membrane permeability and leakage of cellular metabolites which inhibits pathogenic diseases (Elshikh et al. 2016; Gudiña et al. 2013; Diaz De Rienzo et al. 2015). This kind of antimicrobial property may help to increase early wound healing.

6.3 *Biosurfactants as Skin Surface Moisturizer*

Microbial derivatives of biosurfactants were suggested as an alternative to chemical surfactants; human skin has been proven to be compatible by providing a successful skin moisturization surface (Lourith and Kanlayavattanakul 2009; Vecino et al. 2017). Ceramide is a type of epidermal lipid that can help the production of skin disturbance and retain epidermal moisture. According to Choi and Maibach (2005), it was reported that the reduction of ceramides that occurs within the stratum corneum can act as an important factor where skin infections originated such as atopic dermatitis, eczema, and psoriasis. Sethi et al. (2016) reported that synthetic or natural ceramides can be effective in improving skin surface roughness, but they can be costly to produce. Hence, mannosylerythritol lipids possess the same characteristics to provide a suitable substitute for a smaller production (Adu et al. 2020). Different studies had reported that mannosylerythritol lipids (MELs) showed water retention, moisturizing, skin cells, and rough skin recovery effects (Lin et al. 2011; Morita et al. 2009; Yamamoto et al. 2012). Paulino et al. (2016) revealed that the use of MELs in the production of cosmetic products was associated with their potential to improve damaged hairs and water retention of the stratum corneum (Fig. 4). Aquaporins are water channels found in the bacteria, plants, and mammalian cell membrane; they can be considered as a family of proteins that forms water channels in the cell membrane. Mammals have 13 aquaporins (0–12) and this AQP allows the passage of glycerol, urea, and water across the external skin layers; hence, they help

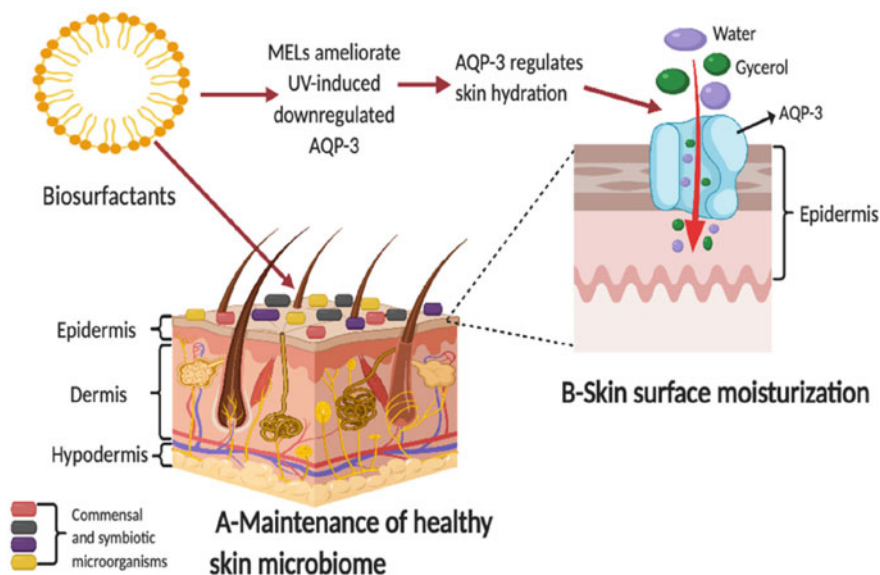


Fig. 4 Potential advantage of GL and LP biosurfactants and their microbiome. (A) It helps in the maintenance of a healthy skin microbiome; (B) It can provide moisturization to the skin surface (Agarwal and Krishnamurthy 2020; Bae et al. 2019; Vecino et al. 2017)

to regulate different skin hydration. Besides, aquaporin-3 in human skin has been considered to be the most abundant and studied aquaporin. Bonté (2011) and Patel et al. (2017) reported that AQP can help to transport uncharged solutes in addition to water and help in regulating the water balance of external skin layers and transportation of smaller solutes. Investigation of the association between disease of age-related skin dryness and AQP-3 expression has proven that there was a decrease in the synthesis of AQP-3 at mRNA and protein levels which can affect skin dryness (Bonté 2011; Ikarashi et al. 2017) (Fig. 4).

Recently, a study revealed the potential of MEL-B to improve ultraviolet induced downregulated AQP-3 in human keratinocytes. It can rejuvenate the skin barrier functions which indicates mammosylerythritol lipids as an ingredient for skin moisturizers to provide a healthy skin microbiome (Fig. 4) (Bae et al. 2019). Another study reported the protective and antioxidant activities of MEL-C against H_2O_2 -induced oxidative stress in human skin fibroblasts (Takahashi et al. 2012). The results indicated that mammosylerythritol lipid-C produced about 50.20% antioxidant effect of 10.10 mg/mL; these results showed the effectiveness of all GLs antioxidant effects (Takahashi et al. 2012). Yoo et al. (2019) reported the potential of mammosylerythritol lipids as a component of skin-whitening formulations that can inhibit the production of melanocytes and increasing of skin tone. In addition, the hypothesis of SLs can mitigate the overload in the subcutaneous part of the skin by the stimulation of leptin synthesis stimulation via adipocytes. Similar studies had reported that RLs are biologically compatible which can better be used in personal

and cosmetic skincare pharmaceutical formulations (Irfan-Maqsood and Seddiq-Shams 2014; Lourith and Kanlayavattanukul 2009). Adu et al. (2020) had established that biosurfactants with lipid ingredients can be used as a moisturizer which enables deeper penetration into the skin by stimulating collagen restoration and controlling other factors that can damage the structure of the skin. Hence, huge data are needed to comprehend the association between the BSs, epidermal layers of the skin, and its constituents such as natural moisturizing factors, keratinocytes, and coenocytes.

7 Commercialization of Biosurfactant Containing Antimicrobial Property

Globally, according to Sajna et al. (2014), BS producers include AGAE Technologies, MG Intobio, Saraya, Soliance, Ecover, and Jeneil Biotech. Jeneil Biotech, Inc. specializes in the production of fermentation-derived natural compounds which can be Generally Regarded as Safe (GRAS) and utilized as sustainable substitutes to traditional synthetics antimicrobials that are utilized in post-harvest preservation of vegetables and fruits, sanitizers, and cleaning solution (Jeneil Biotech 2022). A German chemical industry known as Evonik was reported as the first company to produce BSs on an industrial scale by applying biotech methods (www.business-standard.com 2016). This industry has been working in the last 5 years to develop biosurfactants by using the combination of professionals in the field of interfacial chemistry, process engineering, and biotechnology. Currently, Evonik is majorly focusing its novel BSs on interesting growth markets for individual and household care products.

Besides, many industries in several nations are beginning to manufacture biosurfactants on different scales. At least about two United States companies have produced rhamnolipids utilizing *P. aeruginosa*. AGAE Technologies are currently employing strains of NY3 to produce little amounts of extremely qualified rhamnolipids BS products at affordable pricing (www.agaatech.com). But, the huge productions are being investigated by Jeneil Biotech, generally known as a food additive firm (www.jeneilbiotech.com). The RLs product provided through the Jeneil Company can vary from the crudest formation consisting of fermentation broth of about 2% RLs to relatively qualified products of about 90% RLs. An Irish firm BioFuture Ltd. manufactures RLs for biological remediation of hydrocarbon-contaminated soil, while Pendragon Holdings Ltd. produces PD5 an additive for fuel based on a mixture of enzymes and RL biosurfactants (Kosaric and Sukan 2014).

SLs are currently manufactured by various industries in Korea, Japan, and France where they are being utilized in Yashinomi vegetable wash and dishwasher formulations (Marchant and Banat 2012). In Japan, Sarava Co. Ltd. produces SLs by utilizing *Pseudozyma* containing palm oil which acts as a major fermentation

substrate (Worldwide.saraya.com). Although sophorolipid yields cannot be announced but are expected to be between 30 and 100 g/L. Ecover is another firm that has commercialized some products that consist of “Methyl Rapeseedate Ferment/ Glucose/ Candida Bombicola” (www.Ecover.com). MG Intobio is a Korean company, it markets soaps consisting of SLs, particularly for the treatment of acne (Kosaric and Sukan 2014). Another company from France Soliance has manufactured SLs from rapeseed fermentation for cosmetic uses in skincare via Sebo regulator and antibacterial activity (Kosaric and Sukan 2014). Moreover, Kosaric and Sukan (2014) have reported Sigma-Aldrich Co. LLC is another American firm that had manufactured surfactin with antibacterial, antifungal, and anticancer properties.

8 Conclusion and Future Perspective

Several characteristics of BS have caused a huge range of potential uses in the field of medicine. They can be useful as antiviral, antibacterial, and antifungal agents, and can serve as adhesive agents and immunomodulatory compounds in gene and vaccine therapy. This chapter has described the roles played by biosurfactants in biocidal activities and wound healing. In addition, this chapter has demonstrated that the LPs produced from *B. subtilis* SPB1 can be an attractive candidate to treat wounds which appears to be in connection with their antioxidant effect as well as their antifungal and antimicrobial activity. Besides, it suggests a new role and potential of surfactin A as an efficient and affordable wound healing drug. Similarly, *in vivo* wound healing examination has opened new ways to produce novel biosurfactant-based ointment which is nontoxic and biodegradable as a transdermal alternative over their traditional chemical counterparts. Currently, the growth rate of the biosurfactant market is expanding and its applications in different avenues, but the microbial-derived biosurfactant is lower in quantity and the downstream production can cost about 70 percent of the total expenditure. Therefore, previous studies had shown that there are limited studies on the investigation of antifungal effects of sophorolipids and there is a need for further study on the use of new strains with strong productivity and large applicability. The development and production of an effective wound healing agent is a very significant field of modern medicine that majorly aims to give quality and competent wound healing therapy within a short period and reduce undesired health complications.

Despite the numerous potential of BSs in this area, their applications are still very limited, which may be a result of their higher extraction and production cost and the absence of knowledge and information on their toxicity regarding the human body. The application of BSs at the level of commercialization is both essential and timely to mitigate the dangerous effects of traditional synthetic surfactants on the surrounding. The problems related to cost-efficiency of their potential uses and availability remain unresolved. More so, there may be a need to develop the modes of genomic regulation which can help to study the biological chemistry of BS biosynthesis

enzymes, and the knowledge of its function in cellular communication within the microorganism community. This might lead to the general ecological indication that can decrease biosurfactant production costs and improvement in BS production. More studies on natural microbiota and human cells can be required to authenticate the application of biosurfactants in different health-associated and biomedical fields. Although biosurfactants show to possess higher potential use in the medical field, they are still waiting for full manifestation. The reality that biosurfactants so far have been added to different commercial products bears witness to their potential for further utilization. Hence, there has not been a report of any major challenge to the application of BSs in an extended range of utilizations and products within the next free year, we might look forward to finding an increase in the number of local products consisting of at least MELs and sophorolipids on supermarket shelves. Nevertheless, there is a need to prioritize the fact that the majority of these BSs activities could interact or affect each other to cause side effects for several uses and might need further study.

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Biosurfactants as Potential Antitumor Agents



C. I. Ukaegbu, S. R. Shah, R. O. Alara, and O. A. Thonda

1 Introduction

Biosurfactants are microorganism-produced molecules with surface activity that can be used in a variety of biomedical applications. They are microbially produced amphiphilic surface-active chemicals with significant implications in medicine, food, and bioremediation. Exopolysaccharide (EPS) sheaths surround bacteria in biofilms, protecting them from harmful circumstances. Chemical surfactants have long dominated the market, but attention has recently shifted to the extraction of biosurfactants with reduced toxicity and increased biodegradability (Peele and Ch 2016). Biosurfactants have an intriguing biological activity profile and could potentially be used as antitumor medicines. Biosurfactants have in-vitro antiproliferative activity as reported against human lung cancer cells, as well as antibacterial activities against certain pathogens (Karlapudi et al. 2018). Although biosurfactants have been identified as potential antimicrobial drug candidates in numerous studies, their role in cancer biology has been understudied.

Biosurfactants' antitumor potential is being investigated, even though data on the mechanisms of such action are still rare (Gudiña et al. 2013; Rodrigues 2011). Several studies have shown that biosurfactants separate at interfaces, influencing the adherence of microbes (Rivardo et al. 2009; Mireles et al. 2001; Velraeds et al. 1998). By breaking and lysing microbial cell membranes, these chemicals can

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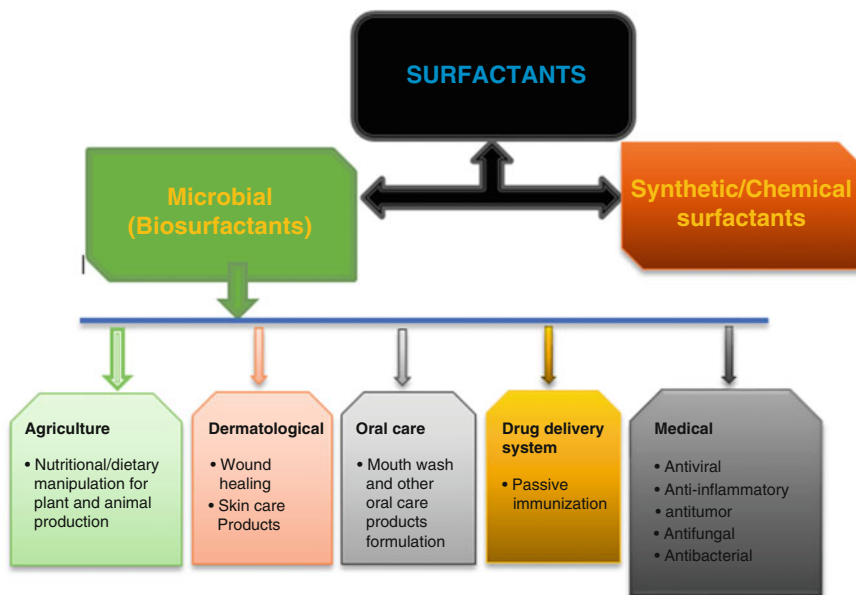


Fig. 1 Broad classification of biosurfactants and their recent applications

increase the membrane permeability (Lee et al. 2012) and can obstruct the progression of some cancers (Fracchia et al. 2012; Rodrigues 2011). The recent applications of biosurfactants in various fields are shown in Fig. 1. This chapter focuses on biosurfactants' possible role as antitumor agents, including their classifications, interaction mechanisms, and potential antitumor activities.

2 Classification, Mode of Action, and Properties of Biosurfactants

Biosurfactants are a large group of amphipathic molecules with varying chemical structures; they are secreted by a variety of microbes as mostly secondary metabolites; they help these microbes to survive by promoting the transport of nutrients, interfering with host–microbe relationship, interrupting microbial quorum sensing mechanisms, as well as acting as biocides (Marchant and Banat 2012). Numerous investigations on their prospective therapeutic applications have been prompted by their recognized potential and biological character (Fracchia et al. 2012; Rodrigues 2011).

Because of their microbial origin, low toxicity, and biodegradability, these molecules outperform manufactured surfactants in many aspects. As a result, they've been extensively researched for uses in the food and cosmetics sectors, as well as improved bioremediation and oil recovery (Marchant and Banat 2012).

Biosurfactants exist as either “low molecular weight biosurfactants (LMWB)” or as “high molecular weight biosurfactants (HMWB)”.

2.1 Low Molecular Weight Compounds (LMWB)

2.1.1 Lipopeptides

Lipopeptides and glycolipids remain the most investigated LMWB molecules. Lipopeptides are mostly secreted by *Bacillus* spp.; they are divided into numerous families, each of which contains numerous variants that vary in their peptide and fatty acid chain moiety (Redhead et al. 2001; Thavasi et al. 2011). Surfactin, iturin A, mannosylerythritol lipids, mono- and di-rhamnolipids, dimycolates, trehalose lipids, acidic and lactonic sophorolipids are the commonly described LMWB. Figures 2, 3, 4, 5, and 6 depict the chemical structures of some common LMWB.

Surfactin is secreted by *Bacillus subtilis* as a cyclic lipopeptide; it was discovered in the culture broth of this microbe by Arima et al. (1968). It is the most biologically active biosurfactant to be exploited (Ron and Rosenberg 2001). Its high surfactant property is the reason for its name (Peypoux et al. 1999). Surfactin is naturally a cocktail of its A, B, C, and D isoforms that are categorized based on their biological effects and variations in their amino acid sequence (Shaligram and Singhal 2010). The structure of surfactin comprised of a 7-amino-acid ring linked by a lactone bond to a fatty acid chain. Surfactin-A contains L-leucine at the amino acid position while surfactin-B and surfactin-C have L-valine and L-isoleucine, respectively as amino acid components; these amino acids are involved in the formation of lactone ring with the C14–C15-hydroxy fatty acid.

There could be differences in the amino-acid residues, and their existence can be linked to changes in the culture conditions, such as culturing the microbe in culture media that contain certain amino-acid residues (Redhead et al. 2001).

Lichenysin is secreted by *Bacillus licheniformis* as a lipopeptide; it is another type of surfactin-related molecule (Horowitz et al. 1990). It has a comparable chemical structure, as well as physiochemical characteristics like surfactin. It differs from surfactin by having glutamine at position 1 rather than glutamic acid of surfactin. Another group of complex acylpeptide antibiotics is pumilacidin A, B, C, D, E, F, and G which have similar characteristics as surfactin; it is secreted by *Bacillus pumilus* and has proven antiviral activity (Naruse et al. 1990; Morikawa et al. 1992).

2.1.2 Iturin

Iturin A is the most researched lipopeptides in the iturin family. It is a heptapeptide that is connected with a β -amino-acid fatty acid; the length of its carbon chain ranges from C14 to C17. It is excreted/secreted by *B. subtilis* and exhibits antifungal properties. Other molecules of the iturin group are iturin C, mycosubtilin, and

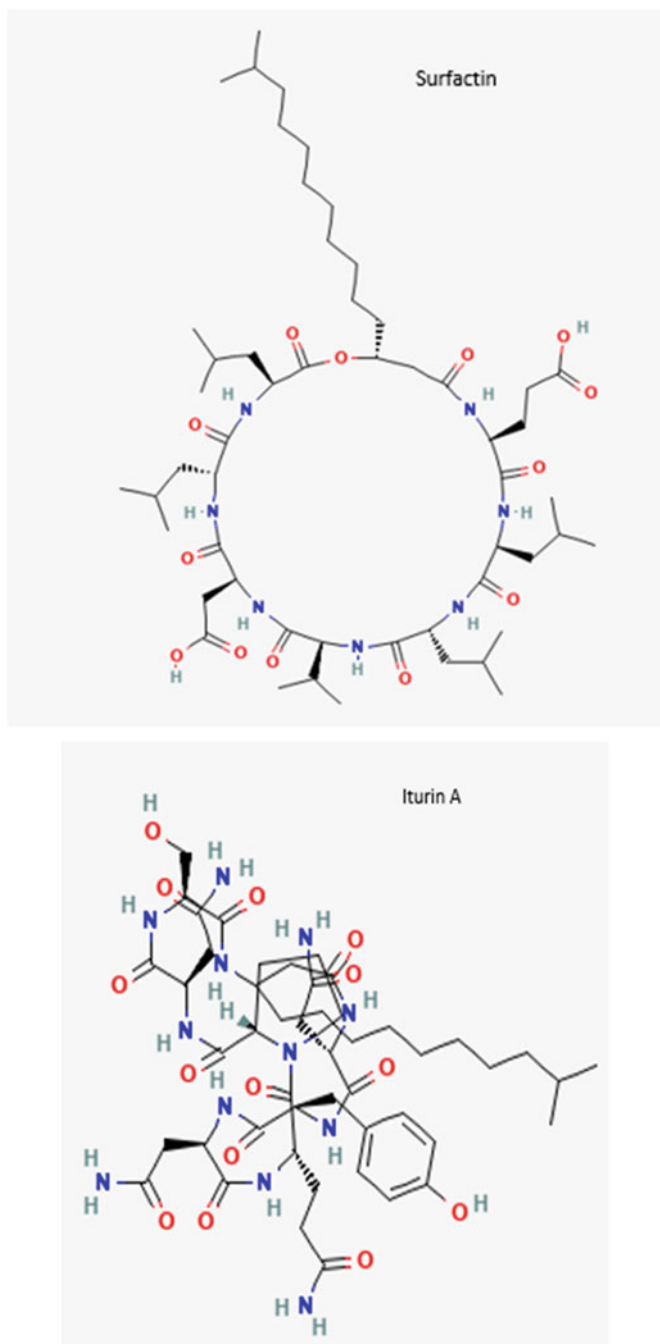


Fig. 2 Structures of Surfactin and Iturin (PubChem)

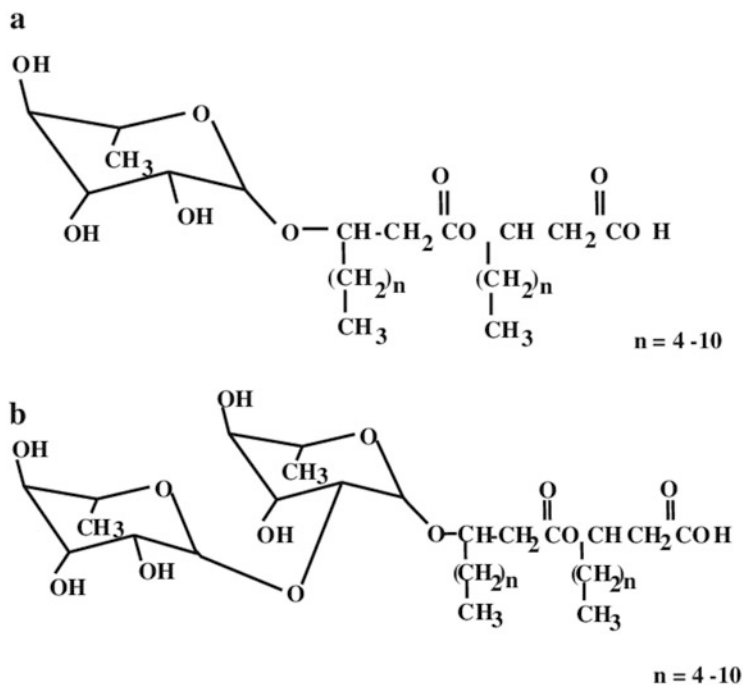
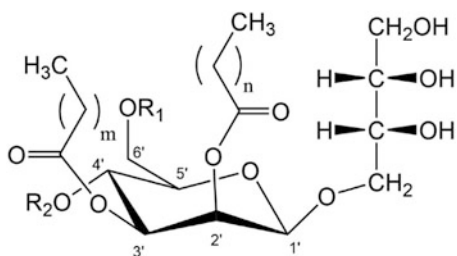


Fig. 3 Structures of (a) Mono-rhamnolipid (b) Di-rhamnolipid (Nereus et al. 2006)

Fig. 4 Structure of di-acylated mannosylerythritol lipids (MEL). A different degree of acylation at C4' and C6' position of mannose leads to the variants MEL-A, -B, -C, and -D (Alexander and Zibek 2020)



bacillomycin D, F, & Lc (Bonmatin et al. 2003). Fengycins A & B are a class of lipodecapeptides that significantly differ in the type of amino acid they contain at position 6; they contain either valine or alanine as their amino acid component and have been experimentally shown to exhibit significant immunomodulating activity and fungitoxic property (Redhead et al. 2001). *Serratia marcescens* produces non-ionic cyclodepsipeptide molecules that are referred to as serrawettins; they have been linked to anti-nematode and antitumor properties (Matsuyama et al. 2010).

Surfactin exhibits a variety of biological activities, including the prevention of fibrin clotting, triggering of ion channel formation in bilayer lipid membranes, inhibition of cyclic adenosine monophosphate (cAMP), inhibition of platelet and

Fig. 5 Structure of trehalose dimycolate (PubChem)

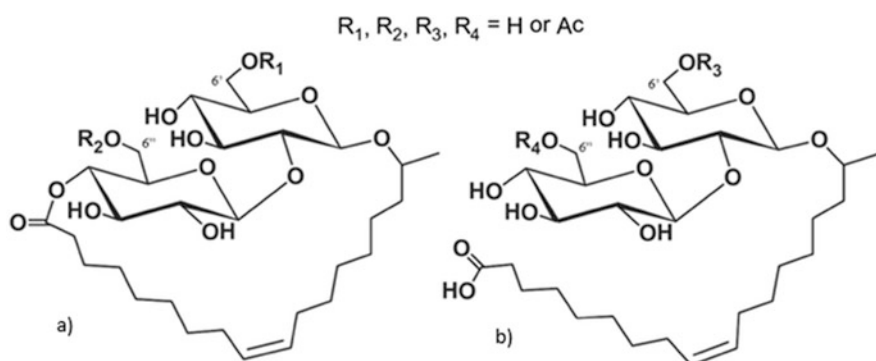
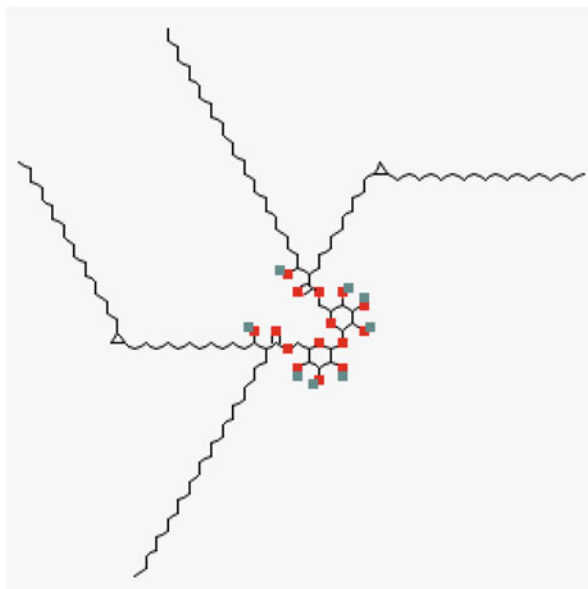


Fig. 6 Structures of (a) lactonic sophorolipid (b) acidic sophorolipid (Amanda et al. 2017)

spleen acytosolic phospholipase A2 (PLA2), as well as antitumor and antiviral properties (Kim et al. 1998). Kim et al. (1998) further noted that the inhibitory effect of surfactin due to direct contact with cytosolic PLA2 can cause selective inhibition of cytosolic PLA2 and can act as a possible anti-inflammatory drug; the study noted that the cytosolic PLA2 inhibition may reduce inflammatory responses. Surfactin therapy boosted proliferation rates and caused morphological alterations in mycoplasma-infected mammalian cells as reported by Vollenbroich et al. (1997). Surfactin's minimal cytotoxicity to cell lines also allowed for selective inactivation of mycoplasmas without causing considerable damage to cell function or the rate of cultured cell proliferation. Surfactin is active against various viruses, such as herpes

simplex virus (HSV), vesicular stomatitis virus, feline calicivirus, murine encephalomyocarditis virus, etc. as reported by another study (Vollenbroich et al. 1997).

Surfactin's antiviral property is mostly due to a physical and chemical interaction between the membrane-active surfactant and the outer viral lipid membrane bilayer, which alters the membrane permeability and inactivates enveloped viruses at higher concentrations. Surfactin C was discovered to boost fibrinolysis in both lab and animal studies via increasing the level of prourokinase activation and causing structural changes in plasminogen (Kikuchi and Hasumi 2002). The role of the plasminogen-plasmin system is in the breakdown of blood clots and other physiological and pathological activities that require localized proteolysis. Surfactin C, when administered with prourokinase in a rat model of pulmonary embolism, boosted the rate of plasma clot lysis. Surfactin may be used in thrombolytic therapy for pulmonary, cardiac, and brain diseases. Biofilms that attach to the surface of biomaterials cause numerous nosocomial infections, including those associated with prosthetic heart valves, central venous catheters, vocal prosthesis, urine catheters, and orthopedic devices. Even though the microbiological origins and host sites differ significantly, many illnesses have certain common traits.

The ability of biofilm-trapped bacteria to avoid host defenses and tolerate antibiotics treatment is the most crucial of these traits. Genetic modification of some popular biosurfactants is a critical aspect in the creation of alternative medicines for therapeutic and prophylactic uses, as antimicrobial resistance is becoming a growing cause of worry in modern medicine. By genetically altering the surfactin production pathway, Symmank et al. (2002) created a new lipohexapeptide with changed antibacterial properties. There was a decrease in observable hemolytic activity that was accompanied by an increased rate of inhibition of the growth of bacterial cells. As a result, surfactin-related compounds may be less hazardous to eukaryotic cells, potentially improving their biomedical applications.

2.1.3 Glycolipids

Glycolipids are the commonest type of biosurfactant secreted by *Mycobacterium* and related species; it contains trehalose lipids when derived from *Mycobacteria*, rhamnolipids when derived from *Pseudomonas* spp., and sophorolipids when derived from yeasts. A two-stage procedure for producing sophorose lipids (SLs) that relies on the use of de-proteinized whey concentrate was established by Otto et al. (1999). A yeast-sourced glycolipid called mannosylerythritol lipid (MEL) can be produced by *Candida* strains from vegetable oils; it has been linked to antibacterial, immunological, and neurological activities.

MEL has antibacterial activity especially against Gram-positive bacteria (Kitamoto et al. 1993). The biological properties of seven microbial extracellular glycolipids [MEL-A, MEL-B, succinoyl trehalose lipids (STL-1 & -3), polyol lipid, rhamnolipid, and SL] have been studied by Isoda et al. (1997). All the glycolipids examined, with the exception of rhamnolipid, caused cell differentiation rather than proliferation in the HL60 cell line. Rather than a basic detergent-like effect, STL and

MEL induced cell differentiation activity by uniquely interacting with the plasma membrane.

Glycolipids are mono- and disaccharide molecules that have been acylated with either hydroxyl fatty acids or long chain fatty acids. The most studied glycolipids are rhamnolipids, MELs, sophorolipids, and trehalolipids. Rhamnolipids are made up of one (mono-rhamnolipids) or two (di-rhamnolipids) rhamnose sugar moieties connected to one or two hydroxy fatty acid chains; they are mostly secreted by *P. aeruginosa* and the *Burkholderia* genus (Raza et al. 2009; Perfumo et al. 2006). Because of their antifungal, antiviral, antibacterial, and antiadhesive capabilities, these compounds have a lot of potential use in the biomedical sector (Remickova et al. 2008; Abalos et al. 2001; Sotirova et al. 2008). They've been employed to synthesize nanoparticles (Palanisamy and Raichur 2009; Xie et al. 2006) and micro-emulsions (Nguyen and Sabatini 2009).

MELs have been secreted by genus *Ustilago* spp. and *Pseudozyma* spp. from *n*-alkane or soybean oil as reported by Arutchelvi and Doble (2010) as a cocktail of partly acylated derivatives of 4-*O*-D-mannopyranosyl-D-erythritol with related hydrophobic groups of C2:0, C12:0, C14:0, C14:1, C16:0, C16:1, C18:0, and C18:1 (Arutchelvi and Doble 2010). MELs are divided into MEL-A, -B, -C, and -D based on the extent of acetylation at the C4 and C6 positions, as well as the order in which they appear on thin layer chromatography (TLC). MEL-A stands for diacetylated, whereas MEL-B and MEL-C are mono-acetylated at positions C6 and C4, respectively. MEL-D refers to a structure that has been totally deacetylated. The environmental compatibility, mild production conditions, structural variety, and varied biochemical roles of MELs have attracted much attention recently. Furthermore, the antimicrobial, anticancer, antifungal, and immunomodulating properties of MELs have been described (Arutchelvi and Doble 2010).

Sophorolipids are glycolipids secreted by *C. bombicola*, *C. batistae*, and *C. apicola* (Van Bogaert and Soetaert 2010). They are made up of two glucose units that are β -1,2 linked. In most cases, the 6- and 6'-OH- are acetylated in these molecules. The attachment of the lipid portion to the reducing end is facilitated by a glycosidic linkage. The fatty acid's terminal carboxyl group can either be lactonic or hydrolyzed to produce an anionic surfactant. Sophorolipids are useful in a variety of biomedical applications as they exhibit antimicrobial, anticancer, and antiviral properties. They've also been employed in cosmetic and pharmaco-dermatological product for the fabrication of metal-bound nanoparticles (NPs) (Van Bogaert and Soetaert 2010). Trehalose lipids are another type of glycolipids with trehalose as sugar component; they have two glucose molecules joined in an α , α -1,1-glycosidic linkage. In *Corynebacteria* and *Mycobacteria*, it is the most basic cell wall glycolipid (Franzetti et al. 2010).

Trehalose 6,6'- dimycolate is a α -branched chain mycolic acid that has been esterified to the C6 position of each glucose; it is the most reported form of trehalose lipids and can be secreted by a host of mycolates, such as *Arthrobacter*, *Rhodococcus*, *Gordonia*, and *Nocardia*. According to Lang and Philp (1998), the *Rhodococcus* genus secretes numerous forms of trehalose lipids. As such, these lipids have received much interest recently due to their roles in cell-to-cell

interaction, as well as their possible antitumor capability (Ortiz et al. 2008; Zaragoza et al. 2009).

Various extracellular glycolipids of microbial origin have been studied for activity on neurite initiation in pheochromocytoma (PC12) cells (Isoda et al. 1997). The ability of PC12 cells to proliferate in a medium with no neurotrophic substance addition made them suitable for examination of several aspects of neural differentiation. The addition of MEL-A, MEL-B, and SL to PC12 cells resulted in a considerable increase in neurite outgrowth. MEL-A had a similar effect on acetylcholinesterase activity by acting as nerve growth factor (NGF). After treating PC12 cells with an anti-NGF receptor antibody that blocked NGF action, MEL-A stimulated neurite outgrowth. Hence, MEL-A and NGF have been found to trigger PC12 cells differentiation via different pathways. MEL was also observed to trigger neurite outgrowth, increase acetylcholinesterase activity, and improve galactosylceramide levels in PC12 pheochromocytoma cells.

Glycolipids can also cause growth inhibition, cell death, and could hamper the progression of malignant melanoma cells as observed in mice, where MEL exposure caused deoxyribonucleic acid (DNA) fragmentation, condensation of chromatin, and a sub-G1 arrest in B16 cells (Zhao et al. 1999, 2000). MEL has also been shown to significantly slow melanoma B16 cells proliferation in mouse in a dose-dependent fashion. Furthermore, MEL activated the secretion of melanoma cell differentiation markers, such as tyrosinase activity and increased/improved production of melanin; this indicates that MEL activated both cell differentiation and apoptotic mechanisms. In addition, MEL has also been reported to increase the acetylcholinesterase activity and arrested the G1 phase of the cell cycle, causing neurite outgrowth and partial cellular differentiation in PC12 cells (Wakamatsu et al. 2001). MEL also promoted neural development in PC12 cells, laying the groundwork for the utilization of glycolipids as therapeutic agents for cancer cells. Nonetheless, more research into the molecular basis of the observed signaling cascade following exposure of PC12 cells to MEL could provide better clue of the processes leading to neurite outgrowth and differentiation of PC12 cell.

Studies have also tested STL-3 analogues for growth inhibition and induction of human leukemia cell line (HL60) differentiation at their critical micelle concentration (Sudo et al. 2000). The activity of STL-3 and its analogues on HL60 cells was discovered to be reliant on STL-3's hydrophobic moiety. Im et al. (2001) also discovered that MEL has a high binding affinity for human immunoglobulin G (HIgG) and suggested that MEL-A could be used as an alternative Ig-ligand after testing the binding affinity of MEL-A, MEL-B, and MEL-C attached to poly (2-hydroxyethyl methacrylate) (PHEMA) beads (Im et al. 2003). The MEL-A-containing composite had the greatest HIgG binding ability among the three studied compounds. More importantly, the bound HIgG was successfully recovered (>90%) at considerably mild elution conditions, showing that glycolipids have a lot of promise as affinity ligand materials. MEL-A greatly enhanced the effectiveness of gene transfection mediated by cationic liposomes with a cationic cholesterol derivative (Inoh et al. 2001, 2004).

Table 1 Some biosurfactants, their class, and their source microorganisms

References	Name of biosurfactant	Class of biosurfactant	Source microorganism
Cao et al. (2010)	Surfactin	Lipopeptide	<i>Bacillus natto TK-1</i>
Zhao et al. (2018)	Iturin	Lipopeptide	<i>Bacillus subtilis</i>
Isoda et al. (1997)	Mannosylerythritol lipid-A	Glycolipid	<i>Candida antarctica T-34</i>
Isoda et al. (1997)	Mannosylerythritol lipid-B	Glycolipid	<i>Candida antarctica T-34</i>
Joshi-Navare et al. (2011)	Sophorolipid	Sophorolipid	<i>Candida bombicola ATCC 22214</i>
Chen et al. (2006)	Di-acetylated lactonic C18:1	Sophorolipid	<i>Wickerhamiella domercqiae</i>
Nawale et al. (2017)	Cetyl alcohol sophorolipid	Sophorolipid	<i>Candida bombicola ATCC 22214</i>
Fu et al. (2008)	Various derivatives	Sophorolipid	<i>Candida bombicola ATCC 22214</i>

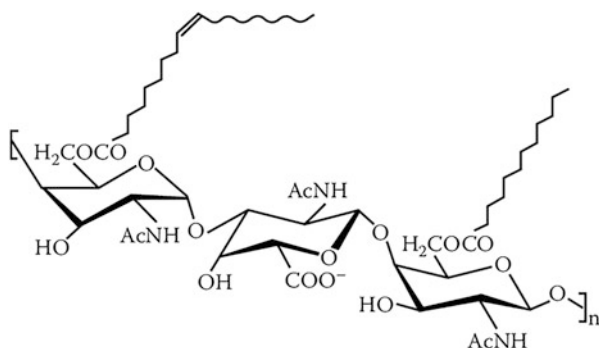
Kitamoto et al. (2002) investigated the synthesis, qualities, and applications of MEL extensively, focusing on its excellent interfacial and differentiation-inducing properties. They also looked at MEL-A's effect on gene transfection utilizing cationic liposomes, as well as its excellent biological and self-assembling properties. Table 1 presents some of the reported biosurfactants and their source microorganisms.

2.2 High Molecular Weight Biosurfactants (HMWB)

The HMWB are generally classified as polymeric biosurfactants and they are made up of polysaccharides, lipopolysaccharides, lipoproteins, lipids, proteins, or complexes that include multiple of these structural forms. They are secreted by a variety of bacteria (Ron and Rosenberg 2001). Emulsan is the commonest HMWB; it is secreted by *A. calcoaceticus* RAG-1 ATCC 31012 as a lipopolysaccharide with a MW of roughly 1000 kDa (Fig. 7). It is the most investigated biopolymer (Rosenberg et al. 1979).

RAG-1 emulsan is a protein-anions heteropolysaccharide compound. Its fatty acids content is about 15% dry emulsan weight and is connected to the polysaccharide backbone via *O*-ester and *N*-acyl linkages; these fatty acids are responsible for the surface activity (Rosenberg and Ron 1999). Alasan is another HMWB that contains a protein and an anionic polysaccharide; its molecular weight (MW) is roughly 1000 kDa and it is secreted by *A. radioresistens* (Smyth et al. 2010). Other polymeric molecules have also been found; however, they are either partially or completely uncharacterized. Apart from the secreting microbe and the general chemical nature of the crude molecule, little is generally known about these

Fig. 7 Chemical structure of Emulsan, the well-known HMWB (Amanda et al. 2017)



biosurfactants. For instance, sulfated heteropolysaccharide is extracellularly secreted by *H. eurihalina* while the acetylated polysaccharide is secreted extracellularly by *P. tralucida* and it is effective in emulsifying various pesticides (Smyth et al. 2010).

3 Biomedical Values of Biosurfactants

Biosurfactants have seen considerable upsurge in their use and prospective commercial value in medicine during the last 10 years. Their biological activities make them promising candidates for use in the treatment of a variety of disorders. Furthermore, because of their biological origin, biosurfactants are typically thought to be safer than manufactured medications. Their usage in these sectors stems from biological features such the capacity to break membranes, resulting in cell destruction and leakage of metabolites via enhanced membrane porosity, and hence antibacterial action. Furthermore, similar to organic-conditioning films, their propensity to partition at interfaces can impact cell/microorganism adhesion qualities (Cameotra and Makkar 2004; Seydlová and Svobodová 2008). Some of the reported bioactivities of biosurfactants include:

3.1 Antimicrobial Activity

The emergence of new harmful microbes and the increasing resistance of the existing ones to most of the existing antibiotics have driven the quest for novel antimicrobial medicines nowadays. In fact, in the past few decades, no new or effective antibiotics have been found (Hancock and Chapple 1999) but the good news is that microbial-sourced metabolites have long been considered a significant source of molecules with novel properties and structures (Donadio et al. 2002).

Some biosurfactants are reportedly suitable substitutes for synthetic medications and antibacterial agents, and as such, may be employed as potential therapeutic

agents (Cameotra and Makkar 2004; Singh and Cameotra 2004). For instance, lipopeptides have the strongest antibacterial action and have been studied extensively in the search for novel therapeutic agents. Lipopeptides exhibit biological activities due to the ability of their molecules to form a pore-bearing channel on lipid membranes (Deleu et al. 2008). Surfactin has been linked to a variety of physical and biological functions, including antibacterial, antiviral, hemolytic, and anti-mycoplasma properties. It can permeate the membrane via hydrophobic interactions, altering the hydrocarbon chain ordering, thereby altering the membrane thickness (Bonmatin et al. 2003). This membrane disruption is a non-specific mechanism of action that is beneficial for action against both Gram-negative and Gram-positive cell membranes (Lu et al. 2007). Surfactin-type peptides have been earmarked as the next generation antibiotics due to their selective activity membrane integrity rather than other critical cellular functions (Rodrigues and Teixeira 2010).

A study by Das et al. (2008) reported that similar bioactive molecules from *B. circulans* had antimicrobial activity against numerous microbes (pathogenic and non-pathogenic), including *B. pumilis*, *M. smegmatis*, *E. coli*, *S. marcescens*, *P. vulgaris*, *C. freundii*, etc. while showing weak activity against methicillin-resistant *S. aureus* (MRSA). This biosurfactant also exhibited non-hemolytic property, implying that it might be used as an antibacterial chemotherapeutic medication. Huang et al. (2011) used a response surface methodology to evaluate the antimicrobial activity of surfactin and polylysine against *S. enteritidis* in milk and found that *S. enteritidis* is susceptible to both molecules, with minimum inhibitory concentrations (MICs) of 6.25 and 31.25 g/mL, respectively. The optimization result showed that *S. enteritidis* load can be reduced by sixfolds at 4.45 °C, requiring a process time of 6.9 h, and 10.03 g/mL concentration. *B. subtilis* strains secrete a wide range of bioactive molecules that include fengycin, iturin, and other compounds of importance (Vanittanakom et al. 1986).

The study by Huang et al. (2008) found that *B. subtilis fmbj* strain secretes a lipopeptide primarily composed of fengycin and surfactin which inactivates the endospores of *B. cereus* by destroying the spores' surface structure. Other antimicrobial molecules secreted by *B. licheniformis*, *B. polymyxa*, and *B. pumilus* are pumilacin, polymyxin B, and lichenysin (Landman et al. 2008). Polymyxin B has been shown to exhibit antibacterial activity against several Gram-negative microbes because of its strong affinity for lipopolysaccharide lipid moieties. As a cation, polymyxin B is attracted to the anionic outer bacterial cell membrane, causing a detergent-like activity that compromises the integrity of the membrane. Polymyxins are effective against most medically important nosocomial microbes like *E. coli*, *P. aeruginosa*, *Acinetobacter* spp., etc. (Landman et al. 2008). Daptomycin (Cubicin[®]) is another potent antimicrobial lipopeptide that is currently being developed for commercial use. It was licensed by the Food and Drug Administration (FDA) in 2003 for managing skin infections. A study by Seydlová and Svobodová (2008) found that daptomycin secreted by *S. roseosporus* is extremely active against MRSA.

Viscosin is secreted by *Pseudomonas* as a cyclic lipopeptide with antibacterial activity and other excellent biological features (Saini et al. 2008). The glycolipids,

rhamnolipids and sophorolipids, are found to exhibit/possess potent antibacterial activity (Benincasa et al. 2004; Abalos et al. 2001; Kim et al. 2002; Van Bogaert et al. 2007). The study by Benincasa et al. (2004) found that a cocktail of six isoforms of rhamnolipids had a very high MIC of 8 g/mL against *B. subtilis*. The antimicrobial activity of MEL-A and MEL-B secreted by *C. antarctica* has been observed against G+ microbes (Kitamoto et al. 1993). *P. aeruginosa* secretes rhamnolipids that exhibited antibacterial activity against *B. cereus*, *S. aureus*, *M. luteus*, *M. miehei*, and *N. crassa* (Nitschke et al. 2010). Flocculosin is secreted by *Pseudozyma flocculosa* as a cellobiose lipid with antibacterial activity against *Staphylococcus species*, including MRSA (Mimee et al. 2009). Excluding the pathogenic *S. aureus* strains, the trehalose lipids secreted by *Tsukamurella* sp. strain DSM 44370 have been shown to inhibit the growth of Gram-positive microbes (Franzetti et al. 2010). This activity was due to the ability of trehalose lipid to increase the fluidity of phosphatidylserine and phosphatidyl ethanolamine membranes, leading to the formation of domains in the fluid state without altering the organization of the macroscopic bilayer membrane (Zaragoza et al. 2009).

3.2 Antiviral Activity

The antiviral activity of surfactin and its structurally related forms has also been reported by Naruse et al. (1990). The activity of these molecules against virus was found to depend on the physico-chemical interactions of the molecules with viral envelope which results in the successful inactivation of enveloped viruses like herpes viruses and retroviruses compared to the non-enveloped viruses (Vollenbroich et al. 1997). Again, some lipopeptides may also exhibit antiviral activity due to the disintegration of viral lipid envelope and capsid as a result of the development of ion channels, resulting in the loss of viral proteins needed for virus adherence and penetration (Seydlová and Svobodová 2008; Jung et al. 2000).

The study by Huang et al. (2006) focused on the antiviral activity of surfactin and fengycin secreted by *B. subtilis fmbj*; the study observed that the molecules effectively inactivated cell-free viral stocks of pseudorabies virus, porcine parvovirus, bursal disease, and Newcastle disease virus and prevented their replication and ability to cause infections. Sophorolipids have also been reported to inhibit the replication of human immunodeficiency virus (HIV) (Shah et al. 2005). Antiviral activity of the rhamnolipid alginate complex was also shown against Type 1 and 2 herpes simplex virus in a dose-dependent manner; this complex effectively inhibited the cytopathic effect of herpesvirus in model kidney cell line (Remickova et al. 2008).

3.3 Antifungal Activity

Biosurfactants have been known for long to have antifungal properties, but their effect against human disease-causing fungus has been poorly documented (Tanaka et al. 1997; Chung et al. 2000). Few studies have focused on the antifungal activities of bio-surfactants secreted by *Pseudozyma flocculosa*, *C. lusitaniae*, *C. neoformans*, *C. albicans*, and *Trichosporon asahii* (Mimee et al. 2005). Under acidic conditions, this cellobiose lipid flocculosin suppressed all pathogenic microorganisms tested and demonstrated synergistic efficacy with amphotericin B. In nature, flocculosin is a bio-control agent used by *P. flocculosa* to combat other fungi.

3.4 Anti-inflammatory Activity

The ability of surfactin to reduce lipopolysaccharide (LPS)-induced nitrogen oxide (NO) generation in RAW264.7 cells via reducing NF- κ B activation has been reported by Byeon et al. (2008), suggesting the potential of surfactin to serve as a microbe-sourced anti-inflammatory agent. Selvam et al. (2009) investigated the impact of a natural probiotic, *B. subtilis* PB6, on the levels of cytokine in people with inflammatory bowel disease (IBD). The role of surfactin in inhibiting phospholipase A2 which is involved in the progression of IBD, was verified as it is secreted by *B. subtilis*. The study also found that the oral *B. subtilis* PB6 administration as a probiotic decreased colitis in rat models of colitis induced by exposure to trinitrobenzene sulfonic acid, as determined by changes in colon shape, mortality rate, and weight gain in animal trials. After oral administration of *B. subtilis* PB6, it was observed that the plasma pro-inflammatory cytokines levels significantly reduced, whereas increases were noted in the levels of anti-inflammatory cytokines, thereby suggesting that *B. subtilis* PB6 inhibits PLA2 via secretion of surfactins.

Furthermore, the anti-inflammatory activity of surfactin isomers secreted by *Bacillus* spp. (No. 061341) was observed (Tang et al. 2010). The study on LPS-induced murine macrophage cell RAW264.7 showed that this class of cyclic lipopeptides strongly prevented overproduction of nitric oxide and release of interleukin-6 (IL-6). Furthermore, the examinations of the structural-activity relationship revealed that the surfactin molecule contains a free carboxyl group that contributed to its anti-inflammatory properties. A study recently explored the mechanism of action of surfactin-induced anti-inflammatory response in periodontitis caused by *P. gingivalis* (Park et al. 2010). It was observed that surfactin suppresses the activity of nuclear factor B in *P. gingivalis* LPS-induced human monocytic (THP-1) cells, thereby reducing the levels of tumor necrosis factor (TNF)-, IL-1, IL-6, and IL-12 (which are pro-inflammatory cytokines) in a heme oxygenase-1 (HO-1)-dependent manner. Surfactin therapy also significantly promotes the secretion of HO-1, which is a key defense against reactive oxygen species (ROS).

4 Potential Use of Biosurfactants as Antitumor Agents

Biosurfactants have exhibited a variety of potentials in biomedicine. For instance, surfactin, MELs, and trehalose lipids have shown immunosuppressive, anti-inflammatory, and immunomodulatory properties, as well as self-assembling, human cell differentiation and stimulation, hemolytic activity, and promoted interaction with stratum corneum lipids. These actions suggest that biosurfactants may have anticancer activity, and various scientists have concentrated on evaluating the antitumor activity of several biosurfactants to explicate this hypothesis. These intriguing microbial compounds, for example, have lately been shown to affect a range of mammalian cell activities. They are thought to play a role in signal transduction, cell immune response, cell differentiation, and other intercellular molecular recognition processes (Osada 1998).

Surfactin has been studied for antitumor activity and found to cause apoptosis in breast cancer cell line (MCF-7) via a reactive oxygen species (ROS)/c-Jun N-terminal kinase (JNK) mediated mitochondrial/caspase pathway (Cao et al. 2010). Recently, the study was furthered by Cao et al. (2011) through evaluating surfactin-induced MCF-7 cell death via the investigation of the impact of ROS and calcium ion on mitochondrial permeability transition pore (MPTP) activity of the cells. Surfactin stimulated the generation of ROS which caused the opening of MPTP and subsequent collapse of the membrane potential of the mitochondria, thereby shooting up the cytoplasmic calcium ion level. Furthermore, the study observed increased release of cytochrome c from mitochondria to the cytoplasm through MPTP which activates caspase-9 and causes cell death.

Another work reported that viscosin secreted by *P. libanensis* M9-3 as a surface-active cyclic lipopeptide blocked the metastasis of PC-3M (prostate cancer cells line) without causing any obvious harm (Saini et al. 2008). Lipopeptides produced by *B. circulans* DMS-2 (particularly isoforms of surfactin and fengycin) have recently shown promising cytotoxic potential against cancer cells as reported by Sivapathasekaran et al. (2010). The study observed that after 24 h of treatment with 300 g/mL of pure lipopeptides, the growth of both HCT 15 and HT 29 colon cancer cell lines was inhibited by >90%; a dose-dependent antiproliferative action of the lipopeptides was observed as well. These compounds also showed significant activity against only malignant cell lines, demonstrating their selective inhibitory function.

Serratamolide AT514 is secreted by *S. marcescens* as a cyclic depsipeptide that belongs to the serrawettins family; this compound has been shown to induce apoptosis in several human cancer cell lines through interfering with the mitochondria-mediated cell death pathway, as well as by interfering with Akt/NF- κ B survival signals. Glycolipids have also been studied for antitumor activities. The diverse interfacial and biochemical actions of MELs are among the properties that made them the most promising biosurfactants of medical importance. MEL-A and MEL-B have exhibited excellent differentiation-inducing and growth inhibition capabilities against various leukemia cell lines such as HL60, K562, and KU812; they have also inhibited the growth of melanoma B 16 cells in mouse (Arutchelvi

Table 2 Anticancer/antitumor activity of biosurfactants against cancer cell lines

Name of biosurfactant	Source microorganism	Class	Effect on cell line	References
Surfactin	<i>Bacillus natto TK-1</i>	Lipopeptide	Induced apoptosis of human breast cancer cell line (MCF-7)	Cao et al. (2010)
Iturin	<i>Bacillus subtilis</i>	Lipopeptide	Prevented the proliferation of leukemia cell line (HL60)	Zhao et al. (2018)
Mannosylerythritol lipid-A	<i>Candida antarctica T-34</i>	Glycolipid	Induced the differentiation of HL60	Isoda et al. (1997)
Mannosylerythritol lipid-B	<i>Candida antarctica T-34</i>	Glycolipid	Induced the differentiation of HL60	Isoda et al. (1997)
Sophorolipid	<i>Candida bombicola ATCC 22214</i>	Sophorolipid	Increased the differentiation of LN-229 (glioblastoma)	Joshi-Navare et al. (2011)
Di-acetylated lactic C18:1	<i>Wickerhamiella domercqiae</i>	Sophorolipid	Induced apoptosis of liver cancer cell line (H7402).	Chen et al. (2006)
Cetyl alcohol sophorolipid	<i>Candida bombicola ATCC 22214</i>	Sophorolipid	Prevented the proliferation of Hela cells	Nawale et al. (2017)
Various derivatives	<i>Candida bombicola ATCC 22214</i>	Sophorolipid	Induced apoptosis of human pancreatic cancer cells	Fu et al. (2008)
Viscosin	<i>Pseudomonas libanensis M9-3</i>		Prevented the metastasis of prostate cancer cell lines (PC-3M)	Saini et al. (2008)
Isoforms of surfactin and fengycin	<i>Bacillus. circulans DMS-2</i>	Lipopeptide	Induced apoptosis of colon cancer cell lines (HCT 15 and HT 29)	Sivapathasekaran et al. (2010).
Serratamolide AT514	<i>Serratia. marcescens</i>	Serrawettin	Induced apoptosis in several human cancer cell lines	Arutchelvi and Doble (2010)
Sophorolipid	<i>Wickerhamiella domercqiae</i>		Induced apoptosis of H7402	Chen et al. (2006)

and Doble 2010). Recently, the study by Chen et al. (2006) found that a sophorolipid derived from *W. domercqiae* induced apoptosis of H7402 cells by interrupting the cell cycle during the G1 and S phases, elevating calcium ion levels in the cytoplasm, and activating caspase-3. A summary of the anticancer/antitumor activity of some biosurfactants is presented in Table 2.

These studies have paved the way for further studies on the antitumor activity of biosurfactants secreted by numerous microorganisms in their habitat. The exploration of the potential antitumor activity of these biosurfactants will add to the pool of the already existing and currently explored natural products for the management of cancerous cells.

5 Conclusions and Perspectives

There is a strong interest in the research of biosurfactants and their possible applications, as indicated by the increasing number of studies on the subject. Biosurfactants, as effective and environmentally friendly substances, completely fulfil the requirement for innovative cancer treatment/management options. The most significant constraint to the commercial application of biosurfactants is the high cost and complexity of the production process, which has hampered their widespread adoption. However, the numerous biological activities of biosurfactants, as well as their recent successes in gene therapy, medical insertion safety, and immunotherapy, imply that they are worth investigating in tumor care.

Due to increased potential economic benefits in the biomedical field, it is believed that the biomedical sector will take the lead in seeing to the development of an optimized and cost-efficient manner of producing these biosurfactants. Advanced biotechnological techniques could also see to the use of recombinant microorganisms that can thrive in a range of cheap renewable substrates to produce biosurfactant in a more profitable and economically feasible manner. In-depth investigations of biosurfactants in terms of their natural involvement in cell-to-cell communication, biofilm development and maintenance, pathogenesis, and cell motility are needed since they could lead to better and more intriguing future biomedical applications of biosurfactants.

6 Companies Working on Biosurfactants as Antitumor Agents

1. Sigma-Aldrich Co. LLC, USA

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Biosurfactants in Oral Cavity Care



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1 Introduction

Biosurfactants are surface-active chemical substances secreted extracellularly by microorganisms such as bacteria, yeasts, and fungi typically present in the oil debased soil (Healy et al. 1996). Biosurfactants have hydrophilic and hydrophobic moieties in their structures and aggregate at the interface between two different polar liquids such as water and oil (Adamczak and Bednarski 2000; Nikolova and Gutierrez 2021). Some important biosurfactant-producing bacterial genera are *Arthrobacter*, *Bacillus*, *Citrobacter*, *Microbacterium*, *Micrococcus*, *Paenibacillus*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Staphylococcus*, and *Streptomyces* (Rani et al. 2020). In dentistry, biosurfactants are recognized for various applications, since they exhibit non-toxicity, biodegradability, antiadhesive, antimicrobial, and high bioavailability properties (John et al. 2022). Biosurfactants tend to have any one of the following structures like glycolipids, mycolic acids, lipoproteins, and phospholipids (Karlupudi et al. 2018). Biosurfactants are commonly utilized in ecological application like oil debasement, medical applications such as anti-cancer agents, and as antimicrobials in the cosmetic industry due to their high surface-activity (Roy

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2017). The bacterial species of *Pseudomonas*, *Bacillus*, *Streptomyces*, and *Stenotrophomonas* present in oil wells (Cai et al. 2015; Yoshida et al. 2005) produce potential biosurfactants using various hydrocarbons as their carbon sources (Varjani and Gnansounou 2017). Biosurfactants inhibit biofilm development through disturbing cell adhesion, disruption of membrane, and electron transport chain (Paraszkievicz et al. 2021; Satpute et al. 2016). On the basis of molecular mass, biosurfactants are classified into low-molecular-mass (glycolipids, phospholipids, and lipopeptides) and high-molecular-mass (Alasan, liposan, emulsan, lipopolysaccharides, and amphipathic polysaccharides) biosurfactants (Satpute et al. 2010; Rosenberg and Ron 1999). Biosurfactants have more advantages than chemical surfactants since they possess higher surface action, highly compatible to the environment, higher selectivity and foaming properties and antimicrobial activities (Roy 2017; Shekhar et al. 2015). In this chapter, the applications of biosurfactants as antimicrobials against oral pathogens, biofilm inhibition and their other uses in dental care are discussed.

2 Types of Biosurfactants

Biosurfactants are classified into glycolipids (rhamnolipids, sophorolipids, and trehalolipids), phospholipids, lipopeptides, lipopolysaccharides, and amphipathic polysaccharides by their chemical composition.

2.1 Glycolipids

Glycolipid biosurfactants are combination of carbohydrates and fatty acids which are linked by an ester or ether group (Santos et al. 2016). *Pseudomonas aeruginosa* producing rhamnolipid (Eslami et al. 2020) and *Candida* sp. producing Sophorolipid are examples for glycolipid surfactants (Kurtzman et al. 2010). The important glycolipids are sophorolipids, rhamnolipids, trehalolipids, mannosylerythritolipids, and cellobiose lipids. They are characterized by decreasing the surface and interfacial tensions, forming pores and destabilizing biological membranes, and possessing antibacterial, antifungal, antiviral, and hemolytic properties. They also act as antiadhesive and antibiofilm agents, detergents, and ingredients in cosmetics (Inès and Dhouha 2015).

2.1.1 Rhamnolipids

Rhamnolipid biosurfactants are glycolipids mainly produced by *P. aeruginosa* (Thakur et al. 2021). Rhamnolipids contain mono or di (L)-rhamnose molecules linked by α -1,2-glycosidic linkage and saturated/unsaturated β -hydroxy fatty acids

linked together by an ester bond (Abdel-Mawgoud et al. 2010). Rhamnolipids are mainly used in food, healthcare, pharmaceutical, and petrochemical industries. Rhamnolipids inhibit the fungal growth and biofilm formation on food products (Jadhav et al. 2011).

2.1.2 Sophorolipids

Sophorolipid biosurfactants are majorly formed by yeasts such as *Candida bombicola* and *Candida batistae*. Sophorolipids are made up of a sophorose molecule linked with a long chain fatty acid by glycosidic linkage. They have minimum of 6–9 different hydrophobic sophorolipids, and their lactone form is preferable for many applications (Casas et al. 1997; Konishi et al. 2008). Sophorolipids are majorly used as detergents in laundry and dishwasher cleaning agents. Sophorolipid-based detergents are biodegradable, ecologically safe, and contain no synthetic detergents (Celligoi et al. 2020).

2.1.3 Trehalolipids

Trehalolipid biosurfactants are glycolipids containing trehalose sugar. Gram-positive bacteria are major producers of trehalolipids especially the species of *Mycobacterium*, *Corynebacterium*, and *Nocardia*. Trehalolipids are also produced by *Rhodococcus erythropolis* and *Arthrobacter* sp. (Franzetti et al. 2010). *Rhodococcus fascians* BD8 produced trehalolipids that exhibit antibacterial activity against *Vibrio harveyi* and *Proteus vulgaris*. They also showed antiadhesive property against bacteria on polystyrene and silicon surfaces, hence they could be used in surface coating products (Janek et al. 2018).

2.2 Phospholipids

Many bacteria, fungi, and yeasts produce phospholipids which exhibit effective biosurfactant properties. *Thiobacillus thiooxidans* and *Rhodococcus erythropolis* are major organisms producing phospholipids (Gayathiri et al. 2022). The most abundant phospholipid is phosphatidylethanolamine which is highly present in the prokaryotic cells. This phospholipid-biosurfactant shows emulsification properties against various hydrocarbons (McClements and Gumus 2016).

2.3 Lipopeptides

Lipopeptide biosurfactants consist of peptide sequences (from 7 to 10 amino acids) connected to a fatty acid chain (between C₁₃ and C₁₈). Major lipopeptides produced by microorganisms are gramicidins and polymyxins. The lipopeptide from *Streptomyces* sp. DPUA1566 (Ramani et al. 2012) has robust application in bioremediation and cosmetic preparations. Another lipopeptide from *Pseudomonas gessardii* had shown metal ion removing properties (Zeraik and Nitschke 2010). Surfactin is the cyclic acidic lipopeptide produced by *Bacillus subtilis*. It has the crucial property of lysing red blood cells. Hemolysis is the important characteristic for initial screening of biosurfactant-producing microorganisms (Arima et al. 1968). *Bacillus licheniformis* produced a lipopeptide called *Lichenysin*, which exhibits tolerance to high temperature, salt, and pH (Purwasena et al. 2019).

2.4 Others

Alasan, liposan, emulsan, lipopolysaccharide, and amphipathic polysaccharides are high-molecular-mass biosurfactants. They are commonly called as emulsifiers or bioemulsans which have high emulsifying efficiency, substrate specificity, and a low critical micelle concentration (CMC). *Acinetobacter radioresistens* KA53 produced a biosurfactant called Alasan which is made up of an amino acid alanine, polysaccharide, and protein (Navon-Venezia et al. 1995). Liposan is produced by *C. lipolytica* and it contains carbohydrate and protein and is used in the food and cosmetic industries (Alizadeh-Sani et al. 2018). *Acinetobacter calcoaceticus* produced a biosurfactant, namely emulsan that degrades crude oil (Amani and Kariminezhad 2016) and it was reported for emulsifying hydrocarbons in water (Gayathiri et al. 2022; Pacwa-Płociniczak et al. 2011; Rosenberg and Ron 1999).

3 Oral Microbiome and Oral Health

The oral microbiome is also known as oral microbiota which colonized in the human oral cavity (Dewhirst et al. 2010). There are different habitats in the mouth like teeth, tongue, inner cheek, hard and soft palates, and gingiva that support colonization of microbes (Kilian et al. 2016). The colonization of normal microflora in the oral cavity is vital for the survival of humans (Elshikh et al. 2016) and it was estimated that the oral microbiome contains more than 700 bacterial species. Most common bacterial genera present in the mouth region are *Firmicutes*, *Bacillus*, *Proteobacteria*, *Actinomycetes*, *Streptococcus*, *Porphyromonas*, *Staphylococcus*, *Prevotella*, and *Lactobacillus* (Mark Welch et al. 2016; Gomez et al. 2017). Even though *S. mutans* is the primary bacterium of the oral microbiome, sometimes it

causes dental plaque (Gomez et al. 2017) (Fig. 1). Microbial colonization could be the planktonic or biofilm states. In planktonic state, microorganisms attach loosely on dental surfaces whereas in the biofilm state they adhere firmly through their specific receptors on dental surfaces (Socransky and Haffajee 2002). The oral microbiome delivers many benefits to humans like maintaining a healthy gut microbiome, maintaining the pH of the oral cavity, neutralizes the acids that cause tooth erosion, reducing colonization of pathogenic microbes, preventing the formation of gum disease, decreasing inflammation in the gum, helps in digestion of food materials, metabolizes nitrates into nitrites, and prevents plaque. The protein-rich dental substratum mainly allows adhesion of bacteria through specific receptors (Kreth et al. 2009) followed by coaggregation and adhesion of initial colonizers (e.g., *Streptococci*, *Fusobacteria* and bacteria from Actinomycetaceae) on the dental surface, which might be leading to develop biofilm (Kolenbrander et al. 2010; Flemming and Wingender 2010; He et al. 2012). Saliva contains rich proteins, minerals, and microbial enzymes which play essential roles in the oral cavity that protects tooth enamel detachment, maintains homeostasis, controls biofilm formation and protects against oral diseases (Amerongen and Veerman 2002).

Deviation and imbalance of the oral microbial community are called dysbiosis of oral microbiome which affects microbiome and host relationship and increases the colonization of pathogenic microbes in the dental plaque. Various factors such as poor oral hygiene, dietary habits, smoking, inflammation in gingiva, and dysfunction of the salivary glands are causing dysbiosis of the oral microbiome. It was found that dysbiosis of oral microbiome might have a link with oral diseases, including dental caries (Costalonga and Herzberg 2014), gingivitis (Kumar 2017), and oral cancer (Wang and Ganly 2014) (Fig. 2). Dental caries arises due to disruption of oral microflora colonization which can be avoided through intake of nutritious and fluoride-enriched foods (Touger-Decker and van Loveren 2003). Oral microbiome dysbiosis has close relationship with rheumatoid arthritis, adverse effects on pregnancy, and cardiovascular diseases (Chen et al. 2018; Cobb et al. 2017; Bryan et al. 2017).

3.1 Links Between Oral and Lung and Oral and Gut Microbiomes

Oral microbiome has a significant relationship with lung microbiome. In a study, the respiratory specimen bronchoalveolar lavage (BAL) from healthy individuals has enriched oral microbiome, especially *Prevotella* or *Veillonella* (Segal et al. 2013). The oropharyngeal microbiome is likely the primary source of the lung microbiome. Metabolites produced by the oral microbiome modulate the host immune responses (e.g., dendritic cells) and determine the host-pathogen interactions in the lung. The oral microbe *Streptococcus salivarius* has a probiotic activity which produces inhibitory molecules that exhibit antimicrobial activity against *Streptococcus*

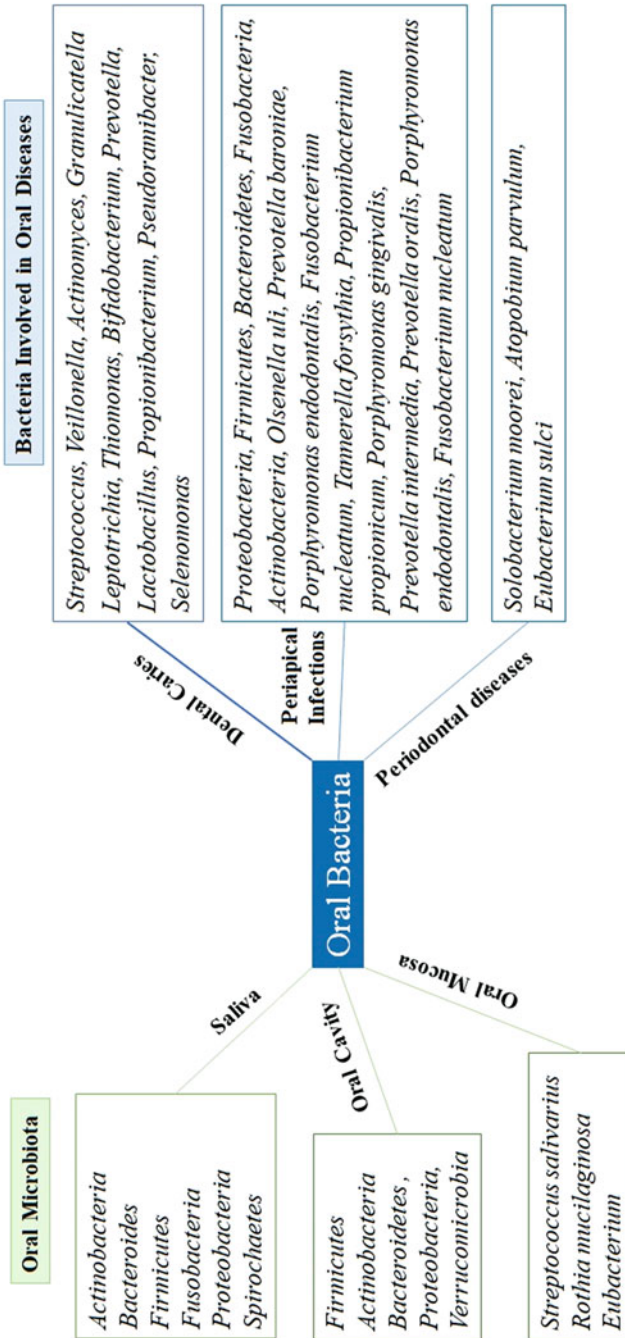


Fig. 1 Bacterial colonization in the oral cavity and bacteria involved in oral diseases

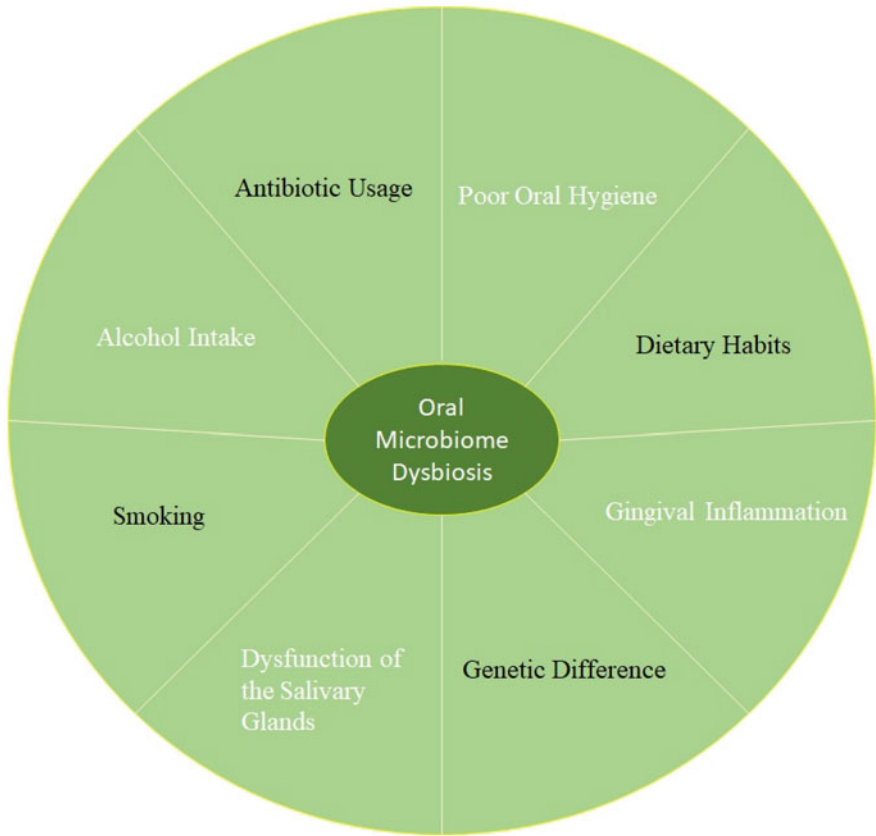


Fig. 2 Factors influencing dysbiosis of the oral microbiome

pneumoniae (Santagati et al. 2012). Gastrointestinal tract is connecting oral and gut regions. Translocation of microbes from oral-to-gut and from gut-to-oral regulates physiological functions and pathological processes and also maintains the microbial ecosystem in both oral and gut regions (Park et al. 2021). Gut microbiome consists of mainly *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. However, *Bacteroidetes* and *Firmicutes* dominate in the gut microbial community, which account for more than 90% of the gut microbiome (Arumugam et al. 2011). These genera are also found in the microbiome of the oral cavity (Avila et al. 2009).

4 Antimicrobial Activity of Biosurfactants Against Oral Pathogens

McCormack et al. (2015) reported that *Staphylococcus aureus* was a frequently isolated organism from the oral cavity. They suggested that the oral cavity would be the source of cross-infection by *S. aureus* and it spreads to other parts of human body. Antimicrobial nanoparticles (NPs) synthesized using rhamnolipid combined with the biopolymer chitosan showed antibacterial activity against *Staphylococcus* strains. In addition, rhamnolipid improved positive charge and stability of chitosan NPs by reducing its size and polydispersity index. Combinations of chitosan and rhamnolipid-nanoparticles (C/RL-NPs) effectively inhibited the *S. aureus* and *S. epidermidis* biofilms due to its increased local delivery on Gram-positive bacterial cell surface (Marangon et al. 2020).

Biosurfactants increase the permeability of the cell membrane of the target organisms. A surface-active biosurfactant complex extracted from *Pseudomonas* sp. PS-17 inhibits *B. subtilis* and this biosurfactant comprises polysaccharide, rhamnose, and 3-oxodecanic acid which forms stable, highly dispersed emulsions with lowered surface tension. However, it fails to disrupt the cell membrane of *P. aeruginosa* and *E. coli* (Sotirova et al. 2009). Biosurfactants produced by *Lactococcus lactis* 53 and *Streptococcus thermophiles* showed antagonistic activity to *Staphylococcus epidermidis*, *S. salivarius*, and *S. aureus*, which colonized on the surface of the artificial voice prosthesis (Rodrigues et al. 2004). Different genes (*swrW*, *swrA*, and *sphA*) are involved in the biosynthesis of biosurfactants that possess antimicrobial activity against bacterial pathogens associated with human health (Fig. 3).

Rhamnolipids are reported to have antibacterial properties against the organisms causing localized invasive periodontitis. Rhamnolipids inhibit biofilm formation ability of the oral pathogens such as *A. actinomycetemcomitans*, *S. mutans*, and *S. sanguinis* (Yamasaki et al. 2020). *Bacillus* sp. produced a lipopeptide called

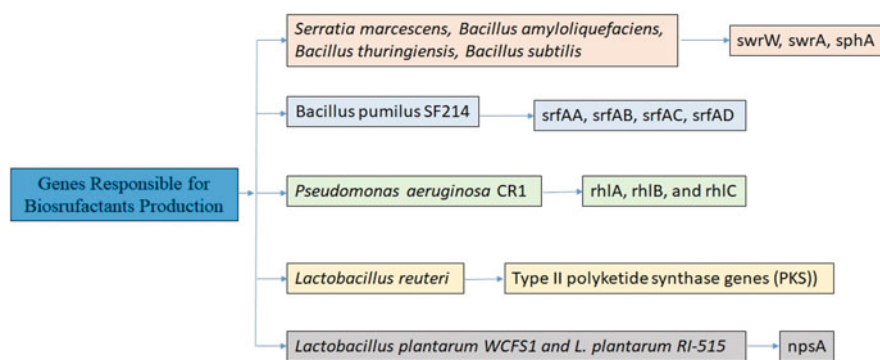


Fig. 3 Genes responsible for biosurfactants production by bacteria (Perez et al. 2017; Saggese et al. 2018; Clements et al. 2019a, b; Mohd Isa et al. 2020; Sood et al. 2020)

mycosubtilin and a combination of mycosubtilin with surfactin was found to be active against food spoilage organisms like *Paecilomyces variotii*, *Byssoschlamys fulva*, and *Candida krusei* (Kourmentza et al. 2021). Biosurfactants produced by microbes and their antimicrobial and antibiofilm activities against pathogenic bacteria and fungi are given in Table 1.

5 Biosurfactants in Controlling Oral Biofilm Formation

Biofilm contains multiple bacterial communities which are attached to the extracellular substances produced by the bacteria themselves. Biofilm formation in medical water supply lines, catheters, surgical items and valves were reported for causing many clinical issues in healthcare receivers. *L. rhamnosus*-derived biosurfactant was evaluated for antibiofilm and antiadhesive activities against the oral pathogen *S. mutans* and found that the biosurfactant downregulated the genes *gtfB*, *gtfC*, and *fff* which are associated with biofilm formation (Tahmourespour et al. 2019). Biofilm disruption and bacterial growth inhibition were observed when biosurfactants are interacted with the cell membrane of bacteria (Busscher and Van Der Mei 1997). It was reported that *B. subtilis* BBK006, *Staphylococcus aureus* ATCC 9144, and *Cupriavidus necator* ATCC 17699 were inhibited by sophorolipids which also disrupt the biofilms (Díaz De Rienzo et al. 2015) (Table 1).

Failure in voice prostheses occurs due to the colonization of oral pathogenic bacteria and fungi. The exo-polymeric substances synthesized by microorganisms cause an unwanted increase in airflow resistance and impede speech (Decho and Kawaguchi 1999). It was also reported that the valve failure is not due to the thickness of biofilms but the presence of exopolysaccharide-producing bacterial and fungal species. Furthermore, *Rothia dentocariosa* was the most commonly isolated bacterium from the patients with prosthetic failure (Elving et al. 2002). Biosurfactant from a *Lactobacillus* sp. isolated from cabbage inhibits *C. albicans*. It also disrupts the biofilm developed on polystyrene by *L. monocytogenes*, *Salmonella arizonae*, *E. coli*, and *S. aureus* and on stainless steel by *L. monocytogenes* (Fracchia et al. 2010).

A study investigated the bio-potential of rhamnolipid isolated from *Burkholderia thailandensis* E264 against oral pathogens such as *Streptococcus oralis*, *Actinomyces naeslundii*, *Neisseria mucosa*, and *Streptococcus sanguinis*. There was a 3–4 logs reduction of bacterial viability and excellent potency in disrupting immature biofilms in a surface coated with the rhamnolipid. It had shown an excellent combination effect with LSS (Lauryl Sodium Sulfate) (Elshikh et al. 2017). A rhamnolipid biosurfactant R89BS produced by *P. aeruginosa* 89 was coated on titanium disks used for dental implantology and the results showed 90% inhibition of *S. aureus* and 70% inhibition of *S. epidermidis* at critical micelle concentration at 4 mg/mL (Tambone et al. 2021).

Biosurfactants formed by *L. jensenii* and *L. rhamnosus* effectively arrest the growth of MDR (multidrug-resistant) *A. baumannii*, *E. coli*, and MRSA

Table 1 Biosurfactants produced by microbes and their effects over pathogenic microorganisms

Organism producing biosurfactant	Biosurfactant produced	Antimicrobial activity against bacteria and fungi	Type of activity	Reference
<i>Lactobacillus casei</i> subsp. <i>rhamnosus</i> 36	Biosurfactant	Uropathogenic <i>Enterococcus faecalis</i>	Antibiofilm	Velraeds et al. (1996)
<i>Streptococcus thermophilus</i>	Biosurfactant	<i>Candida albicans</i> and <i>Candida tropicalis</i>	Antibiofilm	Busscher and Van Der Mei (1997)
<i>L. lactis</i> 53	Biosurfactant	<i>S. epidermidis</i> , <i>S. salivarius</i> , <i>S. aureus</i>	Antimicrobial and Antibiofilm	Rodrigues et al. (2004)
<i>P. aeruginosa</i>	Rhamnolipids	<i>Bordetella bronchiseptica</i>	Antibiofilm	Irie et al. (2005)
<i>Escherichia coli</i>	Polysaccharide	<i>Klebsiella pneumoniae</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , and <i>E. faecalis</i>	Antibiofilm	Valle et al. (2006)
<i>Lactobacillus acidophilus</i>	Biosurfactant	<i>S. aureus</i> and <i>S. epidermidis</i>	Antibiofilm	Walencka et al. (2008)
<i>B. subtilis</i> and <i>B. licheniformis</i>	Lipopeptides	<i>E. coli</i> CFT073 and <i>S. aureus</i> ATCC 29213	Antibiofilm	Rivardo et al. (2009)
<i>Lactobacillus paracasei</i> ssp. <i>paracasei</i> A20	Biosurfactant	<i>C. albicans</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , <i>S. agalactiae</i>	Antimicrobial and Antibiofilm	Gudiña et al. (2010)
<i>Lactobacillus</i> sp. CV8LAC	Biosurfactant	<i>C. albicans</i>	Antibiofilm	Fracchia et al. (2010)
<i>C. lipolytica</i> UCP 0988	Rufisan	<i>S. agalactiae</i> , <i>S. mutans</i> , <i>Streptococcus sanguis</i> 12, <i>Streptococcus oralis</i> J22	Antimicrobial	Rufino et al. (2011)
<i>P. aeruginosa</i>	Biosurfactant	<i>Sarcina lutea</i> , <i>Micrococcus luteus</i> , <i>Bacillus pumilus</i> , <i>Penicillium chrysogenum</i> , <i>C. albicans</i>	Antimicrobial	El-Sheshtawy and Doheim (2014)
<i>Lactobacillus jensenii</i> and <i>Lactobacillus rhamnosus</i>	Biosurfactants	Multi drug-resistant (MDR) strains of <i>Acinetobacter baumannii</i> , <i>E. coli</i> , and <i>S. aureus</i>	Antimicrobial and Antibiofilm	Sambanthamoorthy et al. (2014)
<i>Bacillus</i> and <i>Paenibacillus</i> spp.	Lipopeptides	<i>K. pneumoniae</i> , <i>Enterobacter cloacae</i>	Antimicrobial	Cochrane and Vederas (2016)
<i>Lactobacillus crispatus</i>	Biosurfactant	<i>Neisseria gonorrhoeae</i>	Antimicrobial	Foschi et al. (2017)

<i>L. casei</i>	Biosurfactant	<i>S. aureus</i>	Antimicrobial and Antibiofilm	Merghni et al. (2017)
<i>Bacillus amyloliquefaciens</i> and <i>Bacillus thuringiensis</i>	Lipopeptides	<i>Listeria monocytogenes, Bacillus cereus</i>	Antimicrobial	Perez et al. (2017)
<i>L. jensenii</i> P6A and <i>Lactobacillus gasserii</i> P65	Polysaccharides	<i>E. coli, C. albicans, Staphylococcus saprophyticus, Enterobacter aerogenes</i> and <i>K. pneumoniae</i>	Antimicrobial and Antibiofilm	Morais et al. (2017)
<i>L. acidophilus</i> NCIM 2903	Biosurfactant	<i>S. aureus</i> NCIM 2079, <i>P. aeruginosa</i> MTCC 2297, <i>B. subtilis</i> MTCC 2423, <i>E. coli</i> NCIM 2065 and <i>Pseudomonas putida</i> MTCC 2467 and <i>P. vulgaris</i> NCIM 2027	Antimicrobial and Antibiofilm	Satpute et al. (2018)
<i>B. subtilis</i>	Mycosubtilin, Fengycin	<i>Venturia inaequalis, Spilocaea pomi</i>	Antimicrobial	Desmyttere et al. (2019)
<i>Bacillus</i> sp.	Mycosubtilin (Lipopeptide)	<i>Paecilomyces variotii, Byssosclamyces fulva, Candida krusei</i>	Antimicrobial	Kourmentza et al. (2021)

(methicillin-resistant *S. aureus*) strains. They showed antimicrobial, antiadhesive, and antibiofilm activities against *A. baumannii*, *E. coli* and *S. aureus* at the concentration of 25–50 mg/mL. Electron microscopic study showed that biosurfactants damaged the cell membrane of *A. baumannii* and cell wall of *S. aureus* (Sambanthamoorthy et al. 2014).

6 Mouthwash and Toothpaste Formulations

Microbial biosurfactants have many essential properties, including antimicrobial, skin surface moisturizing, and low toxicity. Hence biosurfactants would be effective alternative to chemical surfactants used in cosmetic and skin care formulations (Adu et al. 2020).

6.1 Biosurfactants in Mouthwash Formulations

Biosurfactants isolated from bacteria are gaining interest in preparing products for oral hygiene and oral care. However, studies on the application of biosurfactants in oral care, especially mouthwash formulations are very limited. In a study biosurfactants formed from *P. aeruginosa* UCP 0992, *B. cereus* UCP 1615, and *C. bombicola* URM 3718 were used along with chitosan and essential oil extracted from peppermint for formulation of mouthwash. Combinations of biosurfactants from *C. bombicola* and *P. aeruginosa* with chitosan exhibit antibacterial activity against microorganisms studied. Toxicity of mouthwashes prepared using biosurfactants was found to be less as compared to mouthwashes prepared using chemical surfactants alone. It was reported that mouthwashes containing biosurfactants are safe and effective and could be the alternative to mouthwashes containing chemical surfactants in controlling oral microorganisms, particularly *S. mutans* (Farias et al. 2019). In another study, the mouthwashes containing biosurfactants showed desirable characteristics like pH 9, 63–95% of foaming ability, and inhibition of viability of cariogenic oral bacteria (Resende et al. 2019).

6.2 Biosurfactants in Toothpaste Formulations

Many kinds of toothpaste are available in the market with different formulations. Toothpaste ingredients must be chosen appropriately, likewise their concentrations. Recently, biosurfactants have been considered as an ingredient in toothpaste formulations. In a study (Bouassida et al. 2017), a chemical surfactant and a lipopeptide extracted from *B. subtilis* SPB1 were separately used as ingredient for toothpaste formulation. The physicochemical properties of the biosurfactant were analyzed for

its spreading ability, water activity, pH, foaming and cleaning tests. Results of the above study revealed that biosurfactant is as efficient as the chemical surfactant, and the study confirmed its suitability in the toothpaste formulation. In that study, the biosurfactant was tested against eight standard bacterial strains such as *E. coli*, *E. faecalis*, *Enterobacter* sp., *L. monocytogenes*, *K. pneumoniae*, *Salmonella enterica*, *Salmonella typhimurium*, and *M. luteus* and it was found that the lipopeptide biosurfactant showed antibacterial activity to *Enterobacter* sp. and *S. typhimurium*. In another study, toothpaste was prepared using a biosurfactant isolated from *Nocardiopsis* sp. which has a pH of 8, comparatively higher than commercial toothpaste and exhibits excellent foaming properties (Das et al. 2013).

7 Conclusion

This chapter discusses biosurfactants' antimicrobial and antibiofilm activities against oral pathogens and their application in industries to formulate mouthwashes and toothpastes. Microbial biosurfactants inhibit biofilm formation by *Aggregatibacter actinomycetemcomitans*, *S. mutans*, *Streptococcus sanguinis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, *Staphylococcus* sp., and *Enterobacter aerogenes*. Moreover, biosurfactants exhibit antimicrobial activity against multidrug-resistant *Acinetobacter baumannii*, *Escherichia coli*, and Methicillin-Resistant *Staphylococcus aureus* and these bacteria have less chance of developing resistance to biosurfactants, unlike antibiotics. Biosurfactants from *Pseudomonas aeruginosa*, *Bacillus* sp., *Nocardiopsis* sp., and *Candida bombicola* find applications in the formulation of mouthwashes and toothpastes. It is concluded that microbial biosurfactants had the potential to be effective antimicrobials, biofilm inhibitors, and potential substances for improving oral cavity health and hygiene.

8 Current Challenges and Future Prospects of Biosurfactants

There is a demand for novel biosurfactants from biological sources for the cosmetic, pharmaceutical, and healthcare industries. Currently, biosurfactants are gaining interest in dental medicine for their usage as antimicrobial agents against oral pathogens, biofilm prevention and coating in the medical instruments and accessories. Biosurfactants have more advantages when compared to chemical surfactants like being readily produced in large quantities, requiring low production costs and less toxic to the environment, animals and humans, and possess many other biological applications. Furthermore, biosurfactants are used for formulations of mouthwashes and toothpastes for maintaining oral hygiene and care. Studies showed that biosurfactants from microorganisms are effective and had the potential to be utilized

in dental medicine. With the advantages listed above, biosurfactants will definitely be the good candidates for treating dental biofilm, dental caries, and other oral-related infections. Importantly many companies are showing interest in developing biosurfactant based products worldwide, but they have not been exploited as much as other commercial products. More studies should be conducted to explore the biological potentialities of biosurfactants in oral cavity care and hygiene through animal experiments and human clinical trials.

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Role of Biosurfactants in Biofilm Prevention and Disruption



Chandana Malakar , Suresh Deka , and Mohan Chandra Kalita

1 Introduction

1.1 Biosurfactant

Biosurfactants are surface-active agents produced by several species of bacteria and yeast. The molecules could be high molecular or low molecular weight cell-bound or cell-free secondary metabolites. Structurally composed of hydrophobic and hydrophilic moieties, biosurfactants are amphiphilic molecules. This structure of biosurfactant facilitates the efficacy of biosurfactant in decreasing the surface tension of various mediums as well as air-water interfacial tension. Owing to their unique structure, biosurfactants are reported to have immense application potential in various sectors such as agriculture, pharmaceutical, cosmetics, food sectors, bioremediation, etc. (Nguyen et al. 2008; Akubude and Mba 2021; Aslam et al. 2021).

The low molecular biosurfactants are classified as glycolipid, lipopeptide, fatty acids, and polymeric surfactants based on the structure. Glycolipids are the biosurfactant that has a carbohydrate moiety attached to a hydrophobic fatty acyl chain consisting of 8–18 carbon. The fatty acyl chain is a long hydroxyl fatty acids chain connected with either an ester or ether group. Based on the carbohydrate

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moieties, glycolipids are classified as rhamnolipid, sophorolipid, mannosylerythritol lipid, and trehalose lipid (Malakar and Deka 2021). Glycolipids are produced by a diverse array of bacteria, and fungi and have tremendous multifarious activities. The lipopeptides consist of peptides attached to a fatty acyl chain. The lipopeptides are classified as surfactin, iturin, and fengycin. Various high molecular weight biosurfactants such as corynomycolic acid, spiculisporic acid, agaricic acid, emulsan, liposan, alasan, and lipomanan are also reported to be produced by several microbial communities (Fujii et al. 1999; Mulligan and Gibbs 2004; Santos et al. 2016; Vijayakumar and Saravanan 2015) (Fig. 1).

These classes of secondary metabolites are produced in response to several environmental conditions by a large number of microbes. Bacteria and yeast belonging to genera of *Pseudomonas*, *Bacillus*, *Rhodococcus*, and *Candida* are reported to produce different types of biosurfactants (Singh et al. 2019). Various species of *Pseudomonas* are reported to produce rhamnolipid, a type of glycolipid structurally composed of one or two rhamnolipids attached to a fatty acyl chain. Species of *Burkholderia* such as *Burkholderia glumae*, *Burkholderia thailandensis*, and *Burkholderia plantarii* are also reported to produce rhamnolipid (Costa et al. 2011; Dubeau et al. 2009; Hörmann et al. 2010). Another form of glycolipid, Sophorolipid is structurally composed of sophorose attached to the lipid chain. They are mainly produced by non-pathogenic yeast such as *Starmerella bombicola*, *Candida batistae*, *Rhodotorula babjevae*, etc. (Costa et al. 2018; Kim et al. 2021; Sen et al. 2017). Sophorolipid has recently been intensely studied owing to multifarious activities. The lipopeptide viz. surfactin, iturin, and fengycin are produced by various *Bacillus* species. Various lipopeptides producing bacteria produce one, two, or all three types of lipopeptides. The lipopeptides are well-known for their antimicrobial activities against a wide range of the pathogen. Surfactin is reported to exhibit more antibacterial activity while fengycin produces effective antifungal activity.

In recent decades, biosurfactants have received enormous interest owing to their multifarious activities. The antimicrobial activities of almost all the discovered biosurfactants have been reported. The presence of a hydrophilic head and hydrophobic tail gives the biosurfactant a structural resemblance with the lipid bilayer of the cell membrane. As a result, biosurfactant exhibits antimicrobial activity by inserting the lipid chain into the lipid bilayer. This results in the cell membrane disintegration and changes in cell membrane permeability. Consequently, the intercellular materials leakage results in cell death (Yalcin and Ergene 2009; Vatsa et al. 2010; Otzen 2017; Sana et al. 2018). Several studies revealed the antimicrobial activity of biosurfactants indicating its efficacy in pharmaceutical fields. An antimicrobial agent needs to exert antibiofilm activity on the pathogen to prevent the recurrence of infection. In this regard, biosurfactants can be a potential alternative as several works of literature report the antibiofilm activity of various types of biosurfactant.

Work involving the antibiofilm strategy of biosurfactants is still in laboratory conditions. The practical antibiofilm application of biosurfactant has not yet developed into a commercial prospect. In the last 5 years, several work has been published

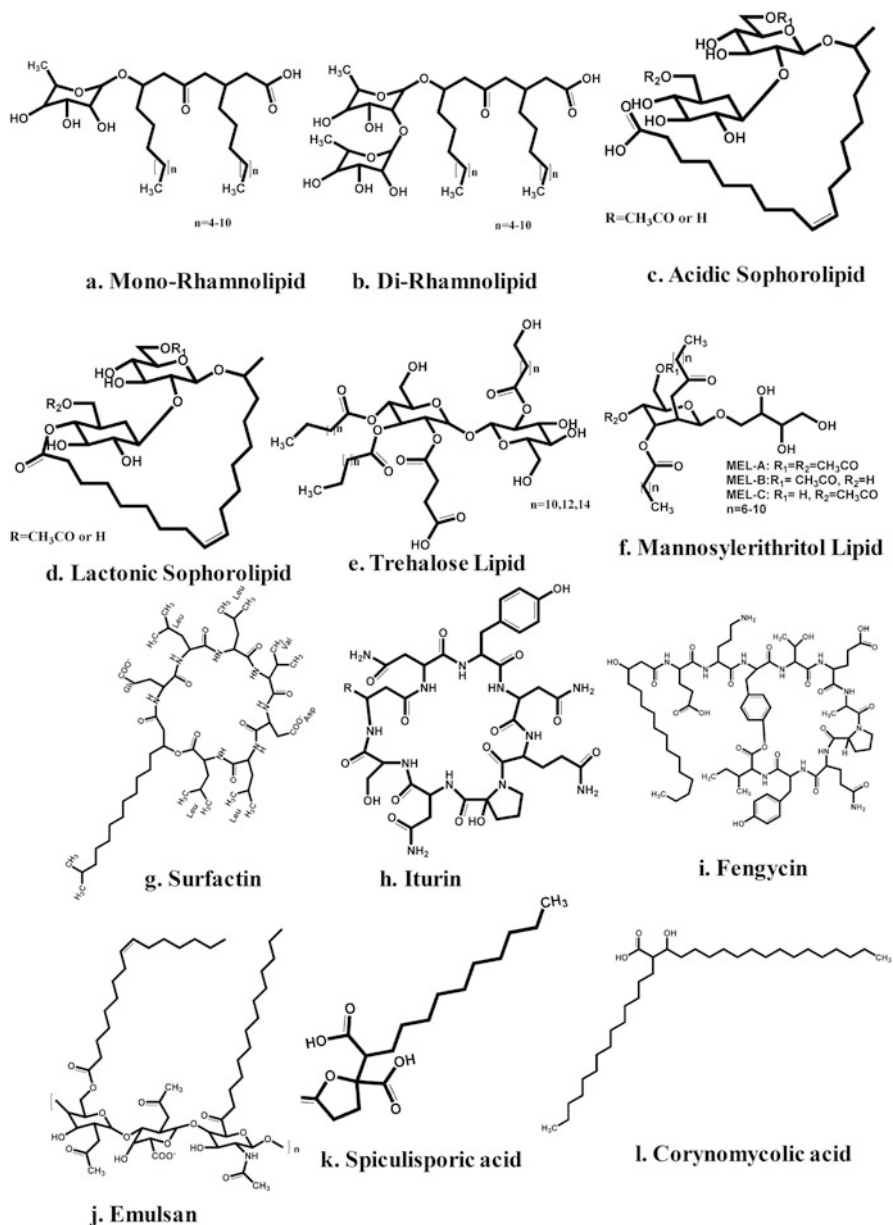
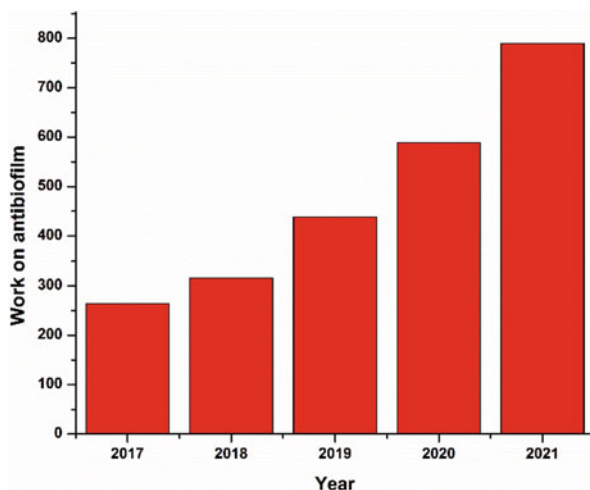


Fig. 1 Structural representation of various types of biosurfactants

which highlights the efficiency of biosurfactant in exhibiting antibiofilm activity. Various types of biosurfactant are investigated, where they have shown efficient antiadhesive, biofilm inhibition and biofilm disruption activity. Figure 2 indicates the increasing amount of work in the biofilm in several sectors.

Fig. 2 Work done on antibiofilm activities of biosurfactant in the last few years



2 Biofilms

The world of microorganisms is very complex. The microbial flora has several impacts on various life forms on the earth. They are an integral part of the food, indigenous flora of several host bodies, and are often part of the gut microflora. The microbes are known to render various beneficial as well as harmful impacts on the host. Although the microbes survive as an individual colony-forming unit, in several cases, they tend to aggregate to form the biofilm. Thus biofilms are an important adaptation and survival strategy commonly employed by bacteria, yeast, and fungal pathogen. Biofilm could be composed of a single type of organism or different microbial colonies, adhered to a given substrate. The biofilm is composed of single or multiple species of the microbes attached while being embedded in an extracellular polymeric substance, known as the exopolysaccharide (EPS). This exopolysaccharide is composed of eDNA, proteins, and polysaccharides (Sharma et al. 2019). Biofilm-associated cells regulate specific genes that have impacts on growth rate. In a complex biofilm, consisting of several species of microbes, the close proximity of the microbes in a biofilm enables the microbes to exchange substrate, various metabolic products, and removal of toxic end products (Hollmann et al. 2022). The formation of biofilm is a multistep approach involving (a) surface adsorption of macro and micro molecules; (b) microbial adhesion to the substratum, (c) EPS production; (d) colony aggregation, and (e) biofilm maturation (Fig. 3).

Biofilm formation is initiated by attachment of microbes to the substratum which is regulated by diverse factors such as growth condition, substratum, and cell surface properties. The type of substratum determines the growth of the biofilm on it. In order to form the biofilm, the planktonic cells must adhere to the substratum. The growth of biofilm is believed to be better on rough and hydrophobic substrates. In addition to this, biofilm formation is also dependent on the type of the microbial

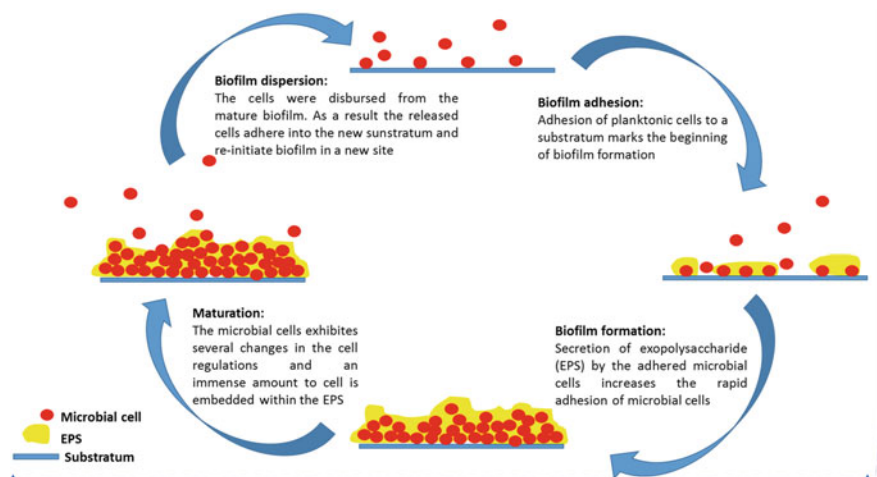


Fig. 3 Steps of biofilm formation

cells. Cells with flagella, pili, fimbriae, or glycocalyx are reported to exhibit efficient attachment of the microbes to the substratum. The cell surface hydrophobicity of the microbial cells is reported to play an important role in microbial attachment to the substratum (Donlan 2001). In certain cases, several microbial colonies form a mobile biofilm that is devoid of the attachment of microbes to the substratum. Cells are thus irreversibly attached to the substratum, which then undergoes cell division to produce micro- and macro-colonies of the microbes.

The attachment phase is followed by the initiation of biofilm formation. Once the cells were successfully attached to the substratum, the microbial cells start to form a monolayer of the microbial cells and secrete exopolysaccharide (EPS) consisting of extracellular polysaccharides, structural proteins, cell debris, and nucleic acids. Initially, the EPS consists of extracellular DNA (*eDNA*) which is ultimately taken over by polysaccharides and structural proteins. Simultaneously there is the formation of microcolonies which exhibits significant growth and quorum sensing. EPS are highly hydrated (98% water) and have micro “water channels” to allow the cells growing within the biofilm to have an access to essential nutrients and oxygen. Biofilm-associated organisms grow more slowly than planktonic organisms.

The microcolonies then start growing on the substratum and eventually develop into a mature biofilm. The biofilm develops in three dimensions. The biofilm architecture of various microorganisms is mediated by EPS molecules, which produces a spatial organization to facilitate cells cluster in microcolonies. The final biofilm formed is a multilayered microbial community. A mature biofilm consists of 10^8 – 10^{11} cells per gram wet weight, which might comprise of the same or several different species (Flemming et al. 2016).

Biofilms are reported to be omnipresent, thus rendering several harmful as well as beneficial effects. Microbial biofilms are reported to be present in tooth enamel surfaces in the oral cavity, ship hulls, medical devices and thus are responsible for

chronic illness, nosocomial infections, industrial pipe fouling, spoilage and contamination of foods, as well as ship hull fouling (Muhammad et al. 2020).

2.1 The Adaptive Beneficial Impact of Biofilm on Microbes

Biofilm renders several adaptive advantages to the microbial colonies involved in the biofilm. Microbes bound to a biofilm tend to resist nutrient deprivation, changes in pH, oxygen radicals, disinfectants, and antibiotics better than planktonic organisms (Jefferson 2004). The biofilm provides a local lifestyle for the microbes affected by stage-specific expression of genes and proteins. The biofilm exopolysaccharide acts as an interface between the biofilm and its environment, enabling its interaction with the surrounding environment. The essential component of the biofilm is the exopolysaccharide which contains water-soluble and water-insoluble components of the matrix. The water-soluble components are gel-forming polysaccharides, proteins, and eDNA, and water-insoluble components are amyloids, cellulose, fimbriae, pili, flagella, etc. (Flemming et al. 2016; Ibanez de Aldecoa et al. 2017). Among these components, eDNA is reported to play an important role in the formation of biofilm and the production of extracellular matrix, which stabilizes the biofilm structure. The eDNA could also be the source of horizontal gene transfer, providing several adaptive capabilities to the microbes within the biofilm. Recent studies have revealed that biofilm is a thousand times better in retreating the effect of antibiotics. Antibiotic resistance has been an emerging global concern as this has failed the effectiveness of several types of antibiotics. Microbes in the biofilm receive protection against antimicrobial drugs, environmental stresses, the host immune system, and shear forces (Santos et al. 2018). In many cases, the biofilm acts as a mediator for horizontal gene transfer, which can sometimes cause the acquisition of antibiotic-resistant genes among the microbes participating in the biofilm.

Biofilm is the microbial society wherein individual microorganisms as well as microbial communities communicate within the biofilm to initiate different physiological processes and cooperative activities. This behavior is influenced by small diffusible autoinducers that are produced by the microbial community within the biofilm (Berlanga and Guerrero 2016). Biofilm offers the opportunity for changes in the microbial cells owing to gene regulation, thereby inciting the formation of novel genetic changes (Fig. 4).

2.2 The Genetic Prospect of Biofilm Formation

Successful production of biofilm is regulated by the up- and downregulation of several genes. Upregulation of *algD*, *algU*, *rpoS*, and genes controlling polyphosphokinase (PPK) synthesis are reported to play a significant role in the biofilm formation of *P. aeruginosa* (Pulcini 2001). Various genes play an important

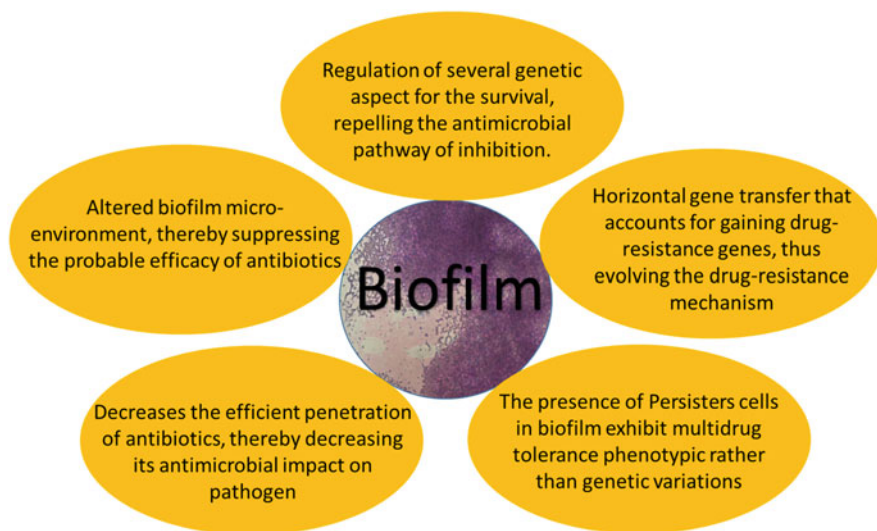


Fig. 4 Beneficial impact of biofilm formation on the microbial community

role in the synthesis of biofilm matrix such as *csgA*, involved in the synthesis and aggregation of colanic acid protein in *E.coli* (Jefferson 2004). *algC* gene, required for alginate synthesis in *Pseudomonas aeruginosa* plays an important role in maintaining the pathogen biofilms (Davies et al. 1993). In the case of gram-positive biofilms such as in the biofilm of *S. mutans*, sucrose-dependent polysaccharide production and biofilm formation are influenced by Glucan binding protein *GbpA* (Loo 2003). Intercellular adhesin locus (*icaADBC*) in *Staphylococcus aureus* and *Staphylococcus epidermidis* are reported to encode the genetic products responsible for the synthesis of a β -1-6-linked poly-*N*-acetylglucosamine polymer called PNAG or PIA (polysaccharide intercellular adhesin) (Heilmann 2003).

Biofilms of *Staphylococcus aureus* were reported to upregulate genes encoding enzymes involved in glycolysis or fermentation due to oxygen limitation in the developed biofilm (Becker et al. 2001). Owing to the upregulation of certain genes and downregulation of other genes, the metabolic activity of the biofilm embedded cells is altered compared to the planktonic cells. Nakamura et al. (2016) reported that in a biofilm, genes involved in the biosynthesis of other secondary metabolites, xenobiotics biodegradation and metabolism, lipid metabolism, membrane transport, amino acid and carbohydrate transport, biosynthesis of secondary metabolites, and stress response are upregulated, while the genes involved in the respiratory chain, nucleotide biosynthesis, fatty acid metabolism, and DNA repair are downregulated. Rumbo-Feal et al. (2013) reported the overexpression of 1621 genes in the biofilm of *A. baumannii* compared to stationary phase cells including 55 genes that were only expressed in biofilms, thereby causing changes in amino acid and fatty acid metabolism, motility, active transport, transcriptional metabolism, and quorum sensing. Thus, with several upregulation and downregulation of the genes, the organisms in

the biofilm community thrive in the biofilm, being protected from several harsh environmental factors.

2.3 *The Beneficial Impact of Biofilm*

Several microbial biofilm and consortia are reported to exhibit various beneficial impacts in day-to-day anthropogenic activities. Rapid industrialization, urbanization, and exponential population growth have created major water contamination. In various cases, bacterial communities have been employed through biofilm-based wastewater treatment technology to neutralize and degrade organic and inorganic compounds in wastewater. (Muhammad et al. 2020). In this technology, biofilm-forming microorganisms are added to the wastewater which then utilizes organic and inorganic compounds present in the wastewater as nutrients. The pathogens present in the wastewater are also trapped by the biofilm, thereby cleaning the water (Sehar and Naz 2016).

Microbial biofilms are also part of various plant, animal, and human body systems. Along with rendering harmful effects, in certain cases, biofilm is reported to exhibit a beneficial impact. In the agriculture system, the biofilm of plant growth-promoting microbes renders efficient protection against several phytopathogens. Rhizobacteria colonize the roots of plants, thereby promoting plant growth through nitrogen fixation, mineral uptake, production of phytohormone, pathogen suppression as well as protection from both biotic and abiotic stresses (Goswami et al. 2020). Goswami and Deka (2020) reported that root colonization of *B. altitudinis* in mustard plants yielded better root architecture along with elevation of the growth factors. The root colonization of microbes mainly involves bacterial isolates belonging to the genera *Bacillus*, *Pseudomonas*, *Streptomyces*, *Serratia*, and *Stenotrophomonas* (Arrebola et al. 2019). Biofilm by *Paenibacillus polymyxa* in the rhizosphere of peanuts was reported to protect against crown root rot disease caused by *Aspergillus niger* (Haggag and Timmusk 2008).

Certain microorganisms can remediate hydrocarbon contaminated sites. The introduction of biofilm producing hydrocarbon-degrading microbes can remove the hydrocarbon from the contaminated sites (Upadhyayula and Gadhamshetty 2010; Rodríguez-Martínez et al. 2006). This formation of biofilm can enhance the rate of remediation of noxious hydrocarbon.

There are reports that certain bacterial strains can be used to prevent the corrosion of many metals. Zuo et al. (2004) reported that a cyclic decapeptide produced by biofilms of *Bacillus brevis* was effective in inhibiting corrosion-causing, sulfate-reducing bacteria (SRB), thereby preventing mild steel corrosion. Aerobic biofilms are reported to better prevent corrosion due to their efficient oxygen consumption (Kip and Van Veen 2015).

A huge number of beneficial microbes are present in the human gut. The gut microbiome plays a vital role in different metabolisms which were found to be present from the oral cavity to the large intestine (Hussain et al. 2020). This

colonization of gut microbes starts at birth or even before when the virtually sterile baby encounters new microbial environments (De Vos 2015). Biofilms formed by the gut microbiota use quorum sensing (QS) to coordinate their social behavior, thereby influencing host cell activities in a non-invasive manner (Deng et al. 2020). The colonization of various beneficial bacteria and yeast on several parts of the host body is reported to provide several benefits along with repelling pathogens (Byrd et al. 2018).

3 Biofilm: A Threat

Although, there are reports that biofilms have some beneficial impact, however, the harmful effect of it cannot be ignored. Biofilms are one of the major reasons for the recurrence of infection in many cases. Their presence is detrimental to several health aspects of the human and life stock. Biofilms have a detrimental effect on the food processing industry as biofilms may lead to food spoilage which would be harmful (Galie et al. 2018). Biofilm formed by *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas* spp., *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Geobacillus stearothermophilus*, and *Campylobacter jejuni* is reported to pose several health threats such as bacterial gastroenteritis, food spoilage, diarrhea, foodborne intoxications, and emetic syndrome (Muhammad et al. 2020). The persistence of various biofilms on drinking water distribution systems can be the cause of severe health hazards (Loveday et al. 2014). Biofilms of phytopathogen are reported to cause a detrimental impact on agriculture. Biofilm of pathogen tends to revert the effect of several antibiotics used in agriculture, thus impacting the agriculture yield. Pierce's disease of grapevines and citrus canker are reported to be caused by the biofilm produced by *Xanthomonas citri* and *Xylella fastidiosa* (FERENCE et al. 2018; Kyrkou et al. 2018). Biofilms produced by *Ralstonia solanacearum* is reported to be involved in the pathogenesis of tomato (Mori et al. 2016; Yao and Allen 2007). Biofilm produced by *Pseudomonas aeruginosa* on roots of *A. thaliana* and sweet basil is reported to kill the plants within 7 days (Danhorn and Fuqua 2007).

The most significant negative role played by the biofilm is its role in several hospital-acquired infections. The persistence of biofilms produced by pathogens in various medical devices such as breast implants, mechanical heart valves, joint prostheses, pacemakers catheters, ventricular shunts, contact lenses, prosthetic heart valves, cerebrospinal fluid shunts defibrillators, and ventricular-assisted devices are reported to exhibit several health threats (Darouiche 2004; Muhammad et al. 2020). Medical devices are often contaminated with biofilms produced by coagulase-negative *Staphylococci*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus* sp., and *Candida albicans* (Kokare et al. 2009). These contaminated devices might expose pathogens to the host internals, thereby resulting in fatal systemic infections. Recurrence of biofilm is reported to be a constant reason for the persistence of various infections. Among several pathogens, *S. aureus* and coagulase-negative *Staphylococci* are reported to

cause two-thirds of implantable device-associated Staphylococcal infections. Among several staphylococcal species, *S. aureus* and *S. epidermidis* are the leading cause of hospital-acquired, surgical site, and bloodstream infections with high hospitalized rates (Khatoun et al. 2018). Biofilm of pathogenic bacteria is reported to be the main cause of diseases such as cystic fibrosis (CF), chronic wounds, infective endocarditis (IE), periodontitis, otitis media, and osteomyelitis (Southey-Pillig et al. 2005; Akyıldız et al. 2013; Masters et al. 2019; Jamal et al. 2018). It is estimated that 65% of all bacterial infections and 80% of microbial infections are associated with biofilm (Jamal et al. 2018; Dhar and Han 2020).

The biofilm retreats the effect of several antibiotics, thereby failing their antimicrobial activity against the pathogens (Vestby et al. 2020). Different pathways are involved in the antimicrobial repelling activity of biofilms such as slow or incomplete penetration of the antibiotics into the biofilm, an altered chemical microenvironment within the biofilm, multicellular properties of the biofilm, EPS-mediated inhibition of the diffusion of the antibiotic into the biofilm. Antibiotic resistance of biofilm is rendered by the multicellular nature of biofilms (Sharma et al. 2019). Persister cells are another type of cells in a biofilm in which the cells are in a dormant state exhibiting multidrug tolerance phenotypic rather than genetic variations (Helaine and Kugelberg 2014; Ayrapetyan et al. 2015).

3.1 Harm Rendered by Bacterial Biofilm

Bacterial biofilms are reported to be present in every inch of the earth. They colonize every living and non-living substratum, thereby becoming an inevitable part of several living and non-living systems. It is known that about 40–80% of bacteria on the planet form biofilm (Flemming and Wuertz 2019). Several superficial, internal, as well as systemic infections are reported to cause increased severity owing to the biofilm of the pathogen. Cystic fibrosis is a pulmonary infection caused by the persistence of *P. aeruginosa* biofilm (Southey-Pillig et al. 2005). Periodontitis is a biofilm-mediated infection that damages the gums, the soft tissues as well as bones supporting the teeth. The infection is reported to be caused by *Porphyromonas gingivalis*, *Actinobacillus*, *Prevotella*, and *Fusobacterium* (Listgarten 1986; Kanwar et al. 2017). *Enterococcus faecalis* and *Enterococcus faecium* are associated with nosocomial infections. These pathogens are well-known for causing biofilm-oriented infections which are often difficult to treat (Paganelli et al. 2012). Another condition such as Cholesteatoma is reported where the keratinizing squamous epithelium is trapped in the middle ear and/or in the mastoid process in which 81.3% of cholesteatomas are reported to be biofilm-associated (Galli et al. 2016; Kaya et al. 2013). Several chronic infections caused by bacteria are often reported to be biofilm-mediated (Wilkins et al. 2014). The biofilms produced by *Staphylococcus epidermidis* and *Staphylococcus aureus* are the causes of nosocomial infections and infections through medical devices frequently (Otto 2008). In a bacterial biofilm, around 1% of cells are antibiotic-resistant (Sharma et al. 2019). Approximately 95%

of urinary tract infections (UTIs) are associated with urinary stent and catheter tubes, while intravascular devices such as pacemakers, left ventricular assist devices, implantable cardioverter defibrillators, and prosthetic vascular grafts are reported to be associated with 87% of bloodstream infections, and 86% of pneumonia are associated with mechanical ventilation (Nandakumar et al. 2013). Twelve percent of hip periprosthetic infections are caused by *Propionibacterium acnes*, *Peptococcus saccharides*, *Peptococcus magnus*, and *Peptostreptococcus magnus* biofilm (Geipel 2009). 78.2% of the chronic wounds are reported to be associated with biofilm (Dhar and Han 2020).

3.2 Harm Rendered by Fungal Biofilm

The association of fungal biofilm has been reported to cause millions of infections yearly. Biofilms formed by *Candida* spp., *Aspergillus* spp., *Fusarium* spp., *Pneumocystis* spp., *Rhizopus* spp., *Rhizomucor* spp., *Cryptococcus neoformans*, *Blastoschizomyces capitatus*, *Malassezia pachydermatis*, and *Trichosporon asahii* have received the most attention due to their pathogenicity (Kernien et al. 2018). The persistence of fungal biofilm on various medical devices can cause fatal harm to patients with a high rate of morbidity. Patients with implanted medical devices or compromised immune systems may be highly susceptible severe, disseminated disease with high mortality caused by biofilms of *Candida* spp. (Douglas 2003). The fungal biofilm defers in the structure compared to bacterial biofilm. Various fungal biofilm is formed by the filamentous hyphae along with the exopolysaccharide. The biofilm formed by *Candida albicans* is reported to be progressed by hyphae formation, followed by the filamentation of the species to form the biofilm. The fungal biofilm protects the pathogen from antimicrobial defenses, such as defensins, and oxidative stress. Owing to their high tolerance of antifungals and immune evasion strategies, fungal infections are difficult to treat. *Candida albicans* and *Candida parapsilosis* biofilms are reported to exhibit anti-fungal resistance against fluconazole, amphotericin B, nystatin, voriconazole, and others, while *Aspergillus fumigatus* biofilms are resistant to itraconazole and caspofungin drugs. *Cryptococcal* biofilms are reported to endure the effect of fluconazole and voriconazole, and biofilms of *Trichosporon asahii* display resistance to amphotericin B, caspofungin, voriconazole, and fluconazole (Fanning and Mitchell 2012). Invasive aspergillosis caused by *Aspergillus fumigatus* is characterized by a high mortality rate (Jayshree et al. 2006). Thus, biofilm-mediated infections of fungus are reported to be a major concern in various hospital-acquired infections as well as surgical infections.

4 The Current Approach to Deal with Biofilm

The biofilm formed by the microorganisms are hard to control due to the inefficacy of several antimicrobial drugs. Biofilm-mediated loss incurred in the health sector as well as agriculture is a problem that needs to be addressed. Several attempts have been made to disrupt the biofilm. Various antibiotics are used to treat biofilm-mediated infections. However, the side effects of antibiotics in the process of treatment cannot be ignored. One such antibiotic, rifampin is reported to exhibit antibiofilm activity against *S. aureus* and *S. epidermidis*; however, the risk of emergence of rifampin resistance during treatment seems to be a hindrance in the process of biofilm management. Several antibiofilm agents are small molecules or enzymes that have the potential to disrupt or inhibit biofilm. Another promising antibiofilm strategy is to modify the biomaterials used in medical devices to prevent biofilm formation (Chen et al. 2013; Schilcher and Horswill 2020). As biofilms resist the inflow of various antibiotics, an increased dose of antibiotics is often given to treat the biofilm-mediated infection. The topical application of antibiotics in surgical wounds is reported to inhibit the biofilm formation of the pathogen (Ciofu et al. 2017). Römling and Balsalobre (2012) reported that nucleotide second messengers, c-di-GMP, (p)ppGpp, and potentially c-di-AMP are major regulators of biofilm formation and associated antibiotic tolerance, and targeting the pathways could hinder biofilm of the pathogens. In cases, where traditional antibiotics fail, coating of the medical devices, vaccination against biofilms, and quorum sensing inhibitors are promising future options for the prevention and treatment of biofilm-mediated infection (Zimmerli and Moser 2012). Adopting one of the mentioned strategies may not effectively control persistent biofilms. An efficient treatment of biofilm infections requires the removal of the infected foreign bodies from the infected site, selection of an effective and well biofilm penetrating antibiotics, systemic or topical administration of antibiotics in high dosage and combinations of different antibiotic, administration of anti-quorum sensing or biofilm dispersal agents (Wu et al. 2015). Owing to the rise in antibiotic resistance, along with the collaborative process, attempts have been made to search for a potent antibiofilm agent that can effectively malfunction the resistant potential of various biofilms.

5 Role of Biosurfactant in Inhibiting and Disrupting Biofilm

Pathogenic biofilms are a global concern as they tend to increase the severity of various diseases and complicate the treatment procedure. Biosurfactant, a potential antimicrobial agent has been held high due to its reported antibiofilm activity. To portray effective antibiofilm efficacy, it is very essential that the agents are capable of inhibiting biofilm formation and disrupting preformed biofilm (Padmavathi and Pandian 2014). The pathogen cells require to adhere to the substratum to initiate the biofilm formation. Biosurfactants are reported to inhibit the biofilm adhesion of

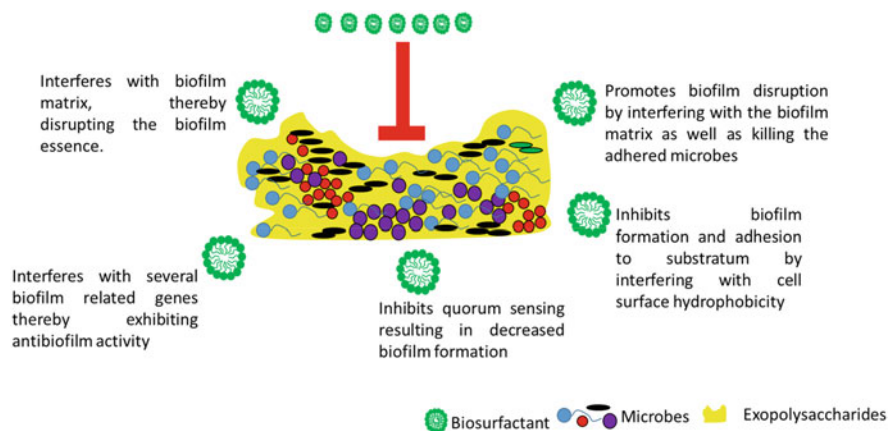


Fig. 5 Antibiofilm activity exhibited by various biosurfactant

the pathogen (Mishra et al. 2020). Adsorption of biosurfactant to the surface of the substratum changes the hydrophobicity of the cells, interfering adhesion. The inhibition of biofilm formation by biosurfactant is also established by enhanced membrane disruption, and electron transport chain inhibition, thereby restricting cellular energy demand (Satpute et al. 2016a). Several reports revealed the effectiveness of biosurfactants in interfering with the genes and the products that play an important role in the formation and maintenance of biofilm (Yan et al. 2019). The biofilm inhibition efficacy of biosurfactant can be utilized in the process of coating agents for medical implants to prevent the biofilm formation of the pathogen. Quorum sensing is reported to be an important mechanism in the process of biofilm formation, which is reported to have interfered with the presence of biosurfactants (Satpute et al. 2016a). There are also reports that the biosurfactants can modify the chemical composition of the exopolysaccharide of the biofilm. Exopolysaccharide is an important constituent of the biofilm which serves the survival strategy of the microbial community. Interference of biosurfactants with the exopolysaccharide can have a detrimental effect on the biofilm (Paraszkievicz et al. 2021). Kim et al. (2015) reported that the interaction of rhamnolipid with protein and carbohydrate of the exopolysaccharide results in the reduction of the amide group and decrease of glucosamine respectively due to their interference in N – H bonds. The antibiofilm efficacy of several types of biosurfactant has been reported against a wide range of fungi, pathogenic yeasts, and bacterial biofilm. The antiadhesive, biofilm inhibition and biofilm disruption property of biosurfactant is facilitated by several mechanism which are summarized in Fig. 5.

6 Antibiofilm Activity Against Bacterial Pathogen

Bacterial biofilm has been a major cause of several medical emergencies in terms of infection. Several glycolipids and lipopeptides are reported to exhibit antibiofilm activity against several bacterial pathogens. Among the glycolipid, rhamnolipid, and sophorolipid are well-known for their effective antibiofilm activity against numerous pathogens. Rodrigues et al. (2006) reported that rhamnolipid applied silicone rubber inhibited 66% adhesion of biofilm produced by *Streptococcus salivarius* and *Candida tropicalis*. Glycolipid from *Burkholderia* sp. has been reported to exhibit antibiofilm activity against *S. aureus* (Ashitha et al. 2020). Biosurfactants produced by *Pediococcus acidilactici* and *Lactobacillus plantarum* were reported to exhibit antiadhesion and antibiofilm activity against *S. aureus* by regulating the expression of biofilm-related genes *cidA*, *icaA*, *dltB*, *agrA*, *sortaseA*, and *sarA* and interfering with signaling molecules (AI-2) in quorum sensing systems (Yan et al. 2019). Several studies have been carried out to establish the synergistic efficacy of biosurfactants with essential oils and antibiotics. Mukherji and Prabhune (2014) reported efficient antibiofilm activity of sophorolipid containing essential oils against *V. cholera*. *Staphylococcus* species are well-known for dwelling in several types of superficial as well as invasive infections. Several species of *Lactobacillus* are reported to produce biosurfactants, known as surface lacticin or surfactin (Satpute et al. 2016b). Biosurfactant secreted by a probiotic strain, *L. fermentum* RC-14 is reported to reduce the adhesion of *S. aureus* on surgical implants, which would be effective in reducing implants-related infections (Gan et al. 2002). Pseudofactin II, a cyclic lipopeptide produced by *Pseudomonas fluorescens* is reported to decrease the adhesion of *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus hirae*, *Staphylococcus epidermidis*, and *Proteus mirabilis* in glass, polystyrene, and silicone surfaces (Janek et al. 2012). Velraeds et al. (1996) reported the inhibition of adherence of uropathogenic cells (pathogen involved in urinary infection) of *Enterococcus faecalis* by 77%. Biosurfactants are reported to exhibit synergistic antibiofilm activity when combined with various antibiotics (Rivardo et al. 2011). Cell bound biosurfactant of *Lactobacillus rhamnosus* has been reported to inhibit pathogen attachment as well as disrupt the preformed biofilm of *B. subtilis*, *P. aeruginosa*, *S. aureus*, and *E. coli* cells within biofilms (Patel et al. 2021). Thus the strong antibiofilm activity of various types of biosurfactant has been reported, which provides a prospect of finding an efficient antibiofilm alternative.

7 Antibiofilm Activity Against Fungal Pathogen

The detrimental effect of fungal biofilm is reported to be far more severe than bacterial biofilm. While the fungal biofilm tends to resist the antifungal activity of several antifungal agents, several types of biosurfactants are reported to exhibit efficient antibiofilm activity against fungal biofilm. The biofilms of dermatophytes

are reported to complicate various soft skin infections. Mařátková et al. (2017) reported the synergistic antibiofilm activity of rhamnolipid and amphotericin B on the biofilm of *Trichosporon cutaneum* and *Candida parapsilosis*. Lipopeptide from entomopathogenic fungus *Beauveria bassiana* was reported to exhibit antibiofilm activity against *M. canis* (Abdel-Aziz et al. 2020). Rhamnolipid produced by *Pseudomonas aeruginosa* SS14 was reported to exhibit promising biofilm dispersive activity against *Candida tropicalis* (Borah et al. 2019). Another glycolipid called Trehalose lipid, produced by *Rhodococcus fascians* BD8 has been reported to exhibit 95% antiadhesion activity against *Candida albicans* against polystyrene surface and silicone urethral catheters (Janek et al. 2018). Sophorolipid along with amphotericin B is reported to exhibit antibiofilm activity against *Candida albicans* (Haque et al. 2017). Surfactin has been reported to exhibit antibiofilm activity against *Candida albicans* by controlling the expression of hyphal-specific genes such as HWP1, ALS1, ALS3, ECE1, and SAP4 (Janek et al. 2020). Among the several lipopeptides, the lipopeptide Iturin is reported to exhibit an antifungal impact on fungal pathogens by disrupting the cell membrane. Iturin is reported to pass through the cell membrane and interacts with the nuclear membrane and other cytoplasmic organelles membrane of the fungal pathogen (Rodrigues and Teixeira 2010). Janek et al. (2012) reported that a cyclic lipopeptide Pseudofactin inhibited 92–99% biofilm adhesion inhibition against *C. albicans* at a concentration of 0.5 mg/ml. With the decreased response of conventional antifungals against the fungal pathogen, biosurfactants can be a promising alternative with efficient antibiofilm activity.

8 Conclusion

Biofilm has a detrimental impact on several anthropological activities. Biosurfactants, with their promising antibiofilm activity, can decrease the pathogen adhesion and biofilm formation and can effectively disrupt the preformed biofilm. This facilitates the utilization of biosurfactants in food sectors to avoid the deterioration of food quality owing to biofilm-forming species. They can be of immense importance in the management of biofilm-mediated infections as well as the biofilm-oriented agricultural infections. With the emergence of antibiotic-resistant strains, the treatment of several infections has become critical. Malakar and Deka (2021) reported the antibiofilm efficacy of various biosurfactants against several bacterial as well as the fungal pathogen. Owing to their non-cytotoxicity, biosurfactant is a potent antibiofilm alternative with a promising prospect. The practical implementation of biosurfactants as an antibiofilm agent in several fields can be a biological alternative to several chemicals, that are used to get rid of the resistant biofilm.

9 Future Perspective

Biosurfactants are microbial non-toxic metabolites with an efficient antibiofilm efficacy. They can be a promising alternative to several chemical antibiofilm agents available on the market. The efficiency of biosurfactants to exhibit antiadhesive activity, biofilm inhibition, and biofilm disruption can be exploited in various fields such as biofilm repellent in biomedical applications, anti-biofouling agents, biofilm inhibitors in packaged food, etc., which would reduce the burden of chemical agents to the environments as well as would decrease the long-term toxicity caused by the chemical agents.

Acknowledgment Chandana Malakar is thankful to the Department of Biotechnology (DBT), Govt. of India for providing financial assistance as Senior Research Fellow (vide letter No. DBT/JRF/BET-17/I/2017/AL/351 dated 01/06/2017) to carry out the research for Ph. D. degree.

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Part IV
**Biosurfactants: Commercialization,
Challenges and Future Outlook**

Advantages and Disadvantages of Biosurfactants over Other Synthetic Surfactants



Jyoti Sharma, D. Sundar, and Preeti Srivastava

1 Introduction

Surfactants are the chemical molecules possessing both hydrophobic and hydrophilic moieties (amphiphilic in nature). They can decrease the surface/interfacial tension, facilitate the solubility of polar compounds in non-polar solvents, resulting in surface activation of liquids (Desai and Banat 1997). This property makes them an exceptional emulsifier, disperser, and foaming agent. Efficacy to decrease surface tension and a lower critical micelle concentration (CMC) determines the effectiveness of a surfactant. An efficient surfactant can drop the water's surface tension from 71 to 35mN/m (Mulligan 2005). Surfactants find their applications in household, chemical industries, petro-chemicals, agriculture, pharmaceuticals, medicine, cosmetics, food, etc. According to the statistics, the global market of the surfactants is likely to reach 44.9 billion dollars by 2022 (Priyashantha and Mahendranathan 2021).

Surfactants can be synthetic or biologically derived (Fenibo et al. 2019). Examples of synthetic surfactants are alcohol ether sulfates, dioctyl sodium sulfosuccinate (DSS), perfluorooctanesulfonate (PFOS), linear alkyl benzenesulfonates, alpha-olefin sulfonates, alcohol sulfates, etc. (Fleurackers 2006). Surfactants produced by microbes or plants are called biosurfactants (Liu et al. 2020). For example, glycolipids, lipopeptides, polymeric, etc. They also possess the same properties like the synthetic ones such as lowering the surface/interfacial tension (Akbari et al. 2018).

At present, most of the chemical surfactants which are commercially available are produced by the petro-chemical industries or synthesized chemically (Campos et al.

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2013). Irrespective of its vast usages, chemical surfactants are also a topic of concern for its impact during its synthesis and disposal processes (Malkapuram et al. 2021). It has been described in many studies that surfactants and their disposal have harmed the macro- and micro-biota of the aquatic environment and soil (Johnson et al. 2021). Chemical surfactants cause serious effects on human health as well (Tmáková et al. 2015). Chemical surfactants persist in the environment because of their non-biodegradable nature (Scott and Jones 2000). The biologically produced surfactants called biosurfactants now have been considered as the best suitable replacement of the synthetic ones (Vieira et al. 2021). Biosurfactants are ecologically safe, chemically diverse, economically viable, biodegradable and have excellent biocompatibility. They are less toxic, easy to produce and stable under a wide range of climatic conditions (Banat et al. 2021). Despite having many advantages, there are some limitations of biosurfactants over the chemical surfactants such as low production from microorganisms and high-cost downstream processes (Safek and Euston 2019).

1.1 Surfactant Classification

Chemical surfactants are classified according to their polar group nature, i.e., anionic, cationic, nonionic, and zwitterionic (Table 1). The anionic surfactants

Table 1 Major types of synthetic surfactants

Type of Surfactant	Structure	Examples
Non-Ionic		Alkylphenol ethoxylates Octyl phenol ethoxylates Alcohol ethoxylates Polyoxyethylene alcohol
Anionic		Linear alkyl benzene sulphonates Secondary alkane sulphonates Alcohol sulphates Sodium stearate
Cationic		Quaternary ammonium-based compounds Laurylamine hydrochloride Trimethyl dodecylammonium chloride
Amphoteric		Dodecyl betaine Cocoamido-2-hydroxypropyl sulfobetaine Lauramidopropyl betaine

have the negative charge. It is most common and commercially most-used type of the surfactant because they have good surface tension reduction ability and high emulsification and wide range of hydrophilic-hydrophobic balance values. They are prominently used in the detergents, soap, cosmetic, and oil industry. Examples of the anionic surfactants are carboxylates, sulfonates, and sulfuric acid esters. Cationic surfactants are positively charged, mostly used for the negatively charged surfaces. Thus, they act as anticorrosion agent, flotation collectors, bactericides, etc. The most-used cationic surfactants are the amine oxides, mono-amines quaternary ammonium salts. The nonionic surfactants have hydrophilic head with uncharged groups. They act as good emulsifiers and used in laundry cosurfactants, personal care and food products. Few examples of the nonionic surfactants are the carboxylic acid and carbohydrate esters, glycerides and their ethoxylated derivatives. The zwitterionic surfactants are the amphoteric. They have low cleansing and emulsifying properties. They are mostly used in cosmetics. Examples of the amphoteric surfactants are alkyl betaines, alkyl dimethylamines, and Imidazolium derivatives (Moldes et al. 2021).

Biosurfactants are amphiphilic in nature. The positively charged part of biosurfactants is made up of amino acids, peptides, mono-di-, polysaccharides. The negatively charged part is made up of fatty acid chains of variable lengths (Shekhar et al. 2015). In terms of molecular weight, they are classified into low-molecular and high-molecular mass substances (Ron and Rosenberg 2001). Low-molecular mass compounds such as glycolipids, lipopeptides, and proteins are effective for reducing the surface tension, while high-molecular mass substances like polysaccharides, lipoproteins, and polymeric particles are best at making constant hydrocarbon emulsions (Muthusamy et al. 2008). Table 2 lists the various types of biosurfactants and respective origins.

1.2 Properties of Synthetic Surfactants and Biosurfactants

1. Surface tension: The primary attribute of surfactants (synthetic or bio-origin) is known for their ability to lessen surface tension. A “good” surfactant can drop the water’s surface tension from 72mN/m to 35mN/m (Mulligan 2005). The biosurfactant (rhamnolipids) can lessen the water’s surface tension to 26 mN/m, comparable to the synthetic surfactants, SDS (25 mN/m) (Sodium dodecyl sulfate) and LAS (31 mN/m) (Linear alkylbenzene sulfonate) (Vijayakumar and Saravanan 2015).
2. Self-assembly: CMC stands for the surfactant concentration needed to make a micelle. Micelle formation is the supramolecular assembly of the surfactant molecules above the CMC, resulting in the creation of a colloidal suspension in the liquid. In nanometers, the length of the surfactant’s hydrophobic chain is equal to micelle radius (Fletcher 1996). Due to the hydrogen bonding, hydrophobic interactions, and van der Waals interactions, surfactant molecules have a propensity to self-assemble (Kitamoto et al. 2009). Biosurfactant CMC values

Table 2 Biosurfactant classes and their production sources (Adapted from (Sharma et al. 2021))

S. no	Biosurfactant class	Biosurfactant type	Biosurfactant producing microbes
1.	Glycolipids		
1.1		Rhamnolipids	<i>P. aeruginosa</i> , <i>Pseudomonas</i> sp. <i>Burkholderia</i> sp., <i>Franconibacter</i> sp.
1.2		Sophorolipids	<i>Torulopsis bombicola</i> , <i>T. apicola</i> , <i>T. petriculum</i> , <i>T. botistae</i> , <i>Candida lipolytica</i> , <i>Candida bombicola</i> , <i>Candida batistae</i>
1.3		Trehalolipids	<i>Nocardia</i> sp., <i>Rhodococcus erythropolis</i> , <i>Corynebacterium</i> sp., <i>Gordonia</i> sp. <i>Tsakamurella</i> spp., <i>Mycobacterium</i> spp.
1.4		Mannosylerythritol lipids	<i>Arthrobacter</i> sp. <i>Candida antarctica</i> , <i>Pseudozyma</i> sp. <i>Ustilago maydis</i> , <i>Candida</i> sp. <i>SY16</i> , <i>P. parantarctica</i>
2.	Lipopeptides		
2.1		Surfactin	<i>Bacillus subtilis</i> , <i>Bacillus pumilus</i> , <i>Serratia marcescens</i>
2.2		Iturin	<i>Bacillus subtilis</i>
2.3		Lichenysin	<i>Bacillus licheniformis</i> , <i>B. subtilis</i>
2.4		Viscosin	<i>Pseudomonas fluorescens</i> , <i>Bacillus brevis</i> , <i>Leuconostoc mesenteroides</i>
2.5		Serrawettin	<i>Serratia marcescens</i>
2.6		Arthrofactin	<i>Arthrobacter</i> sp.
2.7		Polymyxin	<i>Bacillus polymyxa</i>
3.	Polymeric		
3.1		Emulsan	<i>Acinetobacter calcoaceticus</i> RAG-1 <i>Arthrobacter calcoaceticus</i>
3.2		Liposan	<i>Candida lipolytica</i>
3.3		Biodispersan	<i>Acinetobacter calcoaceticus</i> A2
3.4		Lipomanan	<i>Candida tropicalis</i>
3.5		Mannoproteins	<i>Saccharomyces cerevisiae</i> , <i>Kluyveromyces marxianus</i>
3.6		Alasan	<i>Acinetobacter radioresistens</i> KA53
4.	Fatty acids, phospholipids, and neutral lipids		
4.1		Corynomycolic acid	<i>Corynebacterium lepus</i> , <i>Arthrobacter paraffineus</i>
4.2		Spiculisporic acid	<i>Penicillium spiculisporum</i>
4.3		Phosphatidylethanolamine	<i>Acinetobacter</i> sp., <i>Rhodococcus erythropolis</i>
5.	Particulate biosurfactants		
5.1		Vesicles	<i>A. calcoaceticus</i> , <i>P. marginalis</i> , <i>P. Maltophilia</i>
5.2		Whole cells	Many bacteria

for surface tension reduction are 10–40 times lower than the chemical surfactants values. This indicates that fewer biosurfactant molecules are required for attachment to the entire surface compared to the synthetic ones. This indicates that the size of biosurfactant molecules is large and have many branching chains to adsorb onto the surface interface (Karlapudi et al. 2018).

3. Emulsification and de-emulsification: Emulsification is the process by which two or more liquid molecules are dispersed as small droplets to create a semistable combination. Surfactant molecules lessen the interfacial tension at oil/water interface, hence decreasing the formation energy required for emulsion (Tadros 2005). The surfactant molecules adsorb at the interface and act as an electrostatic or steric barrier against any coalescence of droplets, thereby increasing the stability (Wasan et al. 1979). Vice versa, surfactant molecules can destabilize or demulsify by promoting the droplets coalescence. Both chemical surfactants and biosurfactants (high molecular weight) can act as emulsifier and de-emulsifiers (Saad et al. 2020; Raya et al. 2020).

1.3 Advantages of Biosurfactants over the Synthetic Surfactants

1. Biodegradability: Complete biodegradation of the surface-active agents to CO₂, water, and other small inorganic products is highly desirable (Lima et al. 2011). As synthetic surfactants are created from chemicals derived from petroleum and its products, hence they are not biodegradable in nature and release various chemical compounds which persist in the environment and cause environmental pollution. Biosurfactants, on the other hand, are of biological origin and have a basic chemical structure that microbes may quickly and easily degrade in the environment. Several biodegradability tests have indicated that sophorolipids generated by *Candida bombicola* are biodegradable in nature (Hirata et al. 2009). A comparative study revealed that rhamnolipids biodegrade quickly under both aerobic and anaerobic conditions, but under the same conditions synthetic ones show non-biodegradability (Mohan et al. 2006).
2. Low toxicity: Surfactants are widely used in detergents and petroleum industries. They are released in either natural waters or oceans, which consequently have an impact in the aquatic environment and become a worldwide concern (Edwards et al. 2003). In literature, it has been reported that biosurfactants are less hazardous than the synthetic surfactants (Maikudi Usman et al. 2016). For example, in naphthalene solubilization tests, the glycolipids from *Rhodococcus* sp. was found to be 50% less hazardous than the Tween 80 (Kanga et al. 1997). Another report has shown through the bioluminescence inhibition test, bio-based surfactants are less toxic than synthetic surfactants against water-soluble fractions of crude oil, phenol, and naphthalene (Poremba 1993). In another report, it is shown that the relative environmental toxicity of the biosurfactants is lesser than the synthetic ones when used in the oil spill remediation (Edwards et al. 2003). In a study,

when synthetic surfactant (Marlon A-350) was compared with the biosurfactant synthesized by the *Pseudomonas aeruginosa*, Marlon A-350 showed high toxicity in all the assays performed (Flasz et al. 1998). In a study, when the surfactin produced by the *Bacillus subtilis* HSO121 was evaluated for its applicability, it was discovered to be non-toxic and non-irritating, allowing it to be employed in a variety of industries including detergent, cosmetics, and medicine (Fei et al. 2020).

3. **Surface and interfacial activity:** The efficiency and efficacy of an effective surfactant are essential qualities. The surface/interfacial values of a surfactant are used to determine its efficacy, whereas the CMC is used to determine its efficiency. An “effective” surfactant can decrease water’s surface tension from 72 to 35 mN/m and α -hexadecane interfacial tension from 40 to 1 mN/m. The rhamnolipids (produced by the *Pseudomonas aeruginosa*) are sufficient to reduce the water’s surface tension (72 to 26 mN/m) and the water/ α -hexadecane interfacial tension (>1 mN/m). Biosurfactants have been shown to be more effective and efficient than the synthetic ones. The CMC values of biosurfactant are also lower than the chemical ones (Campos et al. 2013).
4. **Biocompatibility and digestibility:** This is an inherent property of the biosurfactants. They are easily converted into smaller compounds and digestible by the ecosystem. While synthetic surfactants are made up of chemical structures that are not easy to break down or not digestible. Several chemically synthesized surfactants are not biodegradable and deposit in nature, and hence cause ecological problems. For example, due to their non-digestible nature and molecular properties, surfactants are not removed from the wastewater and kill the natural ecosystem, by which they disrupt the biological processes causing greater level of pollution in the environment (Nguyen et al. 2010; Maier and Soberón-Chávez 2000; Gudiña et al. 2013; Zhou et al. 2015).
5. **Specificity:** Structurally, biosurfactants have particular groups that enable them to work with precision. This makes them compatible for various applications, such as in cleansing of the different pollutants, in de-emulsification of the commercial chemical emulsions, food industries, pharmaceuticals, and cosmetics. For example, in a study it was reported that emulsan was specific towards the combination of aliphatic and aromatic hydrocarbons (Rosenberg 1993). In another study, it was shown that biosurfactant produced by the *Pseudomonas* PG1 worked as a solubilizing factor towards pristane (Chayabutra et al. 2001).
6. **Availability of raw materials:** Producing biosurfactants from inexpensive substrates is an economical and promising alternative to the chemical surfactants (Satpute et al. 2017). Type of raw materials utilized has an impact on the operating costs. It is anticipated that cost of raw materials accounted for 10 to 30% of the entire cost of the production operations. Thus, it is preferable to select inexpensive raw materials (Rosenberg 1993). For biosurfactant manufacturing, numerous inexpensive raw materials were used. For example, agro-based raw materials, vegetable oils (Esteban and Ladero 2018), oil wastes (Li et al. 2016), starchy substrates, lactic whey (Makkar and Cameotra 1999), and distillery wastes (Rosenberg 1993). All these raw materials are renewable (Paraszkiewicz

Table 3 Different low-cost waste substrates utilized in the production of biosurfactants

S. no	Industry/waste type	Producing microorganisms	References
1	Agro-industrial wastes (date molasses, cassava waste, orange peel, corn steep liquor, sugarcane bagasse)	<i>B. subtilis</i> B20, <i>B. subtilis</i> LB5a, <i>P. cepacia</i> CCT6659, <i>Lactobacillus paracasei</i> subsp. <i>Tolerans</i> N2	(Geetha et al. 2018; Hippolyte et al. 2018)
2	Fruits and vegetables juices (cashew apple juice, banana peels, carrot peels, lime peels)	<i>Halobacteriaceae archaeon</i> , <i>Pseudomonas aeruginosa</i>	(Chooklin et al. 2014; Kalia and Kumar 2017; Kumar et al. 2016; Rocha et al. 2007)
3	Starch-rich waste	<i>Bacillus subtilis</i>	(Bhange et al. 2016; Gurjar and Sengupta 2015)
4	Waste oils	<i>Geotrichum candidum</i> , <i>Candida tropicalis</i> , <i>Candida utilize</i> , <i>Corynebacterium aquaticum</i>	(Eldin et al. 2019; Kaur et al. 2017)
5	Lignocellulosic waste	<i>Serratia nematodiphila</i> , <i>P. aeruginosa</i> TGC01, <i>Achromobacter sp.</i>	(Joy et al. 2019; Bezerra et al. 2019; Panjjar et al. 2020)
6	Frying oil wastes	<i>Pseudomonas aeruginosa</i> , <i>Bacillus stratosphericus</i>	(Panjjar et al. 2020; Chen et al. 2018)
7	Vegetable oils wastes	<i>Candida sphaerica</i> , <i>Starmerella bombicola</i> , <i>Trametes versicolor</i>	(Konkol et al. 2019; Luna et al. 2015)
8	Dairy industrial wastes	<i>Candida bombicola</i> , <i>Cryptococcus curvatus</i>	(Daniel et al. 1998; Daverey et al. 2011)
9	Sugar industrial wastes (molasses)	<i>Corynebacterium spp.</i> , <i>P. aeruginosa</i>	(Daverey et al. 2011; Martins and Martins 2018; Tan and Li 2018)

et al. 2018). However, chemical surfactants are made up partially or fully from petrochemical products. The use of petrochemical resources is one of the major concerns for environment as they are less or non-degradable. Some of the raw materials used to produce surfactants are listed in Table 3.

- Physical factors: Biosurfactants can tolerate various ecological conditions such as extremes of temperatures, pH (ranging from 2 to 12), and salt concentrations. Microorganism and their produced biosurfactants have many industrial, medical, and environmental applications (Schultz and Rosado 2020). These processes include their exposure to the extremes of pH, ionic strength, and temperature (Cameotra and Makkar 1998). Biosurfactants demonstrated resilience in all environmental extremes, whereas the bulk of synthetic surfactants are unsuitable in such settings like, 2% NaCl can inactivate the activity of the synthetic ones and some cannot withstand high temperatures.

Many studies have demonstrated the synthesis of stable biosurfactants from the extremes of temperature. For example, it is shown that organisms isolated from

the extreme cold conditions produce the stable biosurfactant with high emulsification activity and high yield. Biosurfactant produced by the *Oleomonas sagaranensis* and *Candida sphaerica* showed a high thermal and pH stability with high emulsification activity and low surface tension value (Saimmai et al. 2012; Luna et al. 2012). A trehalolipid produced by the *Rhodococcus* sp. showed the stability with wide range of temperatures (20–100 °C), wide range of pH (2–10), and wide range of salt concentrations (5–25%)(White et al. 2013). A novel biosurfactant (Triterpenoid saponins) produced by *Bacillus* sp. IITD106 showed the stability under extreme values of pH (4–10), temperature (30 °C–70 °C), and salt concentrations (2%–15%)(Zargar et al. 2022). In a study, it has been shown that biosurfactant (rhamnolipids) produced by *Franconibacter* sp. IITDAS19 was highly stable at extreme environmental conditions such as temperatures (4 °C–110 °C), pH (1–10), and salt concentrations (0–15%) (Sharma et al. 2022). It has been shown that rhamnolipids could replace the synthetic surfactants for low-temperature washing, which increases the environmental sustainability (Otzen 2017). It has been reported that *B. subtilis* strains isolated from an oil reservoir can produce different biosurfactants under anerobic and aerobic conditions which show good surface activity and emulsification (Nikolova and Gutierrez 2021). In another study, biosurfactant from *B. subtilis* (isolated from crude oil sample) was compared with the commercially available chemical surfactants (Glucopone 215, linear alkylbenzene sulfonates, and Findet 1214 N/23) over the different temperatures and pH (Vaz et al. 2012). Biosurfactant was found to be stable across a wide range of temperatures (20–121 °C) and pH (3–10).

8. Emulsification: Surfactants are known for their emulsification property, which makes it useful in the petroleum industry. The surface/interfacial tension of the gas/water can be altered by the surfactants. Synthetic and bio-based surfactants both have same activity in emulsification, but it was shown that the biosurfactant has lower CMC values and high emulsifying activity as compared to the synthetic ones. This makes them more attractive for many applications where low concentration of the surfactant is required (Perfumo et al. 2010; Pacwa-Płociniczak et al. 2011). According to a study, rhamnolipids and sophorolipids in mixture were able to produce stable and strong microemulsions as compared to the synthetic surfactants for a wide range of oils. These can be applied in various applications such as cleaning, drug delivery, petroleum, etc. (Nguyen and Sabatini 2011). A study has shown the stability of rhamnolipids for emulsion formation at a wide range of pH in comparison to SDS, which shows that rhamnolipids could replace the synthetic surfactants (Lovaglio et al. 2011). Biosurfactant (surfactin) in water has been shown to improve the kinetics parameters for the production of methane hydrate with the shorter induction time than the SDS (Bhattacharjee et al. 2017).

1.4 Disadvantages of Biosurfactants over Synthetic Surfactants

Despite having so many advantages, biosurfactants also have some limitations which restrict their usage in different applications. Some of the disadvantages of the biosurfactants over the chemically synthesized surfactants are discussed below:

1. **Toxicity:** Despite being less toxic and biodegradable to the environment, some findings suggest that the microorganisms producing the biosurfactant are toxic and have virulence effects (Sharma et al. 2021). For example, *Pseudomonas aeruginosa* and *Burkholderia* sp. cause various infections in the humans, and are the main producers of rhamnolipids (Ghibu et al. 2010; Meza-Radilla et al. 2021). Other examples of biosurfactant producing microorganisms which are pathogenic are phospholipid-producing *Klebsiella pneumoniae*, lipopeptide-producing *Serratia marcescens*, heteropolysaccharide-producing *Cronobacter sakazakii*, etc. (Uzoigwe et al. 2015). Production of glycolipid biosurfactant by *Nocardia otiidiscaviarum* is responsible for pulmonary nocardiosis and brain abscess. Lipopeptide produced by *Serratia marcescens* is responsible for urinary tract infection, etc. (Uzoigwe et al. 2015). This raises the concern of health safety. Biosurfactants such as lipopeptides produced by *B. subtilis* have been found to rupture erythrocytes in hemolytic activity tests, but their effect is lower than the synthetic ones such as HTAB (hexadecyltrimethylammonium bromide), TTAB (tetradecyltrimethylammonium bromide), BC (benzalkonium chloride), and SDS (sodium dodecyl sulfate) (Dehghan-Noude et al. 2005). This factor limits the use of biosurfactants in many applications, but studies are going on to produce biosurfactants from the non-pathogenic strains with high yields (Marchant and Banat 2012).
2. **Large-scale production:** Biosurfactant production on a massive scale is not cost-effective. This is the major reason behind low market growth of the biosurfactants. Generally, high-cost raw materials are required to produce biosurfactants (Rosenberg 1993). The substrate compositions contribute around 30 to 50% of the entire manufacturing cost. Many researchers had already experimented with different cheap and renewable substrates for the microbial growth. However, the technology for using these low-cost and renewable substrates at industrial scale is still being developed. Many industries have attempted to minimize production costs and enhance microbial growth and biosurfactant yield by utilizing carbon-rich trash. Many industries have been trying to combat this problem and balance the overall production costs (Jimoh and Lin 2019).
3. **Difficulty in producing pure biosurfactants:** The purity of the biosurfactant after the final step in production depends on the application for which it is required. For example, like in MEOR (microbial enhanced oil recovery), agriculture, bioremediation, etc., the purity of the final product required is low while in several other applications like in food, cosmetic and pharmaceuticals, pure biosurfactants are required. Multiple sequential processes are required in the downstream

processing of the diluted broths at industrial scale, this causes difficulty in obtaining the pure product. This also effects the overall production cost (Kosaric 1992; Twigg et al. 2021).

4. **Low productivity:** One of the major reasons of the low biosurfactant global market is its low production yields. Many researchers have shown high production yield at laboratory scale, but yield required for industrial scale remains a challenge for researchers. Most of the biosurfactant producing strains display low production and overproducing strains are very rare. Also, complex media formulations are required for their growth. Many researchers are working to find the solution to this problem by constructing a recombinant strain or overproducing strain for biosurfactant production (Jimoh and Lin 2019; Kapadia and Yagnik 2013; Sanches et al. 2021).
5. **Lack of knowledge on regulation of biosurfactant synthesis:** Biosynthesis and regulation of many different types of biosurfactants are still not known properly. For industrial-scale production, knowledge about biosurfactant production kinetics is required. The following types of production kinetics parameters exist for biosurfactant production on industrial level: (i) production linked with growth (growth, substrate utilization, and biosurfactant production are linked together), (ii) growth-limiting conditions (one or more medium components are in limited supply), (iii) production by resting or immobilized cells (no multiplication of cells), (iv) production with precursor supplementation (addition of biosurfactant precursors for qualitative and quantitative product changes) (Desai and Banat 1997). For example, if a batch culture is considered, secondary metabolite production starts in a stressed culture condition due to decreasing levels of the nutrients. This can be related with the transition phase (slow growth rate and morphological changes) (Santos et al. 2016). These factors govern the biosurfactant production at a large scale and lack of knowledge about all these factors makes the biosurfactant market lower than the synthetic ones.
6. **Foam formation:** Strong foam formation is observed during the biosurfactant production processes. This also adds significantly to the cost of production. The biosurfactant generated adsorbs to the bubble's air-water interface. This lowers the liquid's surface tension and gives bubbles more stability, increasing the development of strong, stable aqueous foam in the fermentation operations. This would alter not only the biosurfactant yield, but also the microorganism's surface characteristics. The foaming behavior is also affected by fermentation process variables such as pH, agitation, aeration, and medium components (Gong et al. 2021; Winterburn and Martin 2012).

2 Conclusions

Biosurfactants are the surfactants synthesized by microbes (bacteria, fungi, and yeast), whereas chemical surfactants are the surfactants synthesized from the synthetic chemicals or petro-chemicals. Above the CMC levels, both surfactants are

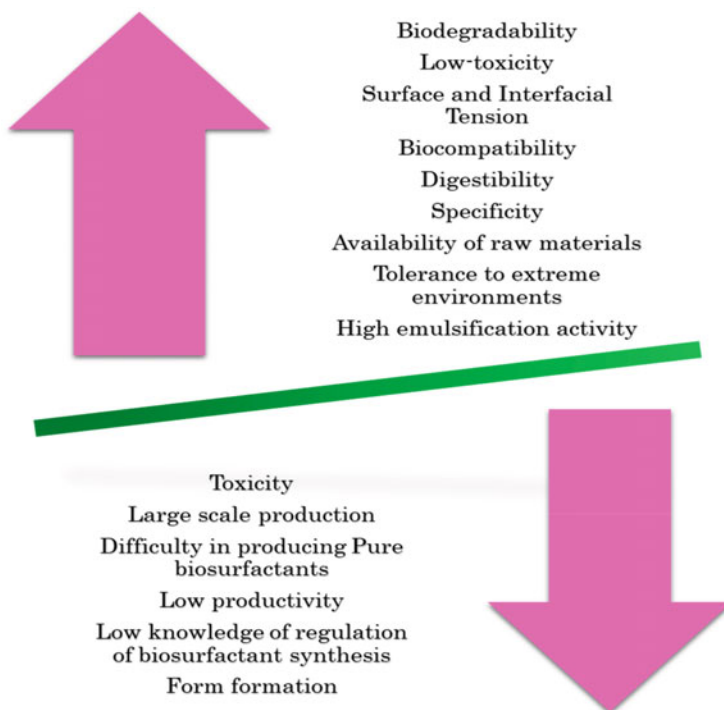


Fig. 1 Advantages and disadvantages of biosurfactants

effective to lessen the surface/interfacial tension of the solvent mixtures by micelle production (Markande et al. 2021). Biosurfactants are gaining attention nowadays due to their wide range of qualities over the synthetic ones such as environmental acceptability, stability in severe atmospheric situations, diversity in chemical structures, specificity, biocompatibility, digestibility, production from cheap raw materials, etc. Biosurfactants are the alternates to replace the synthetic surfactants in numerous industrial sectors including oil/gas, bioremediation, pharmaceuticals, medicine, food, cosmetics, agronomy, personal care, etc. The most produced and used biosurfactants produced by various microorganisms are rhamnolipids, surfactin, sophorolipids, mannosylerythritol, and emulsan. Despite the fact that the biosurfactants have numerous advantages over synthetic ones, the latter are commonly employed in industries due to their commercial availability (De et al. 2015; Fenibo et al. 2019). Synthetic surfactants are non-biodegradable and toxic, they have a large environmental impact and produce unintended ecological concerns. Green surfactants or eco-friendly surfactants can be used to offset these disadvantages (Medrzycka and Karpenko 2009). However, due to high production costs, using biosurfactants on an industrial scale remains a difficulty. The substrate composition and recovery are the key operating costs of any manufacturing process. Process for production of biosurfactants at large industrial scale is an expensive process (Fig. 1).

Table 4 Biosurfactant producing industries around the globe

S. No	Commercially produced biosurfactant	Manufacturing company	Applications	References
1	Emulsan	Petroleum fermentations (Netherlands)	Cleaning oil-containment vessels, microbial enhanced oil recovery	(Shete et al. 2006)
2	Rhamnolipids	Jeneil biosurfactant—USA	Enhanced oil recovery, lubricants, agriculture	(Sachdev and Cameotra 2013)
3	Rhamnolipids	AGAE technologies—USA	Enhanced oil recovery	(Banat et al. 2000)
4	Rhamnolipids	BioFuture—Ireland	Washing fuel oil tanks	(Chakrabarty 1985)
5	Sophorolipids	Synthezyme—USA	Crude oil emulsification	(Shete et al. 2006)
6	Rhamnolipids	EcoChem Organics Company—Canada	Water-insoluble hydrocarbons dispersive agent	(Xu et al. 2011)
7	Sophorolipids	Ecover—Belgium	Cleaning products, cosmetics, bioremediation, pest control, pharmaceuticals	(Shete et al. 2006)
8	Glycolipids, Cellobiose lipids, MELs	Fraunhofer IGB—Germany	Cleansing products, shower gels, shampoos, washing-up liquids, pharmaceutical (bio-active properties)	(Rahman et al. 2002)
9	Sophorolipids	Groupe Soliance—France	Cosmetics	(Muthusamy et al. 2008)
10	Sophorolipids (ACS-Sophor)	Allied Carbon Solutions (ACS) Ltd—Japan	Agricultural products, ecological research	(Sachdev and Cameotra 2013)
11	Sophorolipids, Rhamnolipids, Mannosylerythritol lipids	Henkel—Germany	Glass cleaning products, laundry, beauty products	(Kaskatepe and Yildiz 2016)

Many researchers are focusing on this problem by using cheap raw materials such as vegetable oils, starchy wastes, soya molasses, distillery wastes, dairy wastes, etc. to solve the problem. Another major disadvantage of the biosurfactants for their use in industrial scale is the downstream processing and purification costs (Marchant and Banat 2012). Another area is to produce biosurfactants under non-sterile conditions and genetic modifications to develop microbial strains with high yield and low toxicity by reducing their virulence factors or using non-pathogenic microorganisms. Globally, only a handful of small enterprises are currently producing biosurfactants for commercial use (Johnson et al. 2021; Jimoh and Lin 2019; Bhadani et al. 2020; Helmy and Kardena 2011; Mohanty et al. 2021). Some of the major producers of biosurfactants are mentioned in Table 4. Advancements in fermentation processes

for the production of biosurfactants can attract the large industries which in turn will help in providing a clean and green environment.

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Commercialization of Biosurfactants



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1 Introduction

Many of our everyday events depend on the application of different types of surfactants such as personal hygiene, cosmetic and toothpaste products, and other pharmaceutical by-products; most of these products consist of emulsifiers and surfactants as one of their constituents. Thus, there is an increase in demand and market for these products. Because of the toxicity, non-biodegradability, and accumulating capacity of some chemical petroleum-based products to the surroundings, it has become a common interest to look for microbial-derived products as an alternative to chemically synthesised surfactants (Marchant and Banat 2012a, b; Satpute et al. 2010). Mainly, these microbial biosurfactants (BSs) originated from microorganisms; they are ecologically safe benign products (Banat et al. 2014). The major physical and chemical activities of BSs are to decrease the interfacial and surface tensions at the interfaces between immiscible gases, solids, and liquids, enabling unique stages to interact and mix (Cowan-Ellsberry et al. 2014; Otzen 2017). They can provide several important roles in different segments of industrial

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marketing, such as current products which are in high demand because of the Covid-19 pandemic (Çelik et al. 2020; Johnson et al. 2021). Various products are made of an important number of surfactants in their components including soaps, fabric softener, detergent, toothpaste, and so on (Otzen 2017). The majority of these chemical surfactants are generally synthesised from petroleum chemical products, but they are environmentally unfriendly and not economically viable (Cowan-Ellsberry et al. 2014; Otzen 2017). Despite this, chemical and biotechnology industries have continuously engaged in the research for more and safer ecologically desirable industrial bioprocesses in which they prefer using environmental biomolecules with quality functional and structural characteristics (Geetha et al. 2018; Industries 2020).

Emulsifiers and surfactants provide a huge market value in the last ten years which looks to be ever-increasing with a molecule of 6% yearly growth rate (Satpute et al. n.d.). As part of the synthetic surfactants, BSs have also started to develop their profit-oriented request with a compound yearly increase rate prediction between 8 to 9% (Farias et al. 2021). It is important to underscore the application of renewable compounds in the area of industrial expression with loving massive competition from other markets (Satpute et al. 2017). Nature has given us several BSs from multiple sources with diverse functional and structural diversities. The saponin extracted from *Sapindus mukorossi*; cereals lecithin from egg yolk and other proteins, cholesterol, gelatin, casein, and wax are some substitutes (Ghagi et al. 2011). In plant-derived surfactants, lecithin had been reported to be largely used in natural low molecular weight BSs for industrial applications (Dickinson 1993). Besides animals and plants that formed BSs, microbes have been one of the major appropriate candidates that are used to produce different types of surface-active molecules.

Current findings had established that the global market welcomes novel initiatives and long to source alternatives to replace synthetic surfactants that had reached USD 1.74 billion in 2011. Figure 1 outlines the global surfactant market between 2012 and 2020. About 344,000 tonnes of BSs were estimated to be produced in 2013 with sales above USD 1.8 billion in 2016. In 2018, an estimate of USD 2.21 billion with about 442,000 tonnes was reported. The projected market growth rate between 2014 and 2020 was 4.3%. In 2021, the World BS Market has been estimated to have cost about USD 4.8 billion. About USD 2.6 billion has been estimated for biosurfactant sales in 2023 while other market sources predicted the global sales of BSs to be above USD 5.52 billion by the end of 2022 (Farias et al. 2021). By 2026, it is predicted to have gotten to USD 6.3 Billion with about a 5.5% increase in a Compound Annual Growth Rate (CAGR).

One of the main factors that influence the growth of the biosurfactant market is the increasing demand for biosurfactant application in industrial cleaning, personal hygiene, and the production of detergent and soap (Farias et al. 2021). The consumer's predisposition regarding by-products can improve the industrial demand above the predicted time frame. An increase in the agricultural events and increasing knowledge of cleanliness because of Covid-19 are additionally rocketing the market growth. Although, an increase in the raw materials and production costs are the two main factors that can impact the market growth negatively (Sari et al. 2019). The

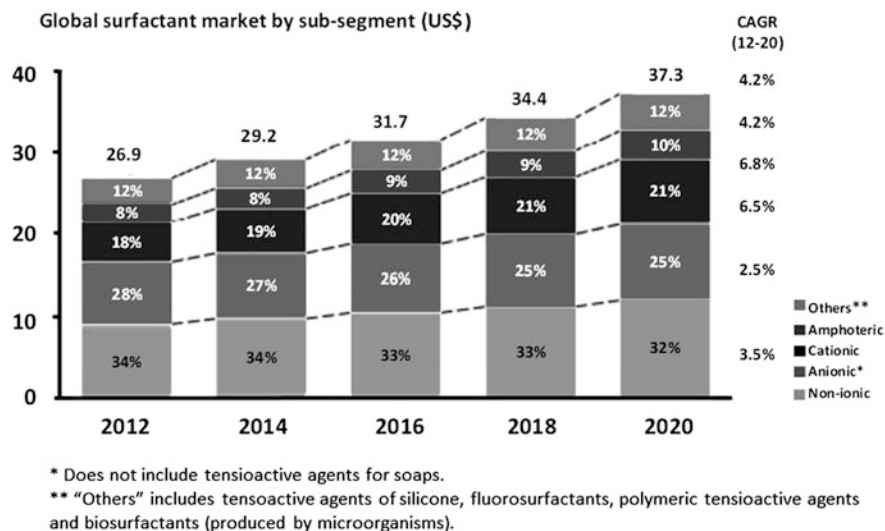


Fig. 1 Global surfactant market by sub-segments (Farias et al. 2021)

major business owners that put money into the production of economical products and wide-reaching commercialization of glycolipid biosurfactants are awaited to provide many chances for the biosurfactant market is generating unexplored opportunities for the market.

Furthermore, the global biosurfactant market is subdivided according to geography, application, and type. According to geography, Northern America has been forecasted in leading the market. Based on the application, the market is categorised as agricultural chemicals, detergent, food processing, personal hygiene, and many others. Based on the type, market can be categorised as lipopeptides, glycolipids, fatty acids, phospholipids, particulate BSs, and polymeric BSs (Markets 2021). Out of all these, glycolipid segments are proposed to control the maximum share of the market. Thus, this chapter was compiled to unveil the importance of BSs and their commercialization potential.

2 Industrial Prospects of BSs in Enabling Commercialization

The industrial use of BSs is rapidly becoming a real life; although, different challenges persist, and universal application can be predicted. These difficulties can be related to cost and yield of production, downstream processing, efforts and time required to change and sort out compounds to certain uses (Marchant and Banat 2012a, b). BS market movements have currently been the objective of in-depth estimation reported by Transparency Market Research™. In 2011, a study reported

Table 1 Some companies and biosurfactant products they are working on and their applications

S/ N	Company	Products	Applications
1.	AGAE Technologies—USA	R90 grade, R95 grade, and R5L	Pharmaceutics/cosmetics, enhanced oil recovery, bioremediation, detergents/home care, household cleaners, and oil tank cleaning
2.	Jeneil Biotech, Inc.—USA	Zonix Biofungicide™, natural rhamnolipids	Agriculture, antimicrobial, bioremediation, household and personal care use.
3.	TeeGene Biotech—UK	TeeGene	Antimicrobial, anti-viral, bioremediation, cosmetic, detergent, food, municipal waste management, water and wastewater treatment.
4.	BIO Cleaners Ltd.—UK	Bio Flush	Abattoirs, animal healthcare range, boat/yacht, chicken and poultry process, dairy, distilleries, fish factories, food processing, hotel and catering, meat processing
5.	EcoCHEM—India	Eco-Green Flo, Eco-Green Marble, Eco-Green Shine, Eco-Green All, Eco-Green Fresh, Eco-Met Tap Shinner	Floor cleaning, marble cleaner, toilet cleaner, glass cleaner, hand wash, metal cleaner
6.	BioFuture—Ireland	BFL oil cept, BFL 6000HC, BFL nutrient mix, BFL A C L, BFL AC, BFL pond clean II, BFL drain clean, BFL septa clean, and others	Aquaculture, bioremediation, eco-cleaning, industrial, and municipal.

worldwide BS market to be USD 1735.50 million and predicted to get as far as USD 2210.5 million in 2018. In 2018, European countries were awaited to benefit 53% of the world BS market revenue share accompanied by the Northern America. Many of the famous surfactants' traders including Ecover and BASF-Cognis have hitherto moved into the biosurfactant business. BASF-Cognis was reported in 2011 to be the leading marketer with more than 20% share of the market. Some of the industries and the products they are working on are presented in Table 1.

2.1 Cosmetic Sector

In the cosmetic company, chemically synthesised surfactants can be utilised for their detergency, foaming, wetting, solubilising, dispersing, and emulsifying characteristics. Persistent application of these chemicals can cause negative effects on the humans and environment. A study revealed that the use of natural substitute molecules has attracted special interest due to the recent demands for animals and

environment-friendly natural-based cosmetics (Corley 2007). Over the past few years, BSs involving patents and marketable products have been revealed for use in cosmetics and healthcare companies (Fracchia et al. 2015). Glycolipid (GL) BSs including methylerythritol lipids (MEL), rhamnolipids (RL), and sophorolipids (SLs) are of high interest in these fields. SL with propylene glycol derivatives can be utilised as softeners or moisturisers in cosmetics due to their hygroscopic activities (Faivre and Rosilio 2010). The study reported a product which contains 12 moles of propylene glycol and 1 mole of SL; the results had shown high positive skin compatibility which made it to be utilised commercially as a skin softener (Fracchia et al. 2015). Kao Co. Ltd. had commercially produced SLs in form of cosmetic makeup brand from humectants like Sofina. This Sofina can be applied in form of lipsticks and as softeners in eye shadow, hair and skin products, aqueous solutions, and compressed powders. The French company Soliance developed and sold active constituents comprising SLs for the cosmetic industries. SLs formed from rapeseed oil fermentations generally contain Sopholiance S which acts as Sebo-regulator formulation and an antibacterial which can be used in face cleansers, deodorants, makeup removers, shower gels, and for treating acne-prone skin (Fracchia et al. 2015).

The sopholine cosmetics is a functional soap that contains SLs which was marketed by the Korean biotech company MG Intobio Co. for the treatment of acne. A study had also reported patents regarding SLs that are utilised as softening to ameliorate the skin physiology, repairing, and restructuring of skin in form of an activator of macrophages; it was also used in fibrinolytic healing for depigmenting and desquamating processes (Maingault 1999). Some germicidal made of a surfactant, fruit acid, and SL biosurfactant are capable of complete eradication of *Shigella*, *Salmonella*, and *E. coli* at the maximum time of 30 s; it had been patented for healthy hair, skin, fruits, and vegetables (Farias et al. 2021). RLs had been reported suitable for healthcare products in several various formulations such as contact lens solutions, antacids, deodorants, toothpaste, acne pads, insect repellents, and nail care product (Maier and Soberón-Chávez 2000). Rhamnolipids had been proposed as antibacterial agents in skincare products, care products (creams, shampoo, soaps, and sprays), personal hygiene, and cleaning animals that are being sold in the market (Ahmadi-Ashtiani et al. 2020). Several studies have patented other applications to make emulsion and liposomes in the cosmetic industry. Methylerythritol lipids (MELs) BSs formed from *Pseudozyma* fungi have currently received awareness because of their favourable structural difference, flexible biochemical uses, production states, and self-assembling features. A study investigated the potential of MELs as an ingredient in cosmetic production for softening dry skin, repairing harmed hair, activating papilla and fibroblast cells, as well as acting as a protecting and antioxidant agent in skin cells (Morita et al. 2013).

MELs shared the same amphiphilic structures as ceramide-3, it is a significant constituent of the intracellular lipid of stratum corneum; hence, proposing the potential of ceramide-like skincare activities (Yamamoto et al. 2012). Using cultured human skin models of 3-dimension showed that methylerythritol lipids produced recovery actions on sodium dodecyl sulphate-injured cell which was compared to

ceramide-3 one (Yamamoto et al. 2012). Besides, MEL-B used in treating human forearm skins significantly improved the water contents of stratum corneum and defeated the exudation on the skin surfaces. This suggestion had demonstrated their possible applications in skin softening. The rights for skincare formulation involving MEL can act as the biosurfactant in antiwrinkle skincare cosmetic, an active component for roughness in cosmetics skincare as well as a constituent for antiaging agent reported in Japan (Fracchia et al. 2015). Besides, Daito Kasei Kogyo Co., Ltd., a Japanese firm had begun to produce a novel foundation powder made of metal oxide particle encapsulated with MEL, this product shows better moisture retention characteristics.

Besides, the application of cosmetics made of lipopeptides (LPs) has been increasing due to their excellent diverse biological and surface effects including moisturising, antiwrinkle, and antimicrobial effects (Naser 2021). LPs were applied in form of emulsifiers and suggested to produce reduced skin irritation acceptable to produce external skin preparations including transparent cosmetics with sequestering functions. Another important use of LPs had been demonstrated in the antiwrinkle cosmetics, prevention and treatment of skin stretch marks, cleansing products with better washability, and very low skin-irritating effect. Another study reported LPs that were produced through SHOWA DENKO into different products which include oil-in-water emulsified components with emollient and moisture retention characteristics in skincare cosmetics (Fracchia et al. 2015). A study revealed the application of emulsifiers from *Acinetobacter calcoaceticus* in personal skincare products, soaps, and shampoos against eczema and acne (Naser 2021). Among other activities of emulsifiers is their capacity to mess with microbial adhesion on hair or skin (Fracchia et al. 2015).

2.2 *Pharmaceutical and Biomedical Sectors*

There are many and most fascinating biological and physicochemical properties of BSs that have caused a huge number of possible biomedical and pharmaceutical. Most especially, their capacity to destroy by attacking the permeability and integrity of the cell membranes, and also their capacity to influence microbial adhesion by altering the surface properties for biomedical applications (Fracchia et al. 2015). Moreover, much literature had suggested that some of the experimental results have shown that BSs are less toxic or non-toxic compared to synthetic surfactants (Gayathiri et al. 2022).

There are numerous literature reports on possible biomedical uses of BSs (Bjerk et al. 2021; Naughton et al. 2019). Although numerous licences have been approved regarding BSs application to improve health, normal pharmaceutical and biomedical industrial applications are still quite limited. Finding new antimicrobial agents that can fight against the emerging microorganisms and stopping the increasing resistances demonstrated against already established antimicrobial medicines has changed the focus to natural-based products with several mechanism actions and

as appropriate substitutes to many synthetic drugs (Banat et al. 2014). LPs have remained the most largely revealed type of BSs with the antimicrobial property because of their capacity to destroy lipid membrane. Some findings on LP modes of action had reported that the occurrence of pores in cell membranes happened after LP oligomer binding, such as Ca^{2+} -dependent multimer (Fracchia et al. 2015). The pore might result in trans-membrane ion influx that leads to disruption of membrane and cell death. These characteristics might cause their use in the pharmaceutical firm, where LPs have been employed in the absent traditional medicine that could no longer fight against resistant fungi or bacteria (Fracchia et al. 2015).

The first LP to be discovered is Polymyxin A, which was isolated from *Bacillus polymyxa*, a soil bacterium comprising antimicrobial activity (Jones 1949). Likewise, bacillomycins, fengycin, mycosubtilins, surfactin, and iturin are formed from *Bacillus subtilis*; pumilacidin, lichenysin, and polymyxin B can be formed by *B. pumilus*, *B. licheniformis*, and *B. polymyxa*, respectively; daptomycin is a cyclic LP produced by *Streptomyces roseosporus* and viscosin is another cyclic LP produced by *Pseudomonas* (Fracchia et al. 2015). All these are well famous antimicrobial LPs. Some types of LPs have gotten to the commercial antibiotic stages such as micafungin, caspofungin, echinocandins, daptomycin, and anidulafungin (Fracchia et al. 2015). Daptomycin can be regarded as a non-ribosomal source of branched cyclic LP antibiotic, isolated as an antibiotic complex from *S. roseosporus* cultures (Baltz et al. 2006). Several studies have decided to interpret its mode of activity which hypothesised that the daptomycin binds to the membrane phosphatidylglycerol head group and takes on a second conformational change that causes membrane oligomerization, and later causes penetration into the membrane (Gray and Wenzel 2020; Huang 2020; Humphries et al. 2013). The daptomycin was endorsed as a non-topical treatment against structural skin diseases caused by gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and was used to treat endocarditis and bacteraemia caused by the strains of *S. aureus* and MRSA (Fracchia et al. 2015). Daptomycin can demonstrate a strong antibacterial effect against medically important resistant microorganisms, including penicillin-resistant *S. pneumoniae*, coagulase-negative *Staphylococci* (CNS), vancomycin-resistant *Enterococci*, and glycopeptide-intermediate-susceptible *Staphylococcus aureus* (GISA) (Manfredi and Sabbatani 2010).

The anidulafungin, micafungin, caspofungin, and echinocandins are modified synthetic lipopeptides, which are generally produced from the fermentation broth of different yeasts from diverse fungi such as *Glarea lozoyensis*, *Aspergillus nidulans*, and *Coleophoma empetri* (Hüttel 2021; Kofla and Ruhnke 2011). Echinocandins help to prevent fungal cell wall formation by non-competitive and specific inhibition of β -(1,3)-d-glucan synthase enzyme (Janeczko 2018). The absence of carbohydrate essential compounds in the fungal cell wall called β -(1,3)-d-glucan destroys the cell wall and consequently leads to cell death (Capoor and Bal 2021). Their main features are fast fungicidal and lower toxicity level against several isolates of *Candida sp.* and their predictable favourable kinetics allow a single dose daily. Besides, their inhibitory spectrum can include the strains of *Pneumocystis carinii*, and *Aspergillus sp.*, but not *Cryptococcus neoformans* (Denning 2002).

Caspofungin had been the first patent echinocandin product. Similarly, micafungin had been utilised to fight fungal diseases such as Aspergillosis and candidiasis in immune-compromised children (Lestner et al. 2013). Anidulafungin had been regarded as the newest antifungal drug. It has a well clinical efficiency and toleration that has been shown for the treatment of fungi in the bloodstream (candidemia) and other types of candidiasis.

A perfect new aspect of using BSs had been shown through some studies that have reported the potential of rhamnolipid BSs in wound healing (De Giani et al. 2021; Long et al. 2012; Randhawa and Rahman 2014; Thakur et al. 2021). The compound which contains rhamnolipids (RLs) has been created to induce re-epithelization in adult skin tissue, providing wound healing to reduce fibrosis, and treating burn shock. Besides, these RLs molecules were reported for their potential in the prevention and treatment of atherosclerosis, schizophrenia, depression, and refusal of the transplanted organ (Stipčević et al. 2006). Currently, approval has been issued regarding antimicrobial biosurfactant peptides formed from the probiotic strains of *Bifidobacterium sp.*, *Streptococcus sp.*, and lactic acid bacteria, which are capable of carefully binding to collagen and inhibiting pathogens surrounding the wounds at the sites of implantation and biofilms related to diseases in mammal. A US company known as Paradigm Biomedical Inc. (the USA) is devoted to producing pharmaceutical products produced from rhamnolipids. This industry has revealed the creation of a design of applications for treating skin diseases including lichen planus, atopic eczema, and seborrheic dermatitis, and similarly, specialised RLs are used for the treatment of burns and wound healing (Fracchia et al. 2015).

Besides their direct effect on fighting microbes, BSs have the capacity of regulating microbial synergy with surfaces, interfering with biofilm formations, and their consequence adhesion property (Quinn et al. 2013). The production of microbial biofilm on medical equipment serves as a significant and the most hazardous situation, this includes bacteria within the biofilms which are greatly resistant to antibiotic and cause side effects on the environment. In addition, a study had published an international approval on this application (Ceri et al. 2010); the BS compounds are utilised together with biocides as an alternative that helps in preventing bacterial growth as a biofilm against or planktonically against abiotic and biotic surfaces (Ceri et al. 2010).

Lactobacillus produced biosurfactant had been approved as an inhibitor of bacterial colonisation and adherence to medical equipment, especially to prevent urogenital disease in mammals. Sugar-derived BSs including rhamnolipids and MELs have been revealed to cause molecular self-assembly, that involves the reversible and spontaneous association of molecular units to form ordered structures through non-covalent bonds without utilising stimulus (Fracchia et al. 2015). MELs have currently been recognised and used during drug delivery and gene transfection in the field of nanotechnology. MELs have been perfectly utilised to improve the efficacy of the gene transfection of cationic liposomes and the mode of action was revealed to show the introduction of exceptional and effective membrane fusion within liposomes of the target cells, the plasma membrane and serial production of

DNA (Fracchia et al. 2015). In 1988, RL liposomes were being approved for drug delivery systems, utilised as microcapsules in dyes, drugs, nucleic acids, proteins, and other compositions, it is used for biological membranes as biomimetic models and as sensors to detect pH differences. These new liposomes have been reported to be biologically safe and decomposable, they have an applicable affinity for microbial pathogens with extended shelf life and stability (Gama et al. 1990). Besides, SLs and RLs had been combined with lecithin to produce biologically compatible microemulsions for drug delivery and cosmetic usages. Another current novelty has been directly focused on the polymeric acylated BSs that could be self-assembled into structural polymeric micellar helpful in topically used skincare products containing antiacne, antimicrobial, and external analgesics (Fracchia et al. 2015).

2.3 *Formulation of Detergents*

Almost 50% of every produced surfactant can be utilised in cleaning and washing sectors (Farias et al. 2021). A huge number of chemical surfactants have been utilised as detergent active substances for many years. Detergents are locally made of one or more detergent active substances with other several constituents including bleaches, detergency perfumes, builders, and fluoresces. Besides, the main uses of detergent compounds include cleaning of cooking utensils, fabric, crockery, and hard surfaces including enamels, glass, plastics, metals, and glazed surfaces (Fracchia et al. 2015). Because of their lower toxicity, carbon footprint, higher biodegradability, and increasing thought; on environmental protection, it currently exhibits a passion to select and include “green” ingredients in the detergent formulations. The difficulties faced by manufacturers depend on the capacity to improve the degree of such constituents in formulations by avoiding higher cost.

The applications of BSs for cleaning with additional antimicrobial features and decreasing solvent utilisation in the productions are being given more attention. Henkel has been regarded as a leading detergent manufacturer, they began to utilise SL biosurfactant in some of their branded glass-cleaning products including Sonasol, Instanet, Tenn, Sidolin, and Breff; these products are selling in Europe (Bouassida et al. 2018). Choosing a biosurfactant-derived detergent compound is usually based on its final application. Face-washing products can cause a surfactant of strong-foaming ability that is benign to the skin, and these washing detergents need a biosurfactant that has strong washing capacity and ability to produce foam that can be easily eliminated (Moldes et al. 2021). SLs are well suitable for less-foaming uses including auto-dish and hard surface cleaning products. A study had patented a low-foaming, biodegradable dish-washing product that contains sophorolipids of better washing capacity with a large range of temperatures (Furuta et al. 2004). It is a novel detergent molecule that is specific to dish-washing machine which uses jet-washing technique that needs strong temperature resistant surfactants and less foam. Moreover, another approval had been issued based on surfactant containing

the carbohydrate group that can produce remarkable cleaning ability using a densified CO₂ dry method.

Cleaning agents made of BSs can be commercialised. The Naturell® is an American firm that has created and customised a product for carpet cleaning, this product contains BSs and enzymes produced by fermenting sea kelps. Besides, another biotechnology firm known as Z BioScience had been reported for developing ecological friendly microbial biosurfactant-based cleaning agents especially for office and commercial surroundings, hospital, and household settings. The study had also suggested that both biosurfactant-based products B1+ low Foaming Biosurfactant Cleaner and A1+ Foaming Biosurfactant Cleaner could show barriers to pathogens by removing the biofilms and preventing the surface re-colonisation. In addition, the HTS BIO Company from France had developed a product line called Ecoway®; this product is being utilised in professional maintenance and cleaning. Likewise, the Akshay Intensive Marketing from India is supplying Acticlean, a cleaning agent that contains non-specific BSs, whose roles have been revealed to improve spreading, wetting, control and modification of foam, solubilisation, emulsification, dispersion, and detergency action. Moreover, Taylor Mclure has produced Drain-Zyme, a liquid product that is used to remove starch, proteins, fats, and so on from the septic tanks that consists of blended pathogens which are specially chosen because of their capacity to form non-specified BSs that help in emulsification of the grease to produce total degradation (Fracchia et al. 2015).

2.4 Food Sector

BSs can provide various encouraging applications in the food industry. Most especially, they can be employed as emulsifiers to process raw materials. Emulsification can be involved to play a significant function to produce the right texture, consistency, and phase dispersion. The food industries across the world have now begun to utilise fatty acid esters, lecithin and its derivatives, and ethoxylated derivatives as emulsifiers. The yearning for increasing the chain of emulsifiers and decreasing reliance on plant emulsifiers such as modified soybeans, the major source for lecithin, and the desire to gain from the favourable biofilm, antimicrobial, antioxidant, and antiadhesive suspension activities had resulted in increasing the attraction to find novel source of amphiphilic molecules as an alternative for the food sector (Rufino et al. 2012). BSs with higher molecular mass can be found as better emulsifiers than those with lower molecular mass. Sophorolipids that are produced from the strain of *Starmerella bombicola* have been found to decrease interfacial and surface tension, although they are not seen as a good emulsifier (Celligoi et al. 2020).

BSs are utilised to manage the agglomeration of fat globules, increase texture and shelf life of starch-based product, stabilise aerated system, modification of the rheological actions of wheat dough and increase the texture and consistency of fat-containing products (Saravanan and Vijayakuma 2015). BSs can also help in retarding staling, solubilising flavour oils, improving organoleptic actions in ice

cream and bakery formulations and acting in form of fat stabilisers during the cooking of fats (Durval et al. 2021). A breakthrough in dough texture, conservation, stability, and volume of bakery products was achieved through the inclusion of rhamnolipid surfactants (Gayathiri et al. 2022). Studies have revealed the application of rhamnolipids in improving croissants, frozen confectionery, and buttercream product properties. Currently, a biological emulsifier from the marine strains of *Enterobacter cloacae* has been suggested to be utilised as a possible viscosity improvement product of attraction in the food company because of their viscosity quality. A study observed that a lower pH helps its utilisation in food product based on ascorbic acid (Saravanan and Vijayakuma 2015). However, it has been confirmed that the addition of rhamnolipids increases the dough properties of bakery products, and the usage of food compositions produced from opportunistic bacteria including *Pseudomonas aeruginosa* is not practically and ethically feasible and acceptable. Rather, a study had confirmed that the application of BSs isolated from the strains of *Lactobacilli* is globally safe and accepted in various food-processing technologies (Fracchia et al. 2015).

A lipopeptide biosurfactant derived from the *Bacillus subtilis* can produce stabilised emulsions with coconut fat and soybean oil, palm, sunflower, olive, linseed, babassu, and Brazilian nut oils (Coasta et al. 2006). Moreover, corn oil and a mannoprotein isolated from the strain of *Kluyveromyces marxianus* produced emulsion that can stabilise for 90 days (Lukondeh et al. 2004). BSs have been reported to show possible antioxidant properties of high influence in the food industry. Studies have revealed that BSs derived from *Bacillus subtilis* RW-1 can scavenge free radicals (Jemil et al. 2017) and a polysaccharide emulsifier produced by the strain of *Klebsiella* was found with potential of inhibiting auto-oxidation of soybean oil through the encapsulation from the medium around (Yalcin and Çavuşoğlu 2010). Furthermore, many BSs had been reported to demonstrate antimicrobial action in fighting viruses, bacteria, fungi, yeast, and algae; they are utilised as additives to prevent direct food contamination or indirectly utilised in the detergent formulations in cleaning surfaces that can come in contact with the food (Fracchia et al. 2015).

Microbial biofilms property found within the food industry surfaces can be a possible origin of contamination that can cause food spoilage and disease transmission. Example of pathogenic bacteria that causes outbreak related to the consumption of contaminated food includes *Salmonella enteritidis*, *Enterobacter sakazakii*, and *Listeria monocytogenes*. Several findings have demonstrated that these bacteria that can produce biofilms and adherence to food-contact surfaces can be more resistant to sanitation than free-living cells (Fracchia et al. 2015). The requirement of surface utilising microbial surface-active molecules can be seen as an encouraging method in preventing the adhesion of foodborne microorganisms to solid surfaces. A biosurfactant produced from the strain of *Streptococcus thermophilus* had been utilised to evacuate foul from the heat-exchanger plate in pasteurizers because it can prevent the colonisation of other thermophilic strains of *Streptococcus* that causes fouling (Satpute et al. n.d.).

Biosurfactants have not been utilised on a huge scale in food processing, because of many directives made by government bodies for new food components and the prolonged endorsement processes. Although, some endorsements are being given on BSs following the recent interest to utilise the microbial-formed product in food industry (Fracchia et al. 2015). Besides, a patent was issued based on the application of BSs as solubilizers or emulsifiers for foodstuffs. It was reported that the addition of nisin to rhamnolipids increased the shelf life and prevented the growth of thermophilic spores in UHT soymilk (Fracchia et al. 2015). More so, the application of ingredients that involves the combination of natamycin and rhamnolipids can increase its shelf life and prevented the growth of moulds in salad dressing. They have also seen that those ingredients containing the combinations of natamycin, rhamnolipids, and nisin can improve the shelf life of cottage cheese through the growth inhibition of gram-positive bacteria and spore-forming bacteria. Rhamnolipids are utilised to maintain the moisture of the bakery texture. A study had reported a formulation which contains rhamnolipids, amylopectin, and amylose to be suitable in the bakery products to prevent retrogradation of amylopectin and amylose or bakery staling (Fracchia et al. 2015).

2.5 Oil Sector

In general, the high-yielding oil wells can be instigated by utilising local primary and secondary renewal methods; these technologies can produce about 20 to 30% of total oil in the well. About two-third of the oil found in reservoir can be in excess if these methods are being adopted. In general, the tertiary oil recovery techniques can be regarded as enhanced oil recovery (EOR). It can be applied at a stage to permit residual oil recovery of about 10 to 15%. These technologies can be microbial-based and chemical methods; the former can be regarded as microbial-enhanced oil recovery (MEOR) (Fracchia et al. 2015). Microbial-enhanced oil recovery lay hold of advantage of various microbial processes, including the development of BSs, selective plugging, creation of gases, and partial breaking of large oil molecules. In the case of chemical methods, it can cause a decrease in the oil/water interfacial tension and the production of an oil-in-water emulsion may result in an increase in the mobility of the oil via rock fractures. The application of BSs in microorganisms to enhance oil recovery may be accomplished via several techniques, these include by injecting nutrients into stimulated in situ production through the indigenous bacteria; production of in situ through the injections of allochthonous microorganism; and production of ex situ into offsite fermenter and injection into the oil reservoirs (Nikolova and Gutierrez 2021).

A major problem that can influence the creation of in situ production methods is the challenge of isolating strains of the pathogen from the utmost surrounding of the reservoirs, which is characterised by high salinity, pressure, pH values, and temperatures of about 85 °C. Furthermore, while injecting microbes into the wells, some operators can encounter corrosion and plugging difficulties. Because of these

problems, several studies had suggested the use of additional *ex situ* derived as an alternative for these applications (Nikolova and Gutierrez 2020). Although, rhamnolipids have been reported as the first candidates for this application, and all other kinds of microbial surface-active molecules were suggested for a microbial-enhanced oil recovery use; nevertheless, a study had reported that lipopeptides including surfactin, emulsan, and lichenysin can be effective to increase oil recovery (Karlapudi et al. 2018). Currently, the reliability of several BSs formed by *P. aeruginosa*, *B. subtilis*, and *B. cereus* was investigated at salinity, pH, and temperature conditions which share the same relationship with those found in oil reservoirs.

Most of the laboratory investigations on microbial-enhanced oil recovery (MEOR) applications utilising BSs were investigated inside the core flooding system to enhance the properties of the oil reservoir. The LP biosurfactant formed by a strain of *B. mojavensis* was utilised in core flooding systems to estimate the recovery of oil from carbonate reservoir; this technique has been revealed to obtain about 60% of the initial oil-in-place of the core (Ghojavand et al. 2012). Till now, there has not been any direct documented injections of BSs to the oil reservoir investigated within the oil field, which has completely utilised this method for MEOR. Moreover, this could be because of an increased production cost of the compounds. An economical efficient substitute could be an *in situ* production of BSs, either through stimulated autochthonous microbes or injected bacteria. Hence, a study has currently changed to the isolation of new surfactant-formed microbial species by utilising utmost situations like oil reservoirs (Gudiña et al. 2012).

There is no conclusive and clear confirmation to pinpoint the excellent biosurfactant-dependent microbial-enhanced oil recovery system. Brown (2010) has given a warning regarding the problem of interpretation of laboratory results and insufficient field experiments. He also suggested that the mode of action required for MEOR systems has been shown only in some minor situations, but this information is still limited. According to the suggestion of Armstrong and Wildenschild (2012), they showed that flooding in a microbial process with both biosurfactant and biomass could serve as the best remedy to oil recoveries because of the combined actions of interfacial tension reduction and biologically clogging of the pore spaces. Another problem that can affect the progress of MEOR methods is the inadequate understanding of the diversity and structure of microbial materials within the reservoirs. Most especially is the deep understanding of the physiological and metabolic ability of the autochthonous pathogens which may bolster the approach to increase *in situ* BS development, without requiring the addition of an external bacteria. Simpson et al. (2011) investigated the potential of producing lichenin or surfactin by detecting the occurrence of *srfA3/licA3* gene in brines obtained from nine wells utilising PCR, and they concluded that a biological stimulation strategy for biosurfactant-enhanced oil recovery could be effective. Some studies have reported that the assessment of micro-community components via cloning of 16S rRNA genes could provide significant understanding, which could assist in estimating the viability of biosurfactant-based MEOR process (Fracchia et al. 2015).

Besides their uses in microbial-enhanced oil recovery, BSs may likely be used for other purposes in the oil company. For instance, the application of various microbial BSs has currently been optimised for extracting oil from sludge; the outcome showed that it has the potential to obtain about 74.6% of oil recovery based on the washing conditions (Zheng et al. 2012). Besides, another study has proved that the rhamnolipid of *P. aeruginosa* F-2 could extract about 92% of oil from oil sludges using the pilot-scale field test (Yan et al. 2012).

2.6 Textile Sector

The textile sector can be described as a finishing industry that requires a lot of water. The pre-treatment of textiles can be a significant stage to produce higher qualitative end-product from the textile finishing activities. Several fibre mixtures and exceptional formulation of lipophilic materials are utilised in the form of lubricants to achieve maximum frictional behaviour when producing fabric; it must be dislodged from the fibre surfaces to produce textiles for the next step of production (Fracchia et al. 2015). Moreover, traditional washing systems using detergent substances show constant undesirable effects on the ecology. Because of this, the application of BSs has been suggested to improve the bioavailability of water-insoluble substrates when producing large ranges of surfactant properties and types than synthetic surfactants. BS application had been revealed in the textile finishing industry for solubilisation, wetting, detergency, emulsification, and dispersing with their potential of reducing environmental pollution (Fracchia et al. 2015).

The possibility of using microbial BSs in textile washing methods to dislodge several lipophilic blends from fibre surfaces had been suggested (Fracchia et al. 2015). Nevertheless, *Rhodococcus glomerulus* was found to have the capacity to break down different pure substrates including triglycerides, polyoxyethylene esters, and fatty acid esters. This is due to some achievements, only spinning oil was broken down completely from different marketable fibre preparations. When they estimated the ability of detergent to remove oils from fibres utilising *Rhodococcus erythropolis* BSs such as trehalose dicorynomycolate and trehalose-tetra ester, the result showed that the oil dislodge from the fabrics was greater in relative to the control experiment where the surfactant-free medium was utilised in form of washing agents. The use of BSs in commercial detergent can also be utilised to clean fabric; however, biosurfactants can also be used in the textile dyeing industry (Fracchia et al. 2015).

Dye solubility is one of the major challenges in the textile industry. Lack of water solubility of the dye can lead to the non-homogenous distribution of the dyes although the fabric solid phases and the preferential aggregation of the dyes on the fibre surface (Berradi et al. 2019). Improvement in the solubility of dye water can generally be accomplished by adding surfactant, which can increase the dispersal of dyes to get enough consistent and successful dye infiltration into the fibre (Velusamy et al. 2021). A study compared the dyeing performance of nylon 6 microfiber by

utilising unrecognised biosurfactant (cHAL), commercialised sodium dodecyl benzenesulfonate, SDS, water-insoluble, and soluble dyes.

2.7 *Agricultural Sector*

Several functions of BSs can depend on their unique property and their roles in biological control to act as either antifungal agent or cause a prompted systemic resistance, which may turn them into possible prospects for subsequent uses in crop protections (Lahlali et al. 2022). Generally, the mechanism of action for BSs in biological mitigation can lead to the creation of mechanisms within the cell wall and barriers to the cell membrane of the pathogens. Because of several categories of BSs, plants can be protected against phytopathogenic yeast via antifungal activities provided majorly by glycolipids including rhamnolipids, cyclic lipopeptides, and cellobiose lipids (Crouzet et al. 2020). The disease of tomato leaves emanated from *Botrytis cinerea* a pathogenic fungus could be controlled through co-inoculation with wild-type *Ustilago maydis* sporidia (Doehlemann et al. 2009). This phytopathogenic fungus was first to produce cellobiose lipids known as ustilagic acid. These lipids can be regarded as natural detergents, as moderately lower concentrations could influence cell death mycelial fungi and yeast, and this action may be a result of their membrane-damaging properties (Fracchia et al. 2015). Stanghellini and Miller (1997) had revealed the possible application of BSs as biological control agent; the results showed how RLs could damage zoospore membranes and lead to zoospore lysis of several oomycete plant microbes. Subsequently, several studies have investigated the significant function of RLs against numerous phytopathogenic fungi. Dessanto (2008) had suggested that RLs formed from the strains of *Pseudomonas sp.* could promote the prevention of *Verticillium microsclerotia* viability.

Sha et al. (2012) proposed that crude RLs like a cell-free culture medium could produce a strong effect on the growth of the colony and biomass accumulation of 7 plant diseases consisting of 2 *Oomycetes*, 3 *Ascomycota*, and 2 *Mucor sp.* fungi. The notable efficiency of antifungal activity of cell-free culture medium of RLs can be ascribed to the di-rhamnolipid which is a major component in this cell-free medium, exhibiting strong lysis behaviours more than that of mono-rhamnolipid to disrupt the spore membranes of zoospore forming plant pathogens. Vatsa et al. (2010) carried out a very good investigation regarding the antimicrobial activities of rhamnolipids and the action of these compounds to boost immunity in plants.

2.8 *Other Industrial Sectors*

Numerous options for industrial application of BSs can be anticipated in various other manufacturing firms such as leather, paint, metals, plastics, pulp, and paper. BSs are utilised in the paper processing industries for the washing and

deresinification of pulp, which can act as colour levelling, defoaming, and dispersing agents; they can also be used for calendaring in form of coating, wetting, colouring, and levelling agents in the paper company (Fenibo et al. 2019). A study investigated the biodispersant formed through *A. calcoaceticus* A2; this biodispersant was reported to be effective in crushing limestones to fine particles and effectively utilised in form of a filler in laboratory-based paper. Another study reported the potential use of extracellular polymeric compounds produced from waste sludges of paper and pulp mills as a wood adhesive. A biosurfactant produced from a compound containing culture medium of *Pseudomonas rubescens* and cellulase enzyme was approved as an agent that allows a decrease in added cellulase and reduction in the cellulosic fibre treating time (Fracchia et al. 2015).

BSs are used in painting and protective coating companies for wetting and dispersing pigment during grinding. It can also be employed for separating, stabilising, dispersing, inhibiting sedimentation, and emulsification of pigment in latex paints (Fracchia et al. 2015). A biosurfactant formed from *Cobetia marina* has been internationally approved to be utilised in aquaculture in form of additives in paint production for easier submersible surface (Dinamarca-Tapia et al. 2012). BSs are known to have developed attraction in biodyes. Moreover, the hybrids of BSs and pyrene were in monitoring the micro-environmental situations of different types of colloidal surfaces and biological interfaces regarding their fluidity and polarity (Gayathiri et al. 2022).

Biosurfactants' possible uses within the leather industry may involve their application as skin detergent, and emulsifier in penetration, wetting, degreasing and as promoter in dyeing and tanning (Fracchia et al. 2015). Kilic (2013) has carried out a study on a saponin BS that can serve as a lower economical and natural alternative to chemical surfactants to degrease sheepskins; this study suggested it as a viable alternative with potential environmental benefits. BS can be utilised as emulsifiers, antistatic, solubilizers, and wetting agents in the plastic industry (Fracchia et al. 2015).

3 Low-Cost Substrates for Producing Commercial-Viable Biosurfactants

For the commercialization of BSs, economical approaches should be employed to make them affordable. BSs are derived from different substrates which are up to 50% of the overall production cost. In an ideal condition, to produce an economical biosurfactant, the system should make sure it employed lower cost substrates associated with a higher yield of a product extracted. Other methods used in producing economical biosurfactants include the production of a lower effective downstream system, optimization of fermentative conditions, and creation of excessive strains production (Banat et al. 2010).

3.1 *Agro-industrial Wastes*

Numerous agro-industrial wastes such as rice, the hull of soy, bran, sugar cane molasses, beet molasses, corn, corn steep liquor, cassava flour and its wastewater, and others are small cost renewable substrates which are utilised to produce BSs at the industrial stage. Some studies have reported orange peel as the best substrate to be employed for the production of biosurfactant by *Bacillus licheniformis* (KC710973) with a yield of 1.795 g/L (Kumar et al. 2016). Potato peel has been effectively utilised as a carbon source of biosurfactant from *Bacillus pumilus* DSVP18; this biosurfactant showed better properties such as stability over a large range of pH, temperatures, and salt stress (Sharma et al. 2015). Similar studies had investigated corn powder as a substrate used to produce biosurfactant by alkaliphilic bacterium *Klebsiella sp.* with higher yields; it could also show distinct stability under adverse conditions (Jain et al. 2013). Cassava wastewater had been reported as a better substrate when *B. subtilis* LB5a was applied in a pilot-scale production of biosurfactant (2.40 g/L) (Barros et al. 2008). In another investigation, a culture medium consisting of corn steep liquor and molasses was revealed to produce a biosurfactant (3.20 g/L) by *P. aeruginosa* strain which contains a combination of 8 different rhamnolipid congeners, where the mono-rhamnolipid Rha-C10-C10 remains the highest in amount (Gudina et al. 2015).

3.2 *Animal Fat*

Meat processing industries can be divided into leather and food industries that produce huge numbers of animal lard, fat, and tallow. Although, there has been low demand for animal fat and its uses; hence, waste has become a prominent issue. Therefore, these wastes may be utilised as a cheap substrate to produce BSs. Sophorolipid had been reported to be formed from *Candida bombicola* in a medium involving glucose and animal fat as a carbon source (Deshpande and Daniels 1995). A study has proposed that lower cost media which consist of animal fat was utilised to estimate the production of glycolipid biosurfactant by *Candida lipolytica* (Santos et al. 2013).

3.3 *Dairy and Distillery Wastes*

Dairy companies generally created many wastes known as whey including lactic whey, cheese whey, whey waste, and curd whey. Studies had proved that lactic whey waste was a relatively more effective substrate than synthetic media to produce rhamnolipid on a commercial scale (Banat et al. 2010). According to Dubey et al. (2012), curd whey was seen to be a better substrate to produce BSs by *Pseudomonas*

aeruginosa strain PP2 and *Kocuria turfanesis* strain-J. These BSs formed were greatly effective to emulsify pesticides under harsh ecological situations. *Streptococcus thermophilus* A and *Lactococcus lactis* 53 strains are two probiotic bacteria that have shown about 1.20 to 1.50 times increase in the amount of biosurfactant produced per gram dry cell weight in media formulation utilising supplemented cheese whey medium and molasses compared to M17 broths and synthetic media MRS (Rodrigues et al. 2006).

Besides, distillery waste which can be regarded as stillage is a by-product of a biological process; it can be seen found to contain lysed fungi cells. It contains every necessary nutrient required to hold the production of pathogens. The production of biosurfactant from an oily sludge isolated *Pseudomonas aeruginosa* strain BS2 had been seen to produce a better outcome with whey waste and diluting distillery waste than with the synthetic medium (Dubey and Juwarkar 2001). More so, another study has reported that dilution of distillery waste with whey waste and sugar industry effluent could produce a successful biomass and biosurfactant yield derived from the bacterial isolates. Similarly, personal wastes could give satisfactory outcomes to produce biosurfactants from two novel bacterial isolates known as *P. aeruginosa* strain BS-P and *Kocuria turfanesis* strain BS-J (Dubey et al. 2012).

3.4 Oil Wastes and Vegetable Oils

Oil production can be generally found in the food industries, and it has accounted for about 2.5 to 3 million tons, producing a large amount of tallow, wastes, lard, soap sticks, marine oil, and free fatty acid during the extraction of oil from seeds. Waste disposal has become a major challenge; thus, it has gained a lot of recognition from the researchers who are now studying the application of the wastes in microbial transformation. In an investigation, the activity of several vegetable oils such as olive, soybean, castor, coconut fat, and sunflower on the production of biosurfactant by *Serratia marcescens* strains was tested and sunflower oil was reported to produce a better result (Ferraz et al. 2002). Based on the investigation done by Nitschke et al. (2005), they found that *P. aeruginosa* LBI produced about 11.70 g/L of rhamnolipid by utilising soybean soap stick. Another investigation had suggested that *Pseudomonas* sp. could be seen to form rhamnolipid successfully utilising olive oil mill effluent which acts as a major carbon source (Ji et al. 2016). Many oils extracted from the plant are good for human consumption; they are found to be inexpensive monetary values and hence, they have been utilised in several studies to produce BSs. *Jatropha* oil is a non-edible oil, and it has been utilised to produce sophorolipids from *Starmerella bombicola* NBRC 10243 to obtain higher concentrations of 123 g/L while rhamnolipids are produced by *Pseudomonas aeruginosa* ATCC 10145 to obtain a yield of 4.60 g/L which can be compared to that of the major common oils (Morita et al. 2013). Furthermore, restaurant oil wastes were utilised in some research works to produce biosurfactants.

4 Techniques for Realistic Commercialization of BSs

Because the utilisation of expensive substrates and low product yield have been the major causes of the high production cost of BSs, the implementation of the following methods are utilised to facilitate the successful commercialization of these compounds:

- Nevertheless, different pathogens can produce BSs, but only *Pseudomonas*, *Bacillus*, and *Candida* species are of high interest. Thus, other hyper-producing genera have been closely investigated to produce large-scale biosurfactants (Nikolova and Gutierrez 2021). Microbes isolated from contaminated soils, wastewater, and effluents sources are examined to produce surfactant because they can use industrial wastes.
- Besides naturally existing biosurfactant-forming species, hyper-producing microbes are engineered by genetic mutation and recombination. Henceforth, not only because the product yield is increased, but it can also improve the properties of the BSs (Saha and Rao 2017).
- System biology has been an attractive strategy that can be utilised to improve the production of biosurfactants by enhancing the metabolic fluxes against the product and mitigating the production of other unwanted metabolites. In addition to randomly targeted genetic changes, understanding the genomics and metabolic engineering strategy can highly increase the production of biosurfactants.
- The amount and type of BSs produced depend on the composition of the medium and ecological situations. Thus, several mathematical and statistical tools are utilised for the optimization of these variables to improve the product yield and volumetric productivity.
- Since the raw materials account for 30 to 80% of the total production cost of BSs, the application of agro-based and industrial wastes and lower cost renewable substrates may cause an important decrease in the operating cost needed for the process.
- Comparing the choice of raw materials and purification steps can be another significant factor to establish an economical system; thus, the application of a cost-effective downstream method can be a better way towards the successful commercialization of BSs.
- Another attractive strategy to produce profitable BSs can be the co-production of these compounds with other metabolites such as industrialised significant enzymes and polyhydroxyalkanoates. Moreover, in situ production of BSs can also be utilised in making the system economically viable since they are employed to enhance oil recovery.

5 Conclusion

Several studies had investigated biosurfactants based on different sectors that can propel commercialization. Thus, this chapter has reviewed several ways and sectors in that BSs can be commercialised. Due to the expensive nature of substrates used in the production of BSs with reduced yield recovery, several techniques are being used to propel the successful commercialization of biosurfactants. Therefore, getting information on the structural nature of BSs and their interactions with cells and contaminants to improve the applicability has been suggested. Furthermore, the use of cheaper materials in the production of biosurfactants has been suggested to facilitate the cost of biosurfactant-based products. This will allow the biosurfactants to compete with the existing chemical surfactants. This can further be achieved through genetic engineering and optimization of process factors to generate low-cost processing.

Conflict of Interest We declare none.

Acknowledgement The authors acknowledge the financial assistance (industrial grant, UIC190806) of Eastern Unity Technology, Malaysia.

Important Websites on Commercialization of Biosurfactants

1. <https://www.agaetech.com/>
2. <https://www.igb.fraunhofer.de/>
3. <http://www.teegene.co.uk/>
4. <http://rhamnolipid.com/>
5. <https://biofuture.ie/>
6. <http://worldwide.saraya.com/>
7. <https://www.ecover.com/>
8. https://www.kaneka.co.jp/en/business/qualityoflife/nbd_002.html
9. <http://saifuusa.com/portfolio-item/mildsurfactants/>
10. <http://www.biochemica.co.uk/>

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Biosurfactants: Challenges and Future Outlooks



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1 Introduction

The demand for the production of surfactants has been expanding continuously since the past few decades due to increase in the application of surfactants in various industries. The worldwide market for surfactants was evaluated at 41.3 billion USD in 2019. The market's growth has been fueled by Asia Pacific's growing population and urbanization. Surfactant markets in China, India, and Brazil have all seen significant expansion in the past decade. In 2019, anionic surfactants because of their low price, accessibility, and broad range of applications dominated the surfactants market. The market was led by home care applications like dishwashing and laundry detergents soaps, carpet cleaners, and floor cleaners. Other applications of surfactants include personal care products, industrial and institutional cleaning, oilfield chemicals, food and beverage industry, agriculture, elastomers, plastics, etc. (Zargar et al. 2022a). The market for surfactants has been predicted to reach \$58.5 billion by 2027. However, growing public awareness of the detrimental environmental hazards of chemical surfactants has prompted the development of environmentally friendly surfactants (Beuker et al. 2014).

Microbial surfactants also called as biosurfactants are green amphiphilic molecules capable of significantly reducing the surface tension of a liquid and the interfacial tensions between fluid phases (Zargar et al. 2022b). Biosurfactants, as opposed to chemically manufactured surfactants, are less toxic, extremely biodegradable, and are active under extreme environmental conditions (Mukherjee et al.

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2006). Furthermore, biosurfactants, in comparison to synthetic surfactants have lower critical micelle concentration and possess different chemical structures that make them useful in various fields like detergent, cosmetic, agriculture, food, energy, environment, and healthcare (Zargar et al. 2022b; Sachdev and Cameotra 2013; Velikonja and Kosaric 1993; Shepherd et al. 1995; Nitschke and Silva e 2018; Geetha et al. 2018; Fiechter 1992; Sharma et al. 2018; Peters et al. 2018; Singh et al. 2019; Kretschner et al. 1982; Christopher et al. 2019; Le Guenic et al. 2019; Desai and Banat 1997; Chandra et al. 2018; Xu et al. 2018; Tortora et al. 2018). In 2020, the global biosurfactant industry was valued more than 3.66 billion USD and is expected to grow at a CAGR of 5.4% to 5.71 billion USD by the end of 2028. This has attracted the attention of several large corporations, like AGAE Technologies LLC (USA), Jeniel Biosurfactants (USA), and Rhamnolipid Inc. (USA) for large-scale and commercial production of biosurfactants. Other companies like Ecover (Belgium) and BASF-Cognis (Germany) have entered the biosurfactant production business and are potential competitors in the biosurfactant market (Hames et al. 2014).

Despite their huge demand and multiple benefits, various factors limit the commercial production of biosurfactants. These include higher production costs associated with raw materials procurement, lower fermentation yield and productivity, fermentation-associated issues like foaming and difficulty in downstream purification of biosurfactants. Besides these, toxicity and immunomodulation reported by some biosurfactants can limit their production and use for medical applications.

This chapter provides an insight into the challenges in commercial production of biosurfactants and the strategies that can be used to address these challenges. Furthermore, the chapter summarizes emerging trends and promising strategies for large-scale production and application of biosurfactants.

1.1 Factors Responsible for Transition from Chemical Surfactants to Biosurfactants

Biosurfactants are biologically derived surface-active chemicals produced mainly as secondary metabolites by bacteria, yeast, and filamentous fungi (Adu et al. 2020). Biosurfactants are emerging as appealing substitutes to chemical surfactants due to global sustainability concerns and special advantages offered by biosurfactants over synthetic surfactants (Zargar et al. 2022a). Commercial surfactants are primarily made from petrochemicals (De Almeida et al. 2016; Farn 2008; Diniz Rufino et al. 2014). The use of petrochemicals for the production of biosurfactants has been associated with increase in the level of environmental pollution. Limited availability of petroleum also escalates concerns about the sustainable production of the chemical surfactants. Therefore, the need for green alternatives (biosurfactants) continues to rise to meet the increasing demand for surfactants for industrial and home applications. Replacement of synthetic surfactants with biosurfactants has been

estimated to result in an 8% reduction in lifetime CO₂ emissions, avoiding the release of 1.5 million tons of carbon dioxide into the environment (Farias et al. 2021; Meira et al. 2019; Banat et al. 2021).

Other advantages of biosurfactants over chemical surfactants include their structural diversity, lower toxicity, higher biodegradability, lower CMC and their application in harsh environmental conditions such as extreme temperature, pH, and salt concentrations (Banat et al. 2014; Varvaresou and Iakovou 2015; Sahnoun et al. 2014; Vijayakumar and Saravanan 2015). Most of the biosurfactants produced by the microorganisms (glycolipids and polymeric biosurfactants) are anionic in nature ((Varvaresou and Iakovou 2015; Banat et al. 2010; Santos et al. 2016). Since anionic surfactants are particularly effective in washing systems, they have applications in soap industry and manufacturing of personal care products (Dave and Joshi 2017; Bratovic et al. 2018). They are also used in the oil industry, agricultural industry, pharmaceutical industry, bioremediation, and bioprospecting due to their broad range of hydrophilic–lipophilic balance (HLB) values, better emulsification capabilities, and strong capacity to lower surface tensions (Banat et al. 2010; Santos et al. 2016). Microorganisms have also been reported to produce cationic, nonionic, and zwitterionic biosurfactants. Cationic surfactants are employed as anti-corrosion agents, flotation collectors, hair conditioners, fabric softeners, and bactericides because they are ideally suited for usage on surfaces with a negative charge. Nonionic surfactants have uncharged head groups forming the hydrophilic part of the surfactant. They are effective as detergents and emulsifiers at low temperatures. They have a mild irritating effect on organic tissue as well. Amphoteric zwitterionic surfactants have poor emulsification and washing properties, but are most compatible with skin due to good dermatological properties.

The presence of particular functional group in the structure of biosurfactant imparts specificity in its action. The structure of the biosurfactant therefore determines its specificity. Biosurfactants have a variety of structures due to their microbial origins, the substrate on which they are produced, and the growth conditions utilized. The diversity in the structure of biosurfactants is another key property that distinguishes them for a specific application and allows them to be used across a wide range of industries.

An additional advantage of biosurfactants over chemical surfactants is that they are less toxic in nature. Less toxicity makes them suitable for cosmetic, pharmaceutical, and food applications. Poremba et al. reported that Corexit (a synthetic anionic surfactant) had an LD₅₀, 10 times lower than rhamnolipids against *Photobacterium phosphoreum*. The group also reported that biosurfactants have greater EC₅₀ values than synthetic dispersants (Poremba et al. 1991). Flasz et al. reported that in terms of toxicity and mutagenesis Marlon A-350 (a synthetic surfactant) was more hazardous and mutagenic as compared to biosurfactant produced by *Pseudomonas aeruginosa* (Flasz et al. 1998).

Microbial biosurfactants exhibit higher biodegradability than synthetic surfactants. This makes them ideal for use in environmental applications like bioremediation (Mohan et al. 2006). Biosurfactants also have low CMC values, typically ranging from 1 to 200 mg/l (Hassan et al. 2016; Marcelino et al. 2019; Abdel-

Mawgoud et al. 2008). These values are comparable to those of low molecular weight alkyl ethoxylate surfactants (Saoares et al. 2008; Konishi and Makino 2018). Vaz et al. compared the CMC of a biosurfactant produced by *Bacillus subtilis* EG1 with CMC values of various synthetic surfactants. The CMC value of biosurfactant was found to be 96% lower than the linear alkylbenzene sulfonate and 83% lower than Glucopon 215. Due to lower CMC values, biosurfactants are more effective at lower concentrations as compared to synthetic surfactants (Vaz et al. 2012). Another advantage of biosurfactants is their activity under extreme environmental conditions (Zargar et al. 2022a). The surface activity of many biosurfactants is unaffected under environmental stress like temperature, salt concentration, and pH. McInerney et al. reported that activity of lichenysin produced by *Bacillus licheniformis* JF-2 remained unaffected across a range of temperature, pH, and NaCl and Ca²⁺ ion concentrations (McInerney et al. 1985). Similarly, another group reported that the surface activity of a lipopeptide produced by *Bacillus subtilis* LB5a remained stable after autoclaving (121 °C, 20 min) and 6 months at −18 °C, pH 5 to 11 and NaCl concentrations up to 20% (Nitschke and Pastore 2006). These advantages have fostered interest in large-scale production of biosurfactants for commercial use across various industries.

1.2 Biosurfactants Market Analysis

Due to increased consumer awareness of the hazardous effects of petroleum-based or synthetic surfactants on human health and its environmental implications, the focus has shifted towards utilization of biosurfactants. The global biosurfactants market was valued at over 3.66 billion USD in 2020 and is expected to rise at a CAGR of over 5.5% to USD 5.71 billion by the end of 2028. Rhamnolipids, sophorolipids, and methyl ester sulfonates are the three most commercially produced types of biosurfactants. In 2020, the global rhamnolipids-based biosurfactants market for oil and gas industry alone was valued at USD 3.95 million, with a CAGR of 9% expected from 2021 to 2027. Because of their outstanding emulsification capabilities, organic surfactants are increasingly being used in enhanced oil recovery, which is projected to further drive rhamnolipids market demand. The global market for sophorolipids-based biosurfactants for personal care is predicted to exceed 1.35 million USD by 2027, with a CAGR of 7.5% from 2021 to 2027. Commercially, sophorolipids are utilized as an active ingredient in skin and body cosmetics. They also stimulate fibroblast metabolism and collagen formation in the skin's dermis, acting as a remodeling and tightening agent. MES, or methyl ester sulfonate, is another category of biosurfactants derived from natural fats and oils and has shown outstanding qualities such as saponification, biodegradability, and increased calcium hardness resistance. The market for these biosurfactants is expected to reach 1.05 billion USD by 2027, rising at a CAGR of more than 6% between 2021 and 2027.

Similarly, the market for other biosurfactants is expected to rise as their application in various industries is increasing continuously. Growing use of household cleaning products as a result of rising personal hygiene awareness is expected to

drive the alkyl polyglucosides (APG) biosurfactants market. The use of APG in surface cleansers, dishwashing detergents, grill cleaners, and bathroom cleaners has increased due to improved wetting capabilities, reduced surface tension, hard water tolerance, and a positive ecotoxicological profile. The expansion of the bakery and confectionary industries is expected to drive the worldwide demand of sorbitan esters. Sorbitan esters are emulsifiers used in salad dressings, ice creams, and other confectionery items.

1.3 Biosurfactant Market: Regional Distribution

Europe emerged as a prominent player in the worldwide biosurfactants market, with a market value of more than USD 801 million in 2020 and a CAGR of 6% over the research period. The biosurfactant market volume in Europe centered on Germany, the United Kingdom, France, and Italy. In Europe, the use of bio-based products rather than traditional petroleum-based products is encouraged by government guidelines and legislation. In the coming years, environmental protection measures and increasing health awareness are likely to continue to evolve. Apart from this, the region's strong consumer awareness and demand for bio-based products has increased local demand for biosurfactants.

Increasing R&D and product innovation investments by industry participants is expected to further accelerate market competitiveness. For instance, Unilever in 2019 utilized Evonik's 100% biodegradable rhamnolipids and launched a new dishwashing liquid under its brand Quix. These biosurfactants have remarkable foam-forming characteristics and provide high-performance cleaning outcomes while still being environmentally friendly. According to the Office for National Statistics in the United Kingdom, the income generated by the manufacturing of soap and detergents, as well as cleaning and polishing preparations, is expected to reach USD 6131.81 million by 2023.

North America has a rapidly growing market and a key market for biosurfactants. Besides the increasing consumer concerns due to increased awareness about the harmful impacts of chemical surfactants, rising demand for biosurfactants in the oil and petroleum sector is likely to drive the biosurfactant market forward. Biosurfactant market development in South America is expected to be driven by high biodiversity in Brazil and other countries, as well as plentiful supply of feedstock and raw materials from the agro-industrial sector that may be used as a substrate for biosurfactant manufacturing. The use of biosurfactants in the recovery and extraction of heavy crude oil is also likely to boost the biosurfactant market in South America.

The market for biosurfactants is also predicted to expand in the Middle East and Africa, owing to the large number of companies making personal care products and household detergents.

The Asia-Pacific region accounted for the majority of the regional biosurfactant market. The region currently accounts for about 32% of the global beauty and

personal care market. Furthermore, among Asia-Pacific countries, the Indian personal care chemicals market is predicted to grow at the highest rate. Thus, the demand for biosurfactants from cosmetics to detergents and industrial cleansers is expected to rise throughout the globe.

1.4 Commercial Production of Biosurfactants

The global biosurfactants industry is highly concentrated, with the top five manufacturers accounting for more than 80% of the market. Evonik Industries (Germany), BASF SE (Germany), Ecover (Belgium), Jeneil (USA), and Givaudan (Switzerland) are among the top companies which dominate the biosurfactants market.

Evonik was the first company in the world to invest in industrial scale biosurfactant production. Evonik teamed up with Unilever to pioneer industrial scale manufacture of fully degradable rhamnolipid biosurfactants. Apart from this, Evonik is also involved in commercial production of sophorolipids. The first Evonik sophorolipids containing household cleaners are already available in supermarkets.

BASF is involved in commercial production of more than 80 biosurfactants for the cosmetic industry, home care and I&I industry. These include: GlucoPON® 650 EC, Dehydol® LT 7/MB, and GlucoPON® 100 DK. BASF and Holiferm, an English biosurfactant company, are collaborating on the development of additional glycolipid surfactants for application in home care, personal care, and industrial formulations.

Ecover's goal is to replace Petro-based surfactants with biosurfactants derived from renewable resources. The company is renowned for producing biosurfactants for use in detergent formulations, fabric softener formulations, stain remover formulations, dishwasher formulations, and surface and glass cleaner formulations.

Where most of the companies producing biosurfactants at a commercial scale focus on a particular field of application, Jeneil biotech on the other hand produces a variety of biosurfactants for agriculture, bioremediation, household, and personal care use and also for antimicrobial applications. Jeneil is the sole manufacturer of Zonix™, a bio-fungicide that protects against plant diseases and zoosporic contamination by *Phytophthora*, *Pythium*, and Downy Mildew without the usage of copper. Jeneil's natural biosurfactant compounds are also employed as growth adjuvants to improve crop output, soil nutrient bioavailability, and root system and general plant health. Jeneil offers a wide range of natural rhamnolipids or co-surfactant solutions for bioremediation and crude oil recovery that are both cost-effective and environmentally friendly. Their rhamnolipid biosurfactants have also been found to be effective in the removal of hydrocarbons and heavy metals from soil and sludge. Jeneil also produces biosurfactants for household and personal care use. The rhamnolipid biosurfactants produced by Jeneil for personal care use show excellent emulsification, wetting, detergency, foaming, biodegradability and are devoid of heavy metals and harsh chemicals and do not produce or leave toxic or persistent residues.

Givaudan commercially produces sophorolipids biosurfactants under the name of Sopholiance® S which has antibacterial and sebum control activity. The biosurfactant targets specific microorganisms that cause acne and body odors. Other companies involved in commercial production of biosurfactants include: AGAE Technologies, Glycosurf, Tensiogreen, Stepan Company, and Holiferm.

1.5 Factors Affecting Biosurfactant Production

Microorganisms produce biosurfactants as secondary metabolites to improve the access of microbial cells to insoluble substrates by lowering the interfacial tension between phases (Sarubbo et al. 2022). Nutritional parameters like water, carbon, nitrogen, inorganic ions, vitamins and oxygen, and physical parameters like temp, pH, salinity, and agitation are all required for microbes to grow and produce commercially valuable compounds (Mulligan et al. 2014; Kosaric and Sukan 2014; Jimoh and Lin 2019; Osman et al. 2019). Biosurfactant production is also heavily dependent on these factors.

1.5.1 Nutritional Factors

The composition of the fermentation medium has a significant impact on biosurfactant synthesis, as the optimum level of medium components such as carbon, nitrogen, metal ions, and other additives are required for maximizing the yield and productivity of biosurfactants (Kosaric and Sukan 2014).

The type of carbon sources utilized in bioprocesses have a significant impact on biosurfactant yields, structure, quality, and quantity (Jimoh and Lin 2019). Ilori et al. identified simple carbon sources like glucose, sucrose and glycerol, and oils like diesel and raw petroleum as potential carbon sources for biosurfactant in their investigation (Ilori et al. 2005). A single microbial strain produces distinct kinds of biosurfactants with different carbon sources (Raza et al. 2007). Vecino et al. reported that when sugars obtained from vineyard pruning waste were utilized as the carbon source, a glycolipopeptide was produced; however, when lactose was utilized as the carbon source, a glycoprotein was produced (Vecino et al. 2017). Different carbon sources, according to Jain et al., affected the amount and quality of biosurfactant produced, resulting in differences in yields and physical–chemical properties (Jain et al. 2013).

Nitrogen is a key component of cellular constituents and for the production of bioactive metabolites. Its source and concentration used in fermentation also affect the overall biosurfactant production. Vigneshwaran et al. reported that high concentrations of nitrogen are detrimental to the fermentation process, with a highest biosurfactant production observed when KNO_3 was used as a nitrogen source (Vigneshwaran et al. 2018). Zargar et al. used yeast extract as a source of nitrogen for the production of saponin (Zargar et al. 2022a). The group reported that saponin

production is inhibited by higher concentration of yeast extract. Abouseoud et al. studied the impact of 3 distinct nitrogen sources on biosurfactant synthesis (NH_4Cl , NaNO_3 , and NH_4NO_3) and concluded that although ammonium nitrate (NH_4NO_3) produced the best results, its excess affected the overall biosurfactant yield (Abouseoud et al. 2008a). Abushady et al. reported that inorganic nitrogen sources especially NH_4NO_3 resulted in higher surfactin yields as compared to organic nitrogen (Abushady et al. 2005). In contrast, Zargar et al. reported that utilization of beef extract as a nitrogen source resulted in increased concentration of saponin as compared to inorganic nitrogen sources (Zargar et al. 2022a). Therefore, the concentration and the type of nitrogen source affect the yield and concentration of biosurfactants.

Apart from carbon and nitrogen sources, various inorganic ions present in the medium affect production of the biosurfactant. Gudina et al. reported that metallic salts FeSO_4 , MnSO_4 , and MgSO_4 resulted in 3–4 times increase in the biosurfactant production (Gudiña et al. 2015). These metals act as cofactors of enzymes which are required for production of surfactin. Improvement in biosurfactant production was observed when they were added to the fermentation medium. Makkar and Cameotra reported that metallic supplements also had a substantial effect on the biosurfactant production. It has also been reported that the presence of a high concentration of inorganic ions in the medium inhibited the synthesis of biosurfactants (Makkar and Cameotra 2002). Abdel-Mawgoud et al. demonstrated that Zn^{1+} , Fe^{2+} , Fe^{3+} , and Mn^{2+} ions boosted *B. subtilis* growth and surfactin production, with superior results obtained when Fe^{3+} was used instead of Fe^{2+} . The investigation demonstrated the Cu^{2+} ion's negative influence on bacterial growth and biosurfactant production (Abdel-Mawgoud et al. 2008). The studies point out to the important role of inorganic ions in promoting growth of the bacteria and in bacterial production of biosurfactants.

1.5.2 Physical Parameters

In addition to nutritional factors, various physical factors affect the overall microbial growth and biosurfactant production. Temperature, pH, mixing, aeration, salinity, and inoculum concentration are common physical factors that influence the yield and concentration of the biosurfactant production.

Temperature is a key factor that not only changes the bioprocess performance but also affects the composition of the biosurfactant. Microbial strains are capable of growing in a range of temperatures, however even if the growth remains unaffected, biosurfactant production occurs only at a particular temperature (Abdel-Mawgoud et al. 2008). Most of the studies have reported the optimum temperature for microbial production of biosurfactants between 30 °C and 37 °C (Hassan et al. 2016; Vigneshwaran et al. 2018; Abushady et al. 2005; Sawant et al. 2021). The pH of a microorganism's fermentation medium is extremely important since it influences both cell growth and the generation of several metabolites. The pH affects the cellular metabolism of the bacterial cells and therefore regulates its growth which

in turn affects the biosurfactant production. Various studies have concluded that optimum pH for biosurfactant production ranges from 4 to 7 (Vigneshwaran et al. 2018; Abushady et al. 2005; Makkar and Cameotra 2002). Aeration and mixing encourage the homogenous distribution of fermentation broth inside the vessel. Silva et al. investigated the effects of aeration and agitation on *P. aeruginosa* biosurfactant synthesis. The group reported that the speed of 200 rpm accumulates higher concentration of biosurfactants (6.5 g/L) than the other two speeds tested (150 and 200 rpm) (Silva et al. 2010). In other investigations, increasing the agitation speed was found to have a favorable influence on biosurfactant accumulation (Ghribi and Ellouze-Chaabouni 2011; Oliveira et al. 2009).

1.6 Challenges in the Large-Scale Production and Commercialization of Biosurfactants

Despite the advantages of biosurfactants, commercialization remains challenging and costly. High cost associated with procurement of raw material and for downstream processing of biosurfactants, low process yields and productivity, and several problems associated with fermentation process, e.g., foaming, are the key obstacles in commercial biosurfactant production (Henkel et al. 2012; Kronemberger et al. 2007; Makkar et al. 2011) (Fig. 1). Challenges in large-scale production of biosurfactants can broadly be categorized into economic constraints, technical constraints, and safety concerns.

1.6.1 Economic Constraints

To be commercially competitive in market, biosurfactants must be priced equally or less than their synthetic counterparts, which are currently valued at USD 2/kg (Santos et al. 2016). The high costs associated with producing biosurfactants are associated with the raw material acquisition and biosurfactant recovery methods, which accounts for up to 80% of total manufacturing expenses (Petrides 2000). Downstream processing of biosurfactants alone is 10–12 times higher than downstream processing of chemical surfactants (Winterburn and Martin 2012). This makes it difficult for these biomolecules to establish themselves in the surfactant and related markets.

Depending on the various variables involved in the production process, some biosurfactants cost around USD 1.0/kg to make, while others cost more than USD 10,000,000/kg. The price of rhamnolipids ranges from USD 1.5/g to USD 1500/g, depending on the purity of the biosurfactant. Lipopeptide biosurfactants are produced in small batches for the use in cosmetics and pharmaceutical industries. Their price ranges from \$20 to \$130/mg (Luna et al. 2012). Fengycin (>90%) and Iturin A (>95%) are two further biosurfactants with total manufacturing costs of USD

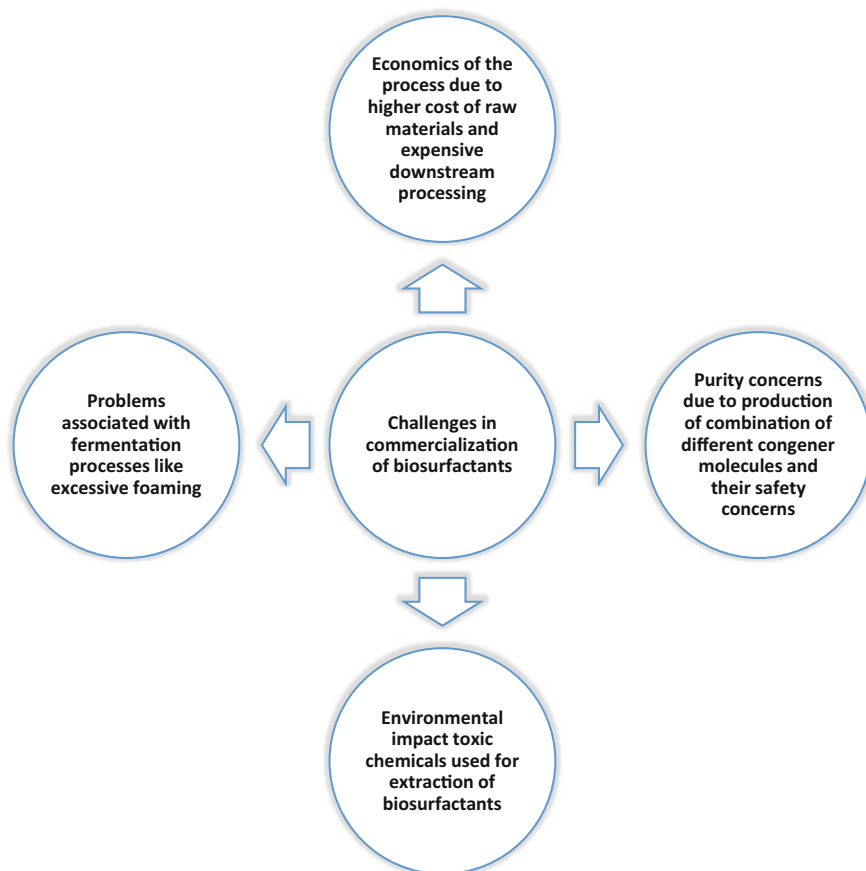


Fig. 1 Challenges in the commercialization of the biosurfactants

114/mg and USD 115/mg, respectively. In contrast, chemical surfactants like sodium dodecyl sulfate (ACS reagent >95%) and saponin, on the other hand, have a total production cost of only USD 1.46/g and 1.59/g, respectively. The difference between the production costs of biosurfactants and the chemical surfactants is one of the key factors that prevents biosurfactants to establish themselves in the market.

1.6.2 Technical Constraints

In most cases, biosurfactant production has been accomplished using batch and fed-batch fermentations. A major limitation in fermentation processes employed for biosurfactant production even at lower scale is the lower yield and the productivity of biosurfactant from low-cost substrates. Hu et al. reported multiscale production of biosurfactant by *Bacillus subtilis* using cheap fish waste as the substrate

(Hu et al. 2021). The surfactin production was successfully scaled from 7 L batch reactor to a 100 L pilot scale reactor and productivity of 0.274 g/L was obtained at pilot scale. Almeida et al. reported the production of biosurfactants by *Candida tropicalis* UCP0996 in a 50 L reactor. The group was successful in achieving biosurfactant concentration of 7.36 g/L. Similarly, Brumano et al. demonstrated biosurfactant production by *Aureobasidium pullulans* in a 5 L stirred tank bioreactor (Brumano et al. 2017). The group reported a maximum tensioactivity of 8.05 mN/m and biosurfactant concentration of 1.5 g/L was achieved. Pagilla et al. reported biosurfactant production using *Gordonia amarae* in an 8 L batch reactor using sodium acetate and hexadecane as primary substrates (Pagilla et al. 2002). The group attained a maximum biosurfactant production of 5.7 g/L. Most of the fermentation studies have reported biosurfactant concentration < 10 g/L. The lower yields obtained limit the large-scale production of the biosurfactant production and hamper the commercialization of the most of the biosurfactants. Other studies have demonstrated a high production yield at the laboratory scale level, but the yield necessary at the industrial scale level on an economical scale remains a difficulty for most of the biosurfactants.

Foaming is a critical aspect in fermentation processes, especially for production of extracellular biosurfactants. Foam forms in the fermenter vessel due to the presence of surface-active compounds in the culture medium, as well as those produced by microbes during fermentation. As bubbles travel through the solution, surfactants in the fermentation broth adsorb to the gas–liquid surface of bubbles and stabilize the foam (Winterburn and Martin 2012). In fermentation processes, foaming that occurs at the outset of a fermentation is usually due to surface-active components present in the growth medium, but foaming that occurs later in the fermentation is most likely due to production of proteins and surface-active compounds excreted into the fermentation broth by the growing cell population (Junker 2007). In fermentation processes for production of biosurfactants, the latter is frequently the source of substantial foaming. The gas flow rate and the agitation required to ensure optimal O₂ mass transfer often result in bulk foam generation (Winterburn and Martin 2012). In fermentations, foaming is undesirable because it disrupts the fermentation process, for example, by carrying over fermentation broth into the off gas, entrapping most of the microbial cells, reducing their fraction in the bulk medium, making the batch more susceptible to contamination, and complicating process control and resulting in lower biosurfactant yields (Winterburn and Martin 2012). Chemical antifoams, which are usually silicone oils and their emulsions, are frequently used to control foam. They work by destabilizing the liquid films in the foam, causing liquid to drain faster and causing the foam to collapse (Garrett 1993; Miller 2008). However, chemical antifoams cannot be used for control of foam during production of the biosurfactants because their presence in the final product may have a negative impact on the performance of biosurfactants.

Another factor that limits the commercial production of the biosurfactants is downstream processing associated with production of pure biosurfactants. One of the most difficult aspects of using biosurfactants is purity of the biosurfactants. Each microorganism produces a unique combination of congener molecules with varying

shapes and properties (Marchant and Banat 2012). The unsaturation of the lipid chains and the quantity of acyl groups in sophorolipids produced by a single microbial strain may vary. This results in two major molecular configurations, acidic and lactonic (Smyth et al. 2010). Similarly, several different molecules of rhamnolipids with differing alkyl chain lengths are produced by *Pseudomonas aeruginosa*. The application of the biosurfactant determines the level of biosurfactant purity required after the final step in downstream processing. For example, the purity of the biosurfactant required in oil solubilization, agriculture, and bioremediation is very low; however, high purity is required for the use in food, cosmetics, and pharmaceutical industries. Although it is possible to extract and segregate the various congeners forms of these biosurfactants, their downstream processing is unlikely to be economically viable on an industrial scale (Marchant and Banat 2012). Multiple sequential processes are required in the downstream processing of diluted broths on an industrial scale, which makes attaining the pure product problematic. This also has an impact on the overall cost of the production.

1.6.3 Safety Concerns

Despite the fact that the biosurfactants are less harmful as compared to chemical surfactants, evidence shows that majority of the common bacteria that produce it are pathogenic in nature and have virulence effects (Dhanya 2021). Along with biosurfactant production, these microorganisms secrete some virulent factors in the culture broth. *Pseudomonas aeruginosa*, the key rhamnolipid-producing bacteria, is an opportunistic pathogen (Cha et al. 2008; Rikalović et al. 2015). Rhamnolipid-producing *Burkholderia* sp., phospholipid-producing *Klebsiella pneumoniae*, lipopeptide-producing *Serratia marcescens*, trehalolipids-producing *Rhodococcus* sp., and heteropolysaccharide-producing *Cronobacter sakazakii* are some other pathogenic biosurfactant-producing bacteria (Toribio et al. 2010; Nwaguma et al. 2016; Kuyukina et al. 2005). Some investigations have found that biosurfactants such as lipopeptides generated by *Bacillus subtilis* have the potential to rupture erythrocytes in hemolytic activity tests; however, their effect is less than that of synthetic biosurfactants such CTAB, TTAB, BC, and SDS. This causes health-related concerns and limits the commercialization and application of these biosurfactants in cosmetic and pharmaceutical industry.

Other factors that also limit the commercialization of the biosurfactants include lack of knowledge for regulation of biosurfactant synthesis to enhance industrial scale production, variation of biosurfactant activity, and antagonistic effect of biosurfactants on other beneficial microbes (Dhanya 2021).

1.7 Future Research Directions to Improve Biosurfactant Yields

Commercialization of biosurfactants is difficult due to the reasons stated in the preceding section. This makes it imperative to focus the current research and developments to increase the yields, lower process economics, and improve the fermentation process for commercial production of biosurfactants. The strategies to enhance biosurfactant production may be targeted at improving biosurfactant yield, improving process economics and improvements in fermentation process for production of biosurfactants (Fig. 2).

1.7.1 Strategies to Improve Biosurfactant Yields

Most of the microbial strains reported for biosurfactant production suffer from a major drawback of low biosurfactant yield and productivity. The metabolic and cellular engineering techniques can help in improving the performance of microbial strains for enhanced biosurfactant synthesis by increasing the metabolic flux towards biosurfactant production (Dhanya 2021). Lee et al. reported successful improvement in the biosurfactant yield by using molecular techniques for upregulating genes encoding enzymes and regulatory proteins for the production of a biosurfactant (Lee et al. 2005). Mulligan et al. (1989) reported that UV-ray-induced mutations at *argC4* and *hisA1* of *B. subtilis* ATCC 21332 resulted in a strain with 3.5-fold increased surfactin synthesis (Mulligan et al. 1989). Similar reports on increased

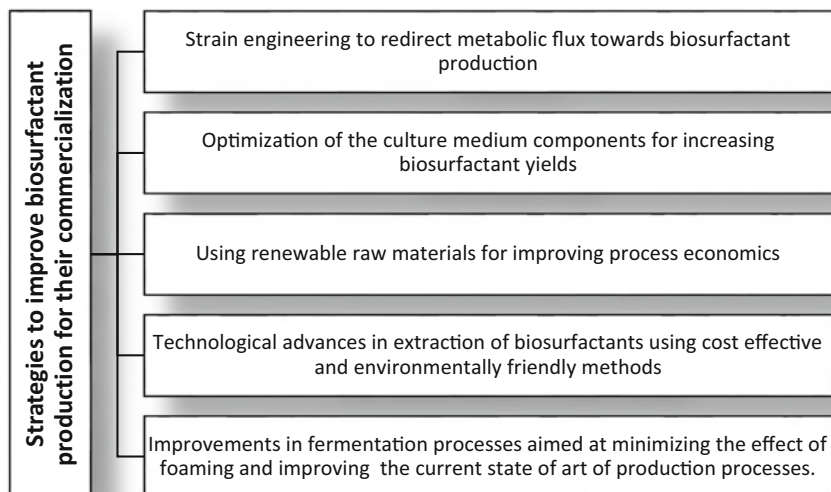


Fig. 2 Strategies for improving industrial production of biosurfactants

sophorolipid production by *Candida bombicola* due to deletion of MFE-2 gene and blocking the β -oxidation pathway exist in literature (Dogan et al. 2006; Koch et al. 1988; Van Bogaert et al. 2009). Another approach is to clone the biosurfactant-producing genes under the control of strong promoters in heterologous hosts and target enhanced production of biosurfactants. Examples of such strategies previously employed include expression of *rhl* genes in *Pseudomonas putida* and insertion of surfactin synthase genes from *Bacillus licheniformis* to *Escherichia coli* M15 (Cha et al. 2008; Wittgens et al. 2017; Anburajan et al. 2015). Another simple strategy for enhancing commercial biosurfactant production may be to try to isolating novel microbial strains with intrinsic capacity to produce high yield of biosurfactants.

As already mentioned, the composition of the culture medium is critical for the production of biosurfactants by bacteria. Therefore, formulation of an optimum medium can result in enhanced yield of biosurfactants. An optimum medium enhances the yield of biosurfactant by supplying all the components in their optimum concentration required for microbial growth and for biosurfactant production (Zargar et al. 2022a). Various studies on the statistical medium optimization using response surface methodology, Taguchi and Plackett–Burman designs for enhancing biosurfactant production have been performed by various research groups (Hassan et al. 2016; Eswari et al. 2016; Dos Santos et al. 2016b). Other than statistical methods, artificial intelligence-based optimizations such as Artificial Neural Networks combined with Genetic Algorithm (ANN-GA) have also been used to optimize the culture medium for production of biosurfactants (Sivapathasekaran and Sen 2013). Such optimizations in the past have successfully resulted in an increase in the yield and the concentration of various biosurfactants. Further research should be directed at optimizing the culture medium components and their concentrations in order to increase the yields of biosurfactants produced by strains that are intrinsically capable of producing high yield of biosurfactants.

1.7.2 Strategies to Improve Process Economics

The cost of the raw materials utilized for industrial production of the biosurfactants, as well as the downstream processing needed in purifying the biosurfactant, determine the economics of biosurfactant production. As a result, attempts to enhance process economics should focus on these two elements.

Ashby et al. discovered that glucose and oleic acid as raw materials accounted for nearly 75% of the total operating cost for the production of 90.7 million kg of sophorolipid (Ashby et al. 2013). The utilization of low-cost, renewable substrates allows production of biosurfactant that is both effective and environmentally friendly (Patil and Rao 2014). Rodrigues et al. used low-cost components to produce a biosurfactant, resulting in 1.5 times higher production of biosurfactant while achieving a 60–80% reduction in the medium cost (Rodrigues et al. 2006). The cost of biosurfactants production can be reduced by switching from expensive raw materials such as glucose, glycerol, vegetable oils, etc. to less expensive raw materials (Santos et al. 2002). Using agro-industrial by-products and renewable

resources for the production of biosurfactants can significantly reduce the overall economics of the process (Henkel et al. 2012). Previously, molasses, sugarcane bagasse, cassava waste, sesame peel flour, potato peel, corn steep liquor, peanut oil cake, orange peel, and banana peel have been utilized for biosurfactant production (Al-Bahry et al. 2013; Chooklin et al. 2014; Sharma et al. 2015; Kumar et al. 2016; Lins et al. 2016; Rubio-Ribeaux et al. 2017; Magalhães et al. 2018). *Aneurinibacillus migulanus*, *Nocardia higoensis*, and *Pseudomonas gessardii* have also been used to explore the synthesis of biosurfactants from animal wastes (Patil and Rao 2014; Ramani et al. 2012; Sellami et al. 2021). Biosurfactants have also been produced from agro-industrial and mill wastes like olive mill waste, palm and soybean oil industry waste (Kiran et al. 2014b; Gudiña et al. 2016; Li et al. 2016; Ramírez et al. 2016; Radzuan et al. 2017). Similarly, other low-cost renewable substrates should be utilized for biosurfactant production to improve the overall process economics.

Apart from the raw materials, improvements in the downstream processing will help in substantially improving the economics of biosurfactant production. Downstream processing of biosurfactants accounts for more than 60% of the total production costs (Desai and Banat 1997). The total cost of downstream processing is often determined by the degree of purity required for a given application. Depending on the microbial strain, the biosurfactant congeners produced, and the field of application, the biosurfactant purification steps differ from one process to another. Conventionally the steps involved in the recovery of the biosurfactants include biomass removal followed by precipitation using acids, solvent extraction using organic solvents, and crystallization. The methods used involve the use of various chemicals, which are not only expensive but also hazardous to the environment. Therefore, commercial application of these chemicals is not a viable option. Recently, various methods based on the surface activity and micelle forming capacity of the biosurfactants have been employed for recovery of biosurfactants. These include foam fraction, ultrafiltration, adsorption, and ion exchange chromatography.

1.7.3 Strategies to Improve Fermentation Process for Production of Biosurfactants

Fermentation techniques have a significant impact on primary and secondary metabolite yield and productivity. The type of reactor utilized and the mode of operation influence the performance with which any bacterial metabolite is produced. There have been no consistent methods used for designing fermentation strategies in biosurfactant production. Batch, fed batch, and continuous modes of fermentation have been utilized by various groups for production of biosurfactants (Beuker et al. 2014).

In literature, many biosurfactant production processes have used batch cultivations (Zargar et al. 2022a; Davis et al. 2001; Yeh et al. 2006; Müller et al. 2010). The limitation of the batch process for biosurfactant production is that no control over the number of substrates in the bioreactor or the pace of growth over time is feasible with

batch fermentation techniques. In addition to batch fermentation, sequential batch techniques have also been employed in the production of biosurfactants (Pornsunthorntawee et al. 2009).

Due to complexity of regulation, fed-batch mode of production based on heuristic approaches has resulted in biosurfactant production comparable to batch operations. Fed-batch strategies can offer certain advantages over batch production such as greater control over substrate concentrations and growth rate by regulating feeding rate. This could result in viable process strategies for obtaining high concentrations of biosurfactants via fermentation. Fed batch mode of operation is a two-step process. In first phase, biomass accumulation can be targeted. In this phase relatively little amount of biosurfactant will be accumulated. In the second phase specific substrate concentration (nitrogen source) can be maintained below a critical level such that biomass production ceases and accumulation of biosurfactant occurs (Davila et al. 1992). This strategy can be used efficiently for production of biosurfactants accumulated during stationary phase. Several research groups have already achieved high biosurfactant yields using this strategy (Davila et al. 1992; Pekin et al. 2005; Van Bogaert et al. 2007). According to Davila et al., this method resulted in the synthesis of 320 g/L of sophorolipids with yield up to 65% relative to the carbon source. However, producing biosurfactants in a fed batch mode may be challenging as sustained nutrient feeding strategy, excessive biomass growth, and limited heat and mass transfer might limit the process efficiency.

Another mode of bioreactor operation used for production of surfactin and rhamnolipid biosurfactants is CSTRs (Chen et al. 2006; Chen et al. 2021; Guez et al. 2021). However, the use of continuous cultures for biosurfactant production is primarily limited by intensive foaming, poor downstream processing for continuous recovery of biosurfactants, and the washout of cells from the reactor at higher dilution rates. To overcome these problems, an alternate strategy would be to utilize immobilized or resting cells. This strategy can be very efficient because microbial cells will utilize the carbon source exclusively for biosurfactant production and not for the growth of the microbial cells. Immobilizing the cells will enable to perform the fermentation at higher dilution rates to enhance the productivity of the process. It will also enable easy downstream processing for product extraction and will circumvent the problem of foaming to a great extent. Therefore, immobilization will result in lower production costs and improved biosurfactant yield and productivity (Srivastava et al. 2021). Various reports on enhanced biosurfactant production due to immobilization are available in literature (Dehghannoudeh et al. 2019; Abouseoud et al. 2008b; Heyd et al. 2011).

Solid state fermentation (SSF) is evolving strategy for the production of biosurfactants, particularly for reducing the foaming problem associated with more extensively used submerged fermentations (Das and Mukherjee 2007; Camilios-Neto et al. 2011). Synthesis of surfactin using SSF by *Bacillus pumilus* UFPEDA 448 has been performed by Slivinski et al. The group used a medium based on okara and sugarcane bagasse in a column bioreactor with forced aeration. They reported that under optimal conditions, 809 mg/L of surfactin was produced, which was comparable to the amounts reported in the literature using submerged fermentation

(Slivinski et al. 2012). The strategy simplified the biomass extraction for extraction of surfactin. Zhu et al. reported the use of soyabean flour and rice straw as substrate in solid state fermentation for production of lipopeptide by *Bacillus amyloliquefaciens* XZ-173 (Zhu et al. 2012). Various other successful stories on solid state fermentation for biosurfactant production are available in literature (Zouari et al. 2014; Velioglu and ÜREK RÖ. 2015).

Another technique for optimizing biosurfactant production is to add specific solid support carriers into the growth medium. Yeh et al. have reported an increase in the growth of the microorganisms resulting in 36-fold increase in the surfactin yield by addition of activated charcoal in the culture medium (Yeh et al. 2005). The enhancement in growth was believed to be due to the formation of activated carbon barriers, that were partially utilized for biofilm-associated cell proliferation.

Another possible strategy to enhance the fermentation process for production of biosurfactants could be to add certain growth inducers to the fermentation medium. Santos et al. reported stimulation of rhamnolipid synthesis by adding endogenous homoserine lactones and recycling a fraction of spent medium (Dos Santos et al. 2016a). This strategy led to a 100% increase in rhamnolipid production. Addition of lactones seems to be promising strategy for increasing the biosurfactant production, particularly glycolipids. Apart from growth enhancement, lactones can also be used for production of a specific type of biosurfactant congener (Singh et al. 2019).

Another strategy to boost production of biosurfactants is the use of nanoparticles (NP). Many metal salts, particularly iron, have been reported to have a major impact on biosurfactant production. As a result, the utilization of low quantities of iron nanoparticles is an emerging prospective strategy for increased biosurfactant production (Fe-NPs). Kiran et al. reported an 80% increase in biosurfactant production by *Nocardiopsis* MSA13A in the presence of 10 mg/L Fe-NP (Kiran et al. 2014a). Similarly, Liu et al. reported 63% increase in glycolipid biosurfactant production by *Serratia* sp. in presence of 1 mg/L Fe-NP (Liu et al. 2013). Sahebnazar et al. have also reported a 57% increase in rhamnolipid concentration by *Pseudomonas aeruginosa* due to addition of 1 mg/L of iron silica nanoparticle (Sahebnazar et al. 2018). More research should be conducted on the role of other metallic nanoparticle in promoting biosurfactant production.

Apart from the above strategies aimed at enhancing the yield and productivity of the biosurfactants, other strategies should be aimed to improve the current state of art of bioreactors used for biosurfactant production. A hydrophobic carbon source is usually advantageous for inducing the biosurfactant production (Henkel et al. 2012). Biphasic fermentation systems can offer certain advantages in those production processes. In such systems, biomass and biosurfactants concentrate in different phases enabling higher biosurfactant yields and simpler downstream processing.

As already stated, most of the biosurfactant production has been performed using stirred tank reactors which faces a huge problem of managing the foam produced during the process. Bioprocess developments aimed at reducing foaming in submerged biosurfactant production processes should be targeted. Since antifoams cannot be used to control foaming, mechanical design of the bioreactor should be modified to enhance foam disruption. Various designs of mechanical foam breakers

which do not cause cell rupture should be tried out for disruption of the foam accumulated in the headspace of the reactor (Beuker et al. 2014). Biosurfactant production in other reactor configurations like rotating disc bioreactor and bubble free membrane bioreactor has been carried out to avoid foaming (Chtioui et al. 2012; Coutte et al. 2010). Other strategy that is proving to be very promising in managing the foam is foam fractionation followed by foam stripping (Winterburn and Martin 2012; Chen et al. 2021). The technique has been shown to be capable of achieving product enrichment and excellent recoveries while retaining biomass within the bioreactor.

Biosurfactant coproduction with other commercially significant chemical is another strategy that can be used for overall process economization. The enzyme lipase is one such chemical that is widely employed in a variety of industries. Microorganisms capable of producing lipases, if grown on water immiscible substrates will be prompted to produce biosurfactants in order to access the hydrophobic substrates. This would result in coproduction of lipases and biosurfactants which could prove to be economically profitable. Such coproduction of enzymes (lipases, alkaline amylase (Colla et al. 2010), and pectinases) and biosurfactants has been reported in the literature (Hmidet et al. 2019; Kavuthodi et al. 2015). Apart from enzymes, commercial biosurfactant production can be coupled with some other bioprocess that uses similar substrates (Raheb and Hajipour 2011; Amin et al. 2013; Zargar et al. 2021).

2 Conclusions and Future Prospects

As a result of the various sustainability initiatives, green agenda, and advantages over chemical surfactants, biosurfactants seem to have reached a very critical stage in their commercialization. The demand for these environmentally friendly compounds in various industries is growing day by day. However, various economic, technical, and safety concerns limit the large-scale production and commercialization of the biosurfactants. Factors such as higher cost, lower yield, and contamination by virulence factors are the primary reasons which drastically affect commercialization of biosurfactants. Downstream processing for extraction and purification of biosurfactants accounts for around 60% of the overall production cost. Its optimization can help to significantly reduce the cost of biosurfactants and bring it closer to that of the chemical surfactants. Failure to achieve higher yields may prevent commercialization of biosurfactants. A number of strategies aimed to enhance the biosurfactant yield and production process have been described. A combination of two or more strategies for a particular biosurfactant could be very effective in improving its large scale production and commercialization. Successful commercialization of the biosurfactants also depends on whether they can be tailored for a specific application (designer biosurfactants) especially in pharmaceutical and cosmetic industries. Potential of biosurfactants for future utilization is clearly reflected in the fact that they are already used in various commercial products.

However, more efforts are required at improving the economics of the biosurfactant production. Despite extensive research over the last two decades aimed at reducing the cost of production, their commercial success in comparison to synthetic competitors still remains a challenge.

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