Nep J Environ Sci (2022), 10(2), 15-23
https://doi.org/10.3126/njes.v10i2.48538

ISSN 2350-8647 (Print) 2542-2901 (Online)

Review Article

# Biosurfactants from renewable sources - A review

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*(**Received**: 29 June 2021; **Revised**: 11 November 2022; **Accepted**: 30 December 2022)*

## Abstract

Biosurfactants have wide applications in pharmaceutical, agriculture and food industries. The research area of biosurfactants is gaining immense attention. The review mentions here the advantages and various substrates used for biosurfactants production. The pre-treatment of substrates for biosurfactants production is also focused. The production of biosurfactants by solid state fermentation is also described. The renewable substrates, yield and microorganisms used for biosurfactant production are also taken into consideration. The screening methods for biosurfactant are also described. The use of renewable sources for biosurfactant production is specially focused in the review. This will be very eco-friendly, easy and economical. More studies need to BE done on large-scale production of biosurfactants using genetically engineered microorganisms.

***Keywords*****:** Agroindustry by-products, economical, eco-friendly, solid-state fermentation, value-added products

## Biosurfactants

Biosurfactants are amphiphilic compounds which can be produced from microbes using cheap renewable substrates. The research on biosurfactants is gaining wide attention by the scientists as because biosurfactants are eco-friendly and economical and can be produced easily using raw substrates or wastes. Biosurfactants have immense applications in medical, pharmaceutical, food, dairy, etc. industries (Akbari et al., 2018). Much of the wastes or by-products are generated from industries such as food, dairy, agricultural, etc. These wastes are disposed to the landfills without any treatment. So, such by-products or wastes can be used to produce biosurfactants. These wastes or by-products include bagasse, press mud, vegetable, fruit wastes such as orange, apple, banana peels, etc. These wastes or by-products generated in huge amounts are lignocellulosic in nature.

**Types of biosurfactants** (Roy, 2017)

The different biosurfactants include glycolipids, rhamnolipids, sophorolipids, trehalolipids, surfactin, lipopeptides and lipoproteins, polymeric and particulate (Fig. 1).

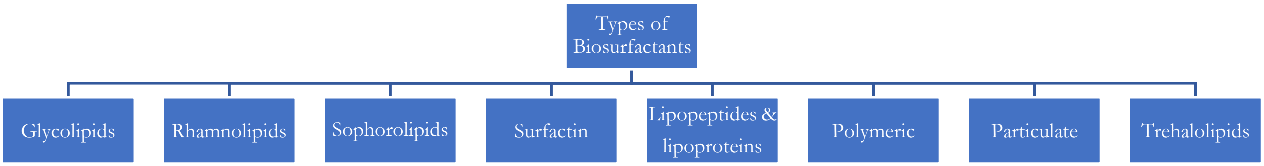

**Figure 1** Types of biosurfactants

**Advantages of biosurfactants** (Fenibo et al., 2019)

- Easy to breakdown
- Non-toxic
- Biosurfactants are compatible and hence have wide applications in various industries.
- They can be produced using economical substrates or wastes.
- They can minimize the surface tension
- Biosurfactants have stability over a wide range of temperature, sodium chloride concentration and pH.
- They have specificity.

**Global biosurfactant market**

Biosurfactants have immense demand in the market. Surfactants have immense demand globally. The global market in 2016 for biosurfactant was USD 30·64 billion which raised to USD 39·86 billion by 2021 (Markets & Markets, 2016). The CAGR for biosurfactant in the market was 4.3% in 2020 (Grand View Research, 2015). By 2023, the biosurfactant market is said to reach USD 2.6 billion (Global Market Insights, 2018). Europe is the biggest market for biosurfactant production to grow as the biggest market (around 53%) and then USA. Sophorolipids, type of biosufactants has the biggest market worldwide. Even

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the synthetic biosurfactants are cheap with the market price \$2 kg$^{-1}$ globally (Santos et al., 2016a).

## Substrates for biosurfactant production

The substrates used for biosurfactant production are agricultural, industrial, starch-rich, vegetable oil processing wastes, and dairy industry, whey, and oil mill waste effluent (Fig. 2).

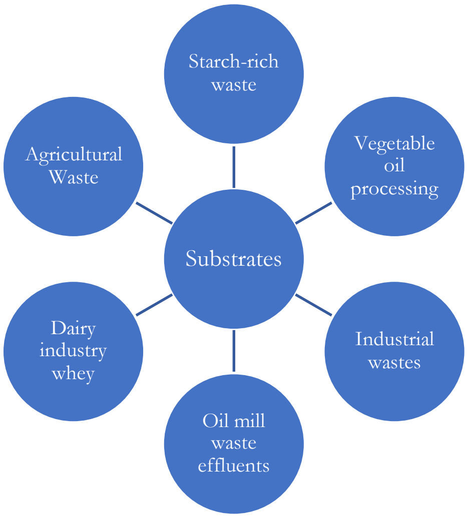

**Figure 2** Substrates for biosurfactant production

## Pre-treatment of substrates used for biosurfactant production

The substrates used for biosurfactant production can be pretreated by using acid such as hydrochloric acid (HCl), sulfuric acid ($H_2SO_4$), alkali such as sodium hydroxide (NaOH), potassium hydroxide (KOH), use of enzymes, drying, etc. (Fig. 3). The pre-treatment of the substrates will reduce the size of the substrates and make it suitable for biosurfactant production. Such pretreated substrates for the biosurfactant production will be useful in solid state fermentation for biosurfactant production.

The production of biosurfactant depends on carbon and nitrogen ratio. The commonly used media include minimal salt medium and usually the yield is in the range of 1.7 mg/ml (Phulpoto et al., 2020). However, the cost of laboratory media is a major constraint for large-scale production of biosurfactant. In order to reduce the cost and make the production economical, lowering the substrate/media cost is essential. At the same time, if the components of media are renewable, the production becomes eco-friendly. There are reports on reuse of agrowastes for biosurfactant production (Kourmentza, 2017; Rivera et al., 2019; Sharma et al., 2020). The present review consolidates renewable resources and proposes cost-effective processes for biosurfactant production.

In recent years, many researchers have shown the possibility of various agricultural, industrial wastes, etc. for utilization of biosurfactant production. They can be categorized as fruit, vegetable, agricultural wastes, starch-rich, lignocellulose, industrial, dairy industry whey, vegetable oil, etc. In order to make the production eco-friendly and cost-effective, low cost substrates and reduced production time is desired along with minimum energy requirements for fermentation process and availability of resources.

## Biosurfactant production using solid state fermentation

Biosurfactants can be produced by solid state fermentation (SSF) using economical and eco-friendly substrates or wastes such as fruit, vegetable, agricultural wastes, etc. SSF is fermentation in the absence or near absence of water. It is a biological process (carried out with the help of microorganisms). The process of biosurfactant production using SSF is represented in Fig. 4. The substrate is inoculated with the microorganism (count- 1x$10^8$cells/ml) and then kept for SSF for 25 days. The leachate obtained is analyzed for biosurfactant production after every 5 days of time period.

## Biosurfactant production using lignocellulosic wastes

Lignocellulose is a carbon source which is generated in huge quantities. They are composed of cellulose, hemicelluloses and lignin. There are reports on biosurfactant production using lignocellulosic wastes (Bezerra et al., 2019; Joy et al., 2019; Konishi et al., 2015; Panjiar et al., 2020). The different lignocellulosic wastes include bagasse, press mud, sugarcane trash, corn cob, orange, apple peel, coconut shell, rice, and wheat straw, etc.

### Fruit and vegetable waste for biosurfactant production

There are several reports where fruits and vegetable wastes such as apples, banana, orange peel, carrot, etc. are used as the best substrates for rhamnolipids biosurfactant (Chebbi et al., 2021; Kourmentza, 2017; Rivera et al., 2019; Sharma et al., 2020).

### Food waste for biosurfactant production

Food waste can be used as substrate for biosurfactant production. The biosurfactant production using food waste as substrate is shown in Fig. 5.

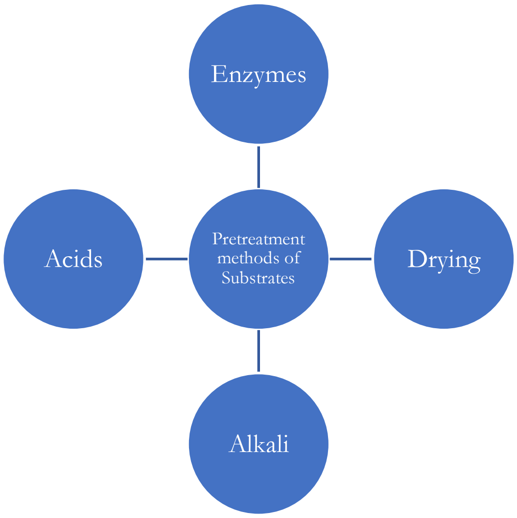

**Figure 3** Pretreatment methods of substrates for biosurfactants production

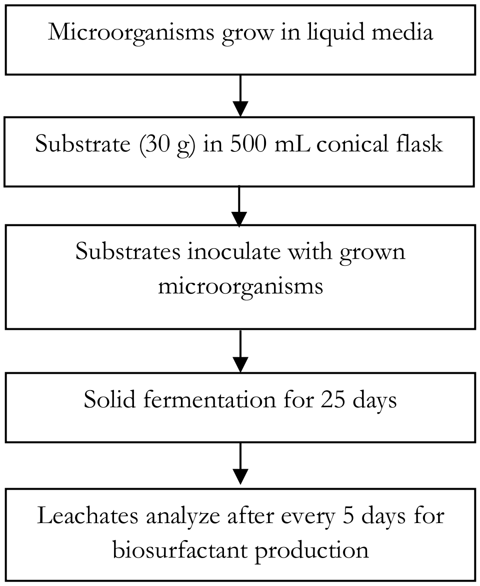

**Figure 4** Biosurfactant production using solid state fermentation

### Advantages of renewable sources for biosurfactant production

The advantages of various renewable sources for biosurfactant production are they are non-toxic, economical, and easily available in huge amounts. These renewable sources include molasses, cassava wastewater, animal fat, frying oil, cashew apple, glycerol, agro-industrial residues, whey, etc. (Fig. 6). The renewable substrates (%) for biosurfactant production (Raza et al., 2007) are shown in Fig. 6.

### Substrates used for biosurfactant production

(Vandana & Singh, 2018)

### Agricultural wastes

The agro-waste as substrate for biosurfactant production is shown in Fig. 7.

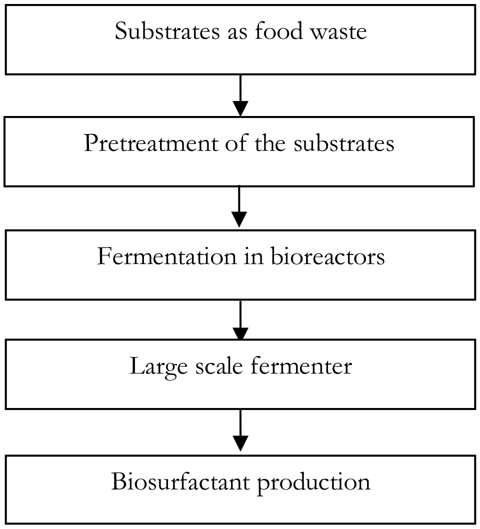

**Figure 5** Biosurfactant production using food waste as substrates

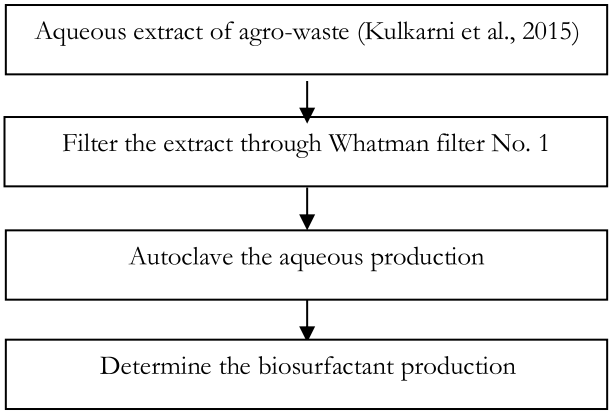

**Figure 6** Renewable sources for biosurfactant production

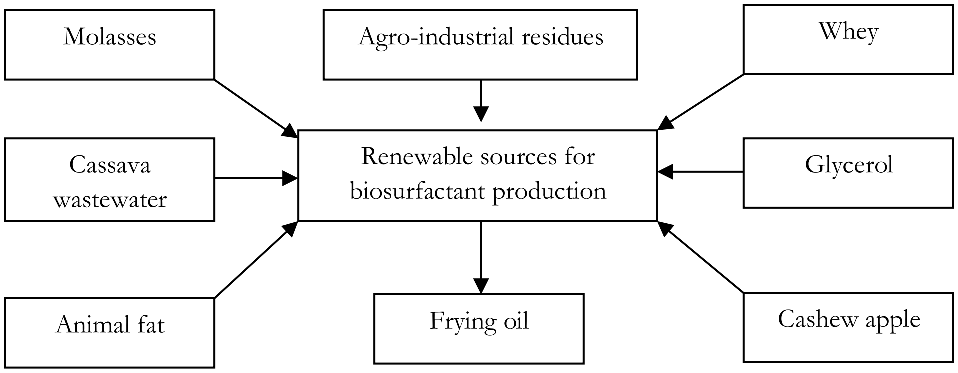

**Figure 7** Agro-waste as substrate for biosurfactant production

### Dairy industry whey

Curd whey is processed by removing casein and then deproteinized whey is used as substrate for biosurfactant production.

### Industrial wastes

Bagasse, distillery waste, sugar industry effluent, fruit processing wastes is used as cheap substrates for biosurfactant production.

### Vegetable oil

Vegetable oil is used as substrate for biosurfactant production.

### Microorganisms producing biosurfactant

The microorganisms (bacteria, fungi, yeast, actinobacteria) producing biosurfactant are *Bacillus*, *Pseudomonas*, *Streptomyces*, *Acinetobacter*, *Candida*, *Aspergillus*, *Paenibacillus*, *Rhodococcus* sp., *Serratia marcescens*, *Yarrowia lipolytica*, etc. The microorganisms and renewable substrates for biosurfactant production are represented in Table 1.

**Microorganisms and biosurfactant yield**

The bacteria and biosurfactant yield in represented in Table 2. And the fungi and biosurfactant yield in represented in Table 3.

**Composition of various substrates used for biosurfactant production**

a) Molasses: Dry matter - 76.8, Glucose - 5.29, phosphates - 2.03, potassium - 1.82, calcium - 1.39, magnesium - 0.43, total sugars - 62.3 (%), nitrates - 464 (mg/kg) (Palmonari et al., 2020).

b) Bagasse - Cellulose - 35.2, hemicelluloses - 24.5, lignin - 22.2, ash - 20.9 (%) (Rezende et al., 2011).

c) Pressmud - Nitrogen - 1.15-3.0, Phosphorus - 0.60-3.50, Potassium - 0.30-1.80, crude wax - 5-14, ash - 9-10, MgO - 0.5-1.5, CaO - 1-4 (%) (Rassppan et al., 2015).

d) Corn steep liquor - Carbohydrates - 5.8, proteins - 24, fats -1, minerals - 8.2 (%) (Joshi et al., 2018).

e) Vegetable oil - fatty acids

f) Rice straw - Cellulose - 49.29, hemicellulose - 11.55, silica - 17.07, organic matter - 80.08, ash - 19.92, crude protein - 4.40 (%)

**Table 1** Microorganisms and renewable substrates for biosurfactant production

| Microorganism | Renewable substrate | Reference |
|---|---|---|
| *Candida lipolytica* | Waste soybean oil and corn steep liquor | (Souza et al., 2016) |
| *Candida glabrata* | Corn steep liquor and whey | (Lima et al., 2017) |
| *Candida glabrata* | Corn steep liquor, whey and cassava wastewater | (Silva et al., 2015) |
| *Cunninghamella bertholletiae* | Waste soy oil and corn steep liquor | (Chebbi et al., 2021) |
| *Rhizopus arrhizus* | Soybean post-frying oil/sodium glutamate | (Pele et al., 2018) |

**Table 2** Bacteria and biosurfactant yield

| Bacteria | Biosurfactant | Substrate | Yield (mg/l) | Reference |
|---|---|---|---|---|
| *Pseudomonas* sp. | Rhamnolipid | | 2070 | (Priji et al., 2017) |
| *B. kururiensis* KP23 | Rhamnolipid | | 780 | (Tavares et al., 2013) |
| *Bacillus subtilis* ATCC 6051 | | Brewery waste (trub) | 100.76 | (Nazareth et al., 2021) |
| *Bacillus subtilis* MTCC 2423 | Surfactin | Rice mill polishing residue | 4.17 | (Gurjar & Sengupta, 2015) |
| *Bacillus licheniformis* AL1.1 | Lichenysin | Molasses | 3.2 | (Coronel-León et al., 2016) |
| *Bacillus pseudomycoides* | Lipopeptide | Soybean oil waste | 56 | (Li et al., 2016) |
| *Bacillus subtilis* DSM 3256 | Surfactin | Olive mill waste | 0.068 | (Maass et al., 2016) |
| *Bacillus nealsonii* S2MT | | Glycerol (2% v/v) and $NH_4NO_3$ (0.1% w/v) | 1300 | (Phulpoto et al., 2020) |
| *Staphylococcus* sp. | Lipopeptides | Residual frying oil | 65-750 | (Hentati et al., 2021) |
| *Bacillus subtilis* | | Corn steep liquor | 1.3 | (Gudina et al., 2015) |
| *Pseudomonas cepacia* CCT6659 | | Canola waste frying oil | 40500 | (Soares et al., 2018) |
| *Pseudomonas aeruginosa* M408 | | Olive oil | 12600 | (Ji et al., 2016) |

**Table 3** Fungi and biosurfactant yield

| Fungi | Substrate | Yield | Reference |
|---|---|---|---|
| *Candida bombicola* | Corn steep liquor, molasses, soybean waste frying oil 5% (v/v) | 61 | (Pinto et al., 2018) |
| *Candida tropicalis UCP0996* | Sugarcane molasses, corn steep liquor, waste frying oil (2·5%) | 7.36 | (Almeida et al. 2017) |
| *Candida lipolytica* UCP 0988 | Animal fat (5%) and corn steep liquor (2·5%) | 40 | (Santos et al., 2017) |
| *Candida bombicola URM 3718* | Molasses, corn steep liquor, soybean waste frying oil 5% (v/v) | 8.4 | (Luna et al., 2016) |
| *Candida sphaerica* | Ground-nut oil refinery residue (9%) and corn-steep liquor (9%) | 21 | (Luna et al., 2015) |
| *Aureobasidium pullulans* | Sucrose (50 $gl^{-1}$), peptone (0.6 $gl^{-1}$) and yeast extract (0.4 $gl^{-1}$) | 15 | (Saur et al., 2019) |
| *Rhodotorula paludigena* | Glucose (150 $gl^{-1}$) and yeast extract (1.5 $gl^{-1}$) | 20.90 | (Garay et al., 2017) |

**Screening methods for biosurfactant production by microorganisms** (Vandana & Singh D, 2018)

The different screening methods for biosurfactant production by microorganisms are represented in Fig. 8.

### Drop collapse test

The drop collapse test is used to check if microorganisms are producing biosurfactant. In this method, a drop of oil is placed on a clean glass slide. To this, 10 µl of microbial culture is added without disturbing the drop of oil. If the drop of oil is found to rupture in 1 min, it indicates production of biosurfactant by microorganism. There is a report on the study of rapid detection of biosurfactants by drop collapse assay on lotus leaf (*Nelumbo nucifera*) (Waghmode et al., 2016).

### Blood agar hemolysis

Blood agar hemolysis is also of the methods to check for biosurfactant production by microorganism. On the blood agar medium, if the microorganism causes lysis of the blood cells, there is presence of a colorless ring around the colony which indicates the production of biosurfactant.

### Oil displacement activity / oil spreading method

This method for screening of biosurfactant requires less volume of sample and is very fast. In this method, the biosurfactant if present, the oil will be displaced, and it will show presence of zone of clearance. The increase in diameter of the zone is calculated.

### Emulsification index assay

Emulsification index assay is also one of the methods for the screening of biosurfactant production by microorganisms.

### Cethyl trimethyl ammonium bromide (CTAB) agar plate method

This method is used to check for the production of anionic biosurfactants. In this method, the microorganisms if producing anionic biosurfactants, it forms blue colored insoluble ion which pairs with methylene blue. The presence of blue-colored halos around the colonies indicates production of anionic biosurfactants by microorganisms.

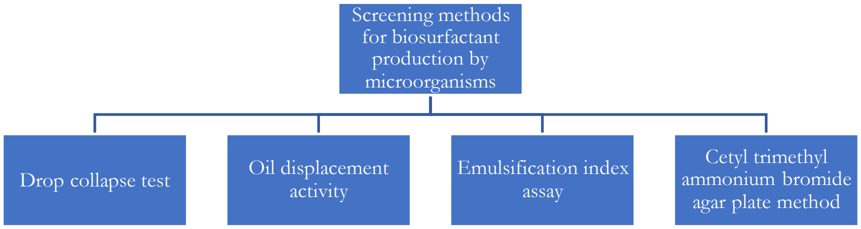

**Figure 8** Screening methods for biosurfactant production by microorganisms

### Factors affecting biosurfactant production

The media composition used for the growth of microorganisms plays an important role in biosurfactant production. Carbon (C) and nitrogen (N) sources are required for the growth of microorganisms. The C: N ratio affects biosurfactant production (Singh et al., 2019). In the media, the carbon sources used are viz., glucose, sucrose, and glycerol, while yeast extract, $NaNO_3$, urea and soya broth as nitrogen sources for the growth of microorganisms (Wu et al., 2018). The carbon and nitrogen sources must be added in proper amount for maximum biosurfactant production. With glucose as carbon and sodium nitrate as nitrogen source, the rhamnolipid production was 5.28 and 4.38 g/l respectively (Onwosi & Odibo, 2012). There is a report on glycolipoprotien biosurfactant production by *Oceanobacillus* sp. BRI 10. The biosurfactant produced was higher (E24 = 55%) in the medium having glucose (3%) and ammonium chloride (0.48%) at 30 °C, pH 8.0 after 48 h (Jadhav et al., 2013). There is a study where glucose in basal salt medium was replaced with sugarcane juice, whey and local commercial table sugar. The maximum emulsification index (E24) of 67.4% was obtained with sugarcane juice (Parhi et al., 2016). The best nitrogen source found for biosurfactant production was sodium nitrate. E24 increased to 68.74% with sugarcane juice and sodium nitrate. The yield of biosurfactant achieved was 14.25 g $l^{-1}$ with a 14-fold increase in the yield and eight times decrease in the production cost (Parhi et al., 2016).

### Genetically engineered microorganisms for biosurfactant production

Genetically engineered microorganisms with optimized cultural conditions and other parameters will be effective to produce good quality biosurfactant. There is a need for research on expression of biosurfactant genes in hosts for improved and good quality production of biosurfactant. The molecular aspects combined with genetic engineering of biosynthetic genes are gaining attention of the researchers and this molecular aspect will improve the synthesis of biosurfactants which is having industrial, agricultural, etc. applications (Jimoh et al., 2021). The recombinants will improve the nature, quality and quantity of biosurfactants. The research on use of genetically engineered microorganisms for biosurfactant production will have wide applications in various industries.

## Conclusions

Biosurfactants produced from renewable sources will be eco-friendly, economical, and easy. The biosurfactants produced will have many industrial applications. The substrates used for biosurfactant production are eco-friendly, economical, and easily available in huge amounts. The production of biosurfactants using microorganisms on a large-scale is very easy. The review is novel as it focuses on the use of renewable sources for biosurfactant production. More studies need to be carried out on genetically engineered microorganisms for biosurfactant production.

## Acknowledgements

I would like to acknowledge Dr. Rama Bhadekar, Department of Microbial Biotechnology, Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth (Deemed to be) University, Pune, Maharashtra, India for her suggestions in this review.

**Conflict of Interest**: The authors declare no conflict of interest.

**Data Availability Statement**: The data that support the finding of this study are available from the corresponding author, upon reasonable request.

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