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# A comprehensive review on production of bio-surfactants by bio-degradation of waste carbohydrate feedstocks: an approach towards sustainable development

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The advancement of science and technology demands chemistry which is safer, smarter and green by nature. The sustainability of science thus requires well-behaved alternates that best suit the demand. Bio-surfactants are surface active compounds, established to affect surface chemistry. In general, microbial bio-surfactants are a group of structurally diverse molecules produced by different microbes. A large number of bio-surfactants are produced during hydrocarbon degradation by hydrocarbonoclastic microorganisms during their own growth on carbohydrates and the production rate is influenced by the rate of degradation of carbohydrates. The production of such biological surfactants is thus of greater importance. This write up is a dedicated review to update the existing knowledge of inexpensive carbohydrate sources as substrates, microorganisms and technologies of biosurfactant production. This is an economy friendly as well as sustainable approach which will facilitate achieving some sustainable development goals. The production is dependent on the fermentation strategies, different factors of the microbial culture broth and downstream processing; these all have been elaborately presented in this article.

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## 1. Introduction

The advancement of science and technology is focused in the proper safety and sustainably of the environment, population and the eco-systems.<sup>1,2</sup> The conventional 'brown chemistry' thus

requires simple changes that must promote 'green chemistry'. To accelerate the 'novel revolution' in chemistry, natural resources have been proved to be the most effective and dependable alternatives.<sup>3-5</sup> In contemporary time, the science community is performing extensive research works and gaining more in-depth understanding of bio-based materials to find sustainable, less pollutant, ecologically beneficiary and economically sound solvents and chemicals. These new age bio-

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alternative species have several attractive properties like less toxicity, biodegradability, chemical stability, inclusive environmental safety and furthermore their ease of recycling.<sup>6</sup>

This write up is to collectively represent the production of such an important bio-based compound, the bio-surfactants from degradation of waste carbohydrate feedstocks. These biologically derived surfactants are interface active agents with amphiphilic nature.<sup>7–10</sup> Production of biosurfactants (BSs) generally occurs extracellularly or as a part of the cell membrane by several bacteria, yeast and filamentous fungi.<sup>11,12</sup> Several substrates such as vegetable oil waste, vegetable wastes, fruit wastes starchy wastes *etc.* are promising source of carbohydrate for production of BSs.<sup>13</sup> The recent article by M. H. Mondal *et al.*<sup>2,4</sup> categorically reported the advantages of BSs over their synthetic or chemical analogues in different industrial sectors, house hold and even in laboratory chemistry due to their lower toxicity, greater environmental compatibility, and the best is they are highly biodegradable and are able to be produced from bio-wastes. Specifically the biocompatible, low toxic and excellent biodegradable nature of bio-surfactant facilitates consumers for direct consumption (in pharmaceuticals, food or skincare cosmetics).<sup>14–17</sup> Thus bio-surfactant itself and its production process obeys the principles of green chemistry.<sup>18,19</sup> More recently the bio-medical advantages of these BSs in drug design and delivery have been reported by B. Das *et al.*<sup>20</sup> Hence, in recent days these BSs are becoming popular in biotechnology products for industrial and pharmaceutical applications. Bio-surfactants are proved to be extensively useful as emulsifiers, de-emulsifiers, wetting and foaming agents, edible food additives, cosmetics and detergents in house hold goods. BSs also have versatility in application in petrochemical industries, waste water treatment, soil remediation and in agro-industries. Recently bio-surfactants are the best choice for cosmetic and personal care products.<sup>4,20</sup>

Despite having lots of attractive features, biosurfactants are still back footed due to its high production cost.<sup>21</sup> The production of bio-surfactant still facing many obstacles including costly substrate, effective bioprocessing methods,

product recovery, purification and so on. So that researchers are investigating both economically and eco-friendly methods for bio-surfactant production.<sup>22</sup> In this regard, agro-industrial wastes are found to be able to act as promising carbohydrate source for the bio-surfactant production in microbial medium. This eco-friendly approach reduces the bio-surfactant manufacturing cost as well as invokes utilisation of waste stream promoting circular bio-economy. In addition to that, it will also facilitate to achieve some sustainable development goals.<sup>10</sup>

## 2. Classification of biosurfactant & microorganism producing bio-surfactants

BSs are generally categorized according to their chemical composition and structure as well as its microbial origin. Rosenberg and Ron in 1999 classified BSs in two main category *viz.* High molecular weight (HMW) BSs and low molecular weight (LMW) BSs. Glycolipids, lipopeptides, phospholipids, fatty acids, neutral lipids belong to LMW group while polymeric (polysaccharide, lipopolysaccharide, lipoprotein) and particulate surfactants belong to HMW group. Rhamnolipids, sophorolipids and trehalolipids are mostly known glycolipids while surfactin, iturin, fengycin, lichenysin are mostly known lipopeptides<sup>23</sup> (Fig. 1).

Varieties of microorganisms including bacteria, fungi, yeasts *etc.* are used in contemporary days for production of varied bio-surfactants (hence forth BSs).<sup>24</sup> As the current write up deals with fermentation only, we will focus on microorganisms which are useful in production of BSs from carbon sources by fermentation process. Huge numbers of microorganisms have been isolated for industrial utilization in the aim of waste management.<sup>25</sup> The Table 1 contains a list of BS producing organism.

## 3. Trends in commercial production of bio-surfactants

It is well established that the production expenditure of BSs is 3–10 times higher than their synthetic prototypes.<sup>53</sup> Detail research over the last decade has suggested four scientifically significant factors that are eligible to reduce the production cost. Many researchers have suggested for increasing the product yield and to make it cost effective, we must look for inexpensive and easily obtainable substrates. Similar to other biotechnologically produced substances also, the amount of BSs yield is primarily effected by factors like type and nature of microorganisms, fermentation condition (pH, temperature, agitation *etc.*) and category of fermentation (sub-merged or solid state).<sup>54,55</sup> A scheme of strategies established in market for production of BSs in shown in Fig. 2 below:

To rise above the high manufacturing cost regarding the BSs production, the fundamental strategies have been adopted universally.



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Fig. 1 (a) Monorhamnolipid. (b) Dirhamnolipid. (c) Lactonic. (d) Acidic sophorolipid. (e) Surfactin.

(i) The utilization and application of cheap and waste feed-stocks for the fermentation process.

(ii) Modification, optimization and development of bio-processes, culture condition and recovery of products.

## 4. Biosurfactant production as a sustainable approach

In recent days the term sustainability has become a burning topic regarding global environmental and socio-economic perspectives and this term arises regarding the parallel economic development with several environmental problems due to rapid industrialization and civilization. Sustainable life means an inclusive wellbeing of life on earth and in water where all live forms are interlinked through environment. Climate change is the main threat to that inclusive sustainability. The concept of sustainability basically consists of three factors: social, economic and environmental and based on these factors there are several sustainable development goals (SDGs). In the field of chemistry before SDGs we already started working by introducing green chemistry.<sup>56</sup> Among them, goal 7 targets at the use of renewable resources replacing the non-renewable ones as well as ensure the access to affordable energy to increase the energy efficiency globally (Department of Economic

and Social Affairs, UN 2016).<sup>57</sup> In our daily life, a lots of product we use in which a variety of chemical surfactants are present which are generally made of non-renewable oleo-chemical compounds as well as they are not biodegradable but its bio alternatives *i.e.* biosurfactants has been found to be a sustainable approach. Hence the demand for such biosurfactants is increasing rapidly which invokes the commercial production of biosurfactants. In various industries the use of biosurfactant are found to be environmentally sustainable compared to its synthetic congeners but the production cost of biosurfactant is not economically sustainable.<sup>58</sup>

In recent past, a drive towards sustainable production approach has found which invokes economically favourable production as well as reduced environmental impact and conserving natural resources and energy too. In this write up the term 'sustainability' also reflects in the production of biosurfactants regarding use of cheap biowaste products as substrate for biosurfactant production. It is worth mentioning that among the SDGs, goal 12 targets at the sustainable management and of waste by recycling and reuse. Processes of production, recycling or reconsumption when follow the principles of green chemistry simultaneously it follows the SDGs like 3, 8, 13, 14, 15 (Department of Economic and Social Affairs, UN 2016). In this regard, the production of biosurfactants from wastages of varied industries having direct green influence in



Table 1 List of biosurfactant – producing organisms

Class	Biosurfactant	Microorganism(s)	Current economic importance <sup>2,26</sup>	References
Low Molecular weight	Glycolipids			
	Cellulose lipids	<i>Ustilago maydis</i> , <i>Sporisorium scitamineum</i>	Antifungal compounds	27
	Rhamnolipids	<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas chlororaphis</i> , <i>Serratia rubidea</i> , <i>Pseudomonas cepacia</i>	Bioremediation, antimicrobial and biocontrol properties	28–30
	Sophorolipids	<i>Candida bombicola</i> , <i>Candida antarctica</i> , <i>Torulopsis petrophilum</i> , <i>Candida botistae</i> , <i>Candida apicola</i> , <i>Candida riidocensis</i> , <i>Candida stellata</i> , <i>Candida bogoriensis</i>	Antimicrobial, antiviral, spermicidal	31 and 32
	Trehalose lipids	<i>Rhodococcus erythropolis</i> , <i>Arthrobacter</i> sp., <i>Rhodococcus qingshengii</i> , <i>Nocardia erythropolis</i> , <i>Nocardia farcinica</i> , <i>Crynebacterium</i> sp., <i>Mycobacterium</i> sp.	Dissolution of hydrocarbons	33–36
	Trehalose dimycolates	<i>R. erythropolis</i>		37
	Trehalose dicorynemycolates	<i>R. erythropolis</i>		38
	Lipopeptides, lipoprotein and others			
	Lichenysin	<i>Bacillus licheniformis</i> , <i>Bacillus subtilis</i>	Microbially enhanced oil recovery (MEOR)	39
	Surfactin/Iturin	<i>Bacillus subtilis</i> , <i>Bacillus pumilus</i> , <i>Bacillus amyloliquefaciens</i>	Antimicrobial properties	40 and 41
	Viscosin	<i>Pseudomonas viscosa</i> , <i>Pseudomonas fluorescens</i> and <i>Pseudomonas libanensis</i>	Surface active lipopeptides	42
	Subtilisin	<i>Bacillus subtilis</i> , <i>Amyloliuefaciens</i> , <i>B. licheniformis</i>	Antimicrobial properties	43
	Polymixins	<i>Paenibacillus polymyxa</i>	Antibiotic agent	44
	Ornithine lipids	<i>Pseudomonas aeruginosa</i>	Bio-emulsifiers	45
	Phospholipids	<i>Acinetobacter</i> sp.	Bioremediation	46
Polyol lipids	<i>Rhodotorula glutinis</i> , <i>R. graminis</i>	Anti-proliferative activity	47	
Mannosylerythritol lipid	<i>Pseudozyma aphidis</i> ZJUDM34	Antifungal compounds	48	
High molecular weight	Polymeric biosurfactant			
	Emulsan	<i>A. calcoaceticus</i>	MEOR	49
	Liposan	<i>Candida lipolytica</i> , <i>C. tropicalis</i>	Bio-emulsan	50
	Alasan	<i>A. radioresistens</i>	Biodegradation of polyaromatic compounds	51
	Particulate biosurfactant			
Whole cell	<i>Cyanobacteria</i>	Bio-flocculent	52	
Vesicles and fimbriae	<i>Acinetobacter calcoaceticus</i> , <i>P. marginilis</i> , <i>P. maltophilia</i>	Bioremediation	52	

the livelihood of live forms both on earth and in water is definitely invoking for inclusive sustainability.<sup>59</sup>

With modernization, the amount of waste matter from different, agricultural fields, households, industries *etc.* is increasing day by day. Most of the time, these waste products are disposed in without any treatment to the environment resulting in contamination. This has harmful effects towards human health furthermore to the ecosystem. But the waste generated from several fields such that, dairy industries, agro industries, sugar industries, fruit industries *etc.* have nutritional values which is very beneficial for microbial growth so that these residues can be very useful for biosurfactant production by creating suitable environment for the microbial growth during fermentation process. This ecofriendly approach not only reduces the biosurfactant production cost but also

invokes utilization of waste stream promoting circular bio-economy which emphasizes the use of renewable natural resources to minimize the waste quantity to reduce its harmful environmental impact.<sup>6,53</sup>

## 5. Agro-industrial wastes as potential substrates for the bio-surfactant production

To stick to the title of the write up, this segment is focused in a detail description of potential sources that includes carbohydrates as the main carbon source.

Agro-industrial wastes are basically organic wastes which are biodegradable by nature and contain several nutritional compounds such as starch, cellulose, hemicelluloses, proteins,



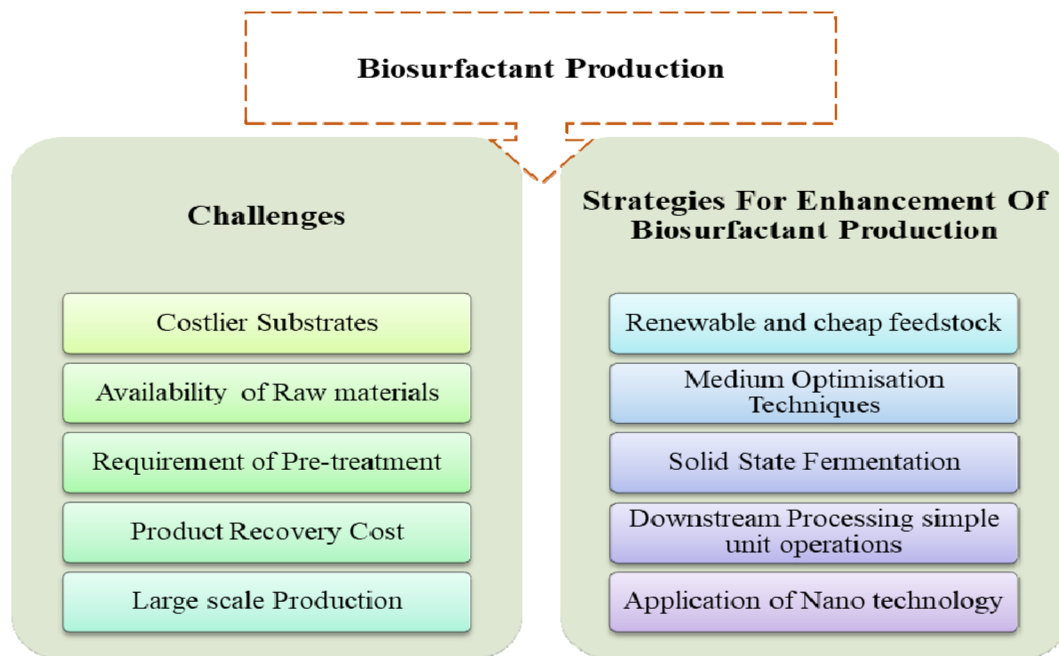


Fig. 2 Scheme of strategies to enhance biosurfactant production.

lignin, fibre, minerals, vitamins, and others. The amount of waste residues from different agro-industries is increasing neck and neck with modernisation and civilisation. It is worth mentioning that every year 4.4 billion tons of waste product is generated in Asia and in which India contributes >350 million tons of waste from several sources.<sup>60</sup> Most of the time these residues are disposed in untreated condition which results in environmental problems.<sup>61</sup> On the other hand many of these agro-industrial residues have nutritional value and due to having nutrients these residues are able to create suitable environment for the microbial growth, thus can be used as substrate for fermentation process by different microorganisms<sup>13</sup> (Fig. 3).

In spite of having several advantages over conventional synthetic surfactants, biosurfactants are still back footed because of its high production cost and low yield. Several researches have been developing by last few decades so that it can be produced commercially using inexpensive substrates

specially the carbon sources replacing the costlier chemical alternatives.<sup>10</sup> Agro-industrial wastes are the most common and least expensive substance with high carbohydrate content, can be obtained from processing industries and agriculture for large scale industrial level production of BSs. Agro-waste such as rice water, cereal processing waste water are potential sources of carbohydrates that could be utilized for the production of BSs.<sup>62</sup>

These cheap agro-industrial waste materials are found to act as potential substrate for BS production with effective reduction in cost of production (Fig. 4).

### 5.1. Agricultural residues

The residues from tropical agronomic crops can be potential inexpensive substrates with high carbon content that facilitates fermentation, culture of microorganisms and indeed production of BSs.<sup>63</sup>

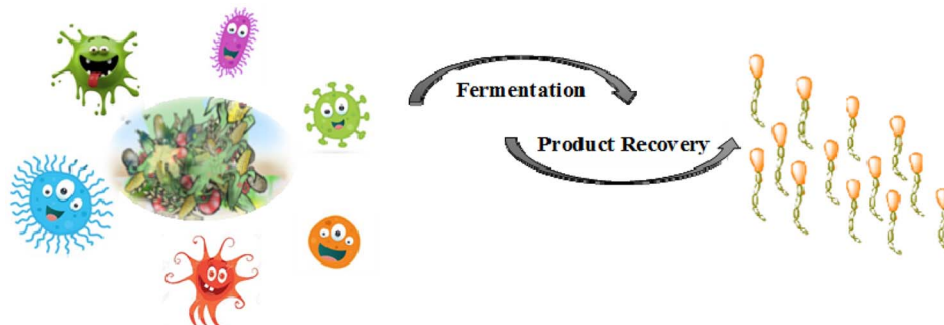


Fig. 3 Biosurfactant production by microorganism using agro-industrial waste as chief carbohydrate source.





Fig. 4 Different agro-industrial residues used as substrate during biosurfactant production.

**5.1.1. Vegetable oil and (oil) wastes.** A large amount of waste residues formation occurs during the oil extraction in various oil industry. These left over products includes oil cakes, oil soap stocks, by-products rich in fat content, semisolid and water soluble effluents, fatty acid residues *etc.*<sup>64</sup>

Oil wastes from different vegetable oil refineries and food industries along with industrial oil waste such as marine oils, lard and free fatty acid have a potentiality to help micro-organism growth and surfactant production.<sup>54,65</sup> Wegerer and co-workers have reported production of rhamnolipids (RLs) from rapeseed oil by *Pseudomonas* sp. (DSM 2874). The by-product as reported by them was L-(+)-rhamnose.<sup>66</sup> Many plant based oils like palm oil, mesua oil, castor oil and jojoba oil *etc.* are inappropriate for human consumption because of their unpleasant aroma, colour and human toxicity but are potential sources for biosurfactant production due to their carbohydrate enriched bio-composition.<sup>67–69</sup> Sunflower seed-oil is hydrolysed directly by secretion of lipase from the bacterial microbes and acts as a preferable source of carbon for RLs production.

*P. aeruginosa* 47 T2 is reported to produce rhamnolipids when grown in olive oil waste water with a yield as much as 8.1 g L<sup>-1</sup>.<sup>67–69</sup> Considering these low-cost cheaper oils and oil wastes can help us overcome high production costs.

As a result of olive oil extraction process a huge quantity of liquid waste called olive oil mill waste effluent (OMWE). The OMWE is a black liquor consist of high organic content (20–60 kg per COD per m<sup>3</sup>).<sup>70</sup> The OMWE is toxic to human health due to presence of polyphenols<sup>71</sup> but is valuable for its chemical composition; it contains important organic substances such as sugars, organic acids and nitrogenous compounds which help in microbial growth. The presence of great amount of carbohydrates, polysaccharides, sugars, phenols and lipids in OMWE make their treatment difficult and thus OMWE is a potential environment carcinogen.<sup>72</sup> The use of OMWE is beneficial for both environmental and economical points.

**5.1.2. Starchy substrates.** During the extraction of starch from different crops like rice, wheat, corn, cassava and potato, a huge amount of starchy wastewater and husks are produced



which are found to be great carbon sources for the production of biosurfactants.

A main resource of low-cost starchy substrate is potato-agro-industry. The wastes of potato industry contain 80% water, 17% carbohydrates along with other minor components. Thus, they are rich source of carbohydrates (sugars and starch). Fox and Bala evaluated Surfactin production by *Bacillus subtilis* ATCC 21332 using potato substrate basically established potato medium and stimulated potato waste effluents.<sup>73</sup> Thomson *et al.* and Noah *et al.* also have investigated different types of potato process effluents and reported production of surfactin. *B. subtilis* 21332 was the microbe for the production of surfactin from potato effluents.<sup>74,75</sup> Das and Mukherjee studied the efficacy of *Bacillus subtilis* strains DM-03 and DM-04 for the production of lipopeptide biosurfactants using potato peel as carbon source in solid state fermentation and submerged fermentation systems.<sup>76</sup> Wang and his co-workers reported the co-production of fengycin lipopeptide and  $\gamma$ -PGA by *Bacillus subtilis* strain B6-1 using soybean and sweet potato wastes in SSF.<sup>77</sup> Sharma and his co-workers reported the production of lipopeptide biosurfactants by *Bacillus pumilus* grown on potato peels.<sup>78</sup> Another study by Ayed *et al.* revealed the production of lipopeptide BSs (isoforms belonging to fengycin and surfactin) by *Bacillus mojavensis* A21 using potato waste as substrate.<sup>79</sup> Das *et al.* studied the production of rhamnolipids by *Pseudomonas azotoformans* AJ15 utilizing sugarcane bagasse and potato peels.<sup>80</sup> Not only the potato peels but also the pulp were used as production medium for the biosynthesis of Surfactin by *Bacillus subtilis* DDU20161 in the study by Pande and his coworker.<sup>81</sup> Another study by Das and Kumar reported the production of lipopeptide BS by *Bacillus licheniformis* strain J1 using petroleum as carbon source while in presence of potato peel powder its efficiency enhances.<sup>82</sup>

In the cassava flour industry, processing of cassava tubers into starch or flour produces a huge amount of Cassava waste water containing several by-products like cassava peels, cassava pomace, cassava sieve and stump rich in carbohydrate content, generally possesses environmental pollutions but they can be used as feedstock for biosynthesis which can be a promising way to alleviate the environmental issues.<sup>83</sup> Cassava waste water are potential substrates for fermentation process to produce surfactin with the help of *B. subtilis*.<sup>84</sup> Siddhartha *et al.* reported production of another biosurfactant rhamnolipids by changing the microbe. His group used *P. aeruginosa* as the

fermenting microbe.<sup>85</sup> A list of microbial strains using starchy substrate as carbon source for biosurfactant production has been given in Table 2.

## 5.2. Industrial wastes from animal origin

### 5.2.1. Dairy industry residues.

A significant amount of dairy waste water, derivatives and by-products (whey, butter-milk) is produced from dairy industry every day.<sup>86</sup> Whey is basically a liquid phase by-product obtained during the manufacturing of casein products and it comprises of significant amount of lactose making it suitable for fermentation. In general, these waste products have high Biological Oxygen Demand (BOD) thus resulting in contamination of water sources if disposed in untreated condition although a considerable amount of this waste products are going through recycling process to obtain other useful products like animal food *etc.* These effluents from the dairy industry are known to grow microbes and thus can be used for biosurfactant production.<sup>85,87</sup> Daniel and co-workers achieved high yield of sophorolipids by two-stage cultivation process of yeast *Cryptococcus curvatus* ATCC 20509 and *Candida bombicola* ATCC 22214 utilising deproteinized whey.<sup>88</sup> Daverey *et al.* also reported production of Sophorolipids by *Candida bombicola* ATCC 22214 using dairy wastewater from dairy industry.<sup>89</sup> Several researches have been reported by a number of researchers on rhamnolipid production by *Pseudomonas aeruginosa* strains<sup>87,90–92</sup> and lipopeptide production by *Bacillus* spp.<sup>93–95</sup> using whey waste as substrate.

A large portion of whey waste disposed as effluent consists of several carbohydrates, peptides, amino acids; thus, are good carbon and nitrogen sources. Whey waste can be incorporated in industrial level as inexpensive substrate for biosurfactant production and laterally facilitates management of dairy wastes. A list of microbial strains using dairy waste as carbon source for biosurfactant production has been given in Table 3.

## 5.3. Industrial wastes from plant origin

### 5.3.1. Molasses.

Molasses is a co-product of sugar industry, produced at the time of sugar manufacturing. Typical sources include sugarcane, sugar beet, date *etc.* It is a very rich source of carbohydrates. Cane molasses contains over 48–56% of sugar. Another interesting substrate is soy molasses which is a by-product obtained from soybean processing industry. It contains 30% (w/v) carbohydrate. Molasses can be potential

Table 2 List of microbial strains using starchy substrate as carbon source for biosurfactant production

Sr. no.	Microorganism	Substrate	Biosurfactant type	Reference
1	<i>Bacillus subtilis</i> ATCC 21332	Potato substrate	Surfactin	73
2	<i>Bacillus subtilis</i> ATCC 21332	Potato effluent	Surfactin	74
3	<i>Bacillus subtilis</i> strains DM-03 and DM-04	Potato peel	Lipopeptide	76
4	<i>Bacillus subtilis</i> strain B-61	Soybean and sweet potato wastes	Fengycin lipopeptide and $\gamma$ -PGA	77
5	<i>Bacillus pumilus</i> DSVP18	Potato peels	Lipopeptide	78
6	<i>Bacillus mojavensis</i> A21	Potato waste	Lipopeptide	79
7	<i>Pseudomonas azotoformans</i> AJ15	Sugarcane bagasse and potato peels	Rhamnolipids	80
8	<i>Bacillus subtilis</i> DDU20161	Potato peels and pulp	Surfactin	81
9	<i>Bacillus licheniformis</i> strain J1	Potato peel powder	Lipopeptide	82



Table 3 List of microbial strains using dairy waste as carbon source for biosurfactant production

Sr. no.	Microorganism	Substrate	Biosurfactant type	Reference
1	<i>Pseudomonas aeruginosa</i> BS2	Distillery and curd whey waste	Rhamnolipids	87
2	<i>Cryptococcus curvatus</i> ATCC 20509 and <i>Candida bombicola</i> ATCC 22214	Deproteinized whey	Sophorolipids	88
3	<i>Candida bombicola</i> ATCC 22214	Dairy waste water	Sophorolipids	89
4	<i>Pseudomonas aeruginosa</i> strain SR17	Paneer whey	Rhamnolipids	90
5	<i>Pseudomonas aeruginosa</i>	Cheese whey or olive oil mill wastewater	Rhamnolipids	91
6	<i>Pseudomonas aeruginosa</i> B189	Milk factory wastewater	Rhamnolipids	92
7	<i>Bacillus methylotrophicus</i> , <i>Bacillus pumilus</i>	Whey	Lipopeptide	93
8	<i>Bacillus licheniformis</i> M104	Whey	Lipopeptide	94
9	<i>Bacillus subtilis</i> 20B <i>Bacillus subtilis</i> R1 <i>Bacillus</i> HS3 <i>Bacillus licheniformis</i> K51	Whey	Surfactin Lichenysin	95
10	<i>Candida bombicola</i>	Canola oil with cheese whey	Sophorolipids	96
11	<i>Lactobacillus pentosus</i> CECT-4023	Whey	—	97
12	<i>Lactococcus lactis</i> 53 <i>Streptococcus thermophiles</i> A	Cheese whey	—	98
13	<i>Yarrowia lipolytica</i> <i>Micrococcus luteus</i> <i>Burkholderia cepacia</i>	Whey wastewater	—	99
14	<i>Pseudomonas aeruginosa</i> Strain-PP2 <i>Kocuria turfanensis</i> strain-J	Curd whey	—	100
15	<i>Candida glabrata</i> UCP 1556	Whey Corn steep liquor	Lipopeptide	101
16	<i>Streptococcus thermophiles</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus rhamnosus</i>	Whey	—	102
17	<i>Yarrowia lipolytica</i>	Butter whey, cheese whey, ricotta whey	Bio-emulsifier	103

substrates for biosurfactant production due to being carbohydrate rich, renewable moreover cheaper and eco-friendly alternative of costlier carbon sources though the clarification process of molasses is quite costly.

Patel and Desai 1997 have reported a good quantity of rhamnolipids biosurfactant by fermentation using *P. aeruginosa* (GS 3).<sup>104</sup> Other studies by many researchers revealed that different strains of *Pseudomonas* sp. produce rhamnolipids using molasses as carbon sources.<sup>105–112</sup> Another study by Al-Bahry *et al.* (2013) highlights the use of date molasses as carbon source in the fermentative production of biosurfactant by *Bacillus subtilis* B20 and *Bacillus subtilis* B30 and other includes different strains of *Bacillus* sp. producing lipopeptide biosurfactants with the help of molasses as carbon source.<sup>95,113–119</sup> Besides that sophorolipids can also be produced by different microbial strains using molasses as carbon source.<sup>120–124</sup> A list of microbial strains using molasses as carbon source for biosurfactant production has been given in Table 4.

**5.3.2. Cashew apple juice residue.** Cashew apple is a part of cashew tree which holds the cashew nut at the end. This is basically a pseudo fruit obtained after being separated from cashew nut. These left over cashew apples are used for making juice, jam, syrup although maximum remains unused. These are rich in carbohydrate content so as it can be used as source of carbon for BS production by different microorganisms.<sup>134</sup> Several researches have been developed in last few decades over fermentation in cashew apple as carbohydrate supplement for production of BSs, some of them are represented in Table 5.

**5.3.3. Bagasse, pomace & peels.** A large quantity of leftover is obtained from food industry during the manufacturing and extraction of juices from several fruits and vegetables which includes residues like banana peels, apple pomace, sugarcane bagasse, different citrus peels, pineapple peels, carrot peels *etc.* Das and Kumar (2018) has studied the production of rhamnolipid biosurfactant by *Pseudomonas aeruginosa* AJ15 using potato peels and sugarcane bagasse as carbon sources under submerged fermentation.<sup>80</sup> A list of microbial strains using these as carbon source for biosurfactant production has been given in Table 6.

## 6. Pre-treatment of the feedstock

In recent past the use of these kinds of cheap and sustainable feedstocks has become widely developed research interest globally. However, these feedstocks are recommended to pass through a series of pre-treatment for commercial scale biosurfactant production. The pre-treatment procedure is very important to achieve higher monosaccharide content and fewer amounts of impurity and inhibitory complexes in final substrate which is involved in the microbial growth and metabolism phase. Accordingly the process helps in increasing the yield of biosurfactant production and facilitates the commercial biosurfactant production.<sup>53,147</sup>

The initial footstep of pre-treatment is corresponding to the decrease of size of the substrate to make sure the improved utilization of the substrate by the microorganisms and the





Table 4 List of microbial strains using molasses as carbon source for biosurfactant production

Sr. no.	Microorganism	Agro-industrial waste (molasses)	Biosurfactant type	Reference
1	<i>Pseudomonas aeruginosa</i> (GS 3)	Molasses	Rhamnolipids	104
2	<i>Pseudomonas aeruginosa</i>	Molasses	Rhamnolipids	105
3	<i>Pseudomonas aeruginosa</i> EBN-8	Blackstrap molasses	Rhamnolipids	106
4	<i>Pseudomonas putida</i> 300-B	Molasses	Rhamnolipids	107
5	<i>Pseudomonas aeruginosa</i> B1	Sugar beet molasses	Rhamnolipids	108
	<i>Pseudomonas aeruginosa</i> B2			
6	<i>Pseudomonas luteola</i> B17	Sugar beet molasses	Rhamnolipids	109
	<i>Pseudomonas putida</i> B12			
7	<i>Pseudomonas aeruginosa</i>	Sugar cane molasses	Rhamnolipids	110
8	<i>Pseudomonas aeruginosa</i> GIM32	Molasses distillery wastewater	Rhamnolipids	111
9	<i>Pseudomonas aeruginosa</i> ATCC 10145	Soy molasses	Glycolipids	112
10	<i>Bacillus subtilis</i>	Molasses	Surfactin	113
	MTCC 2423			
	MTCC 1427			
11	<i>Bacillus subtilis</i> 20B	Molasses	Surfactin	95
	<i>Bacillus subtilis</i> R1		Lichenysin	
	<i>Bacillus</i> HS3			
	<i>Bacillus licheniformis</i> K51			
12	<i>Bacillus subtilis</i> SA9	Molasses	Lipopeptide (similar to surfactin and lichenysin)	114
	<i>Bacillus licheniformis</i> TR7			
13	<i>Bacillus subtilis</i> ATCC 6633	Sugar cane molasses	Lipopeptide	115
14	<i>Bacillus subtilis</i> B20	Date molasses	Lipopeptide (similar to surfactin)	125
15	<i>Bacillus subtilis</i> B30	Date molasses	Lipopeptide (similar to surfactin)	126
16	<i>Bacillus licheniformis</i> W16	Cane molasses	Lipopeptide (similar to Lichenysin-A)	116
17	<i>Bacillus subtilis</i> ANR 88	Molasses	Lipopeptide	117
18	<i>Bacillus subtilis</i> RSL-2	Molasses	Surfactin	118
19	<i>Bacillus subtilis</i> Al-Dhabi-130	Date molasses	Surfactin	119
20	<i>Candida bombicola</i> ATCC 22214	Soy molasses	Sophorolipids	120
21	<i>Candida bombicola</i> ATCC 22214 or <i>Starmerella bombicola</i> NRRLY17069	Sugarcane molasses	Sophorolipids	121
22	<i>Starmerella bombicola</i> NBRC10243	Sugarcane molasses	Sophorolipids	122
23	<i>Candida tropicalis</i>	Sugarcane molasses	Sophorolipids	123
24	<i>Starmerella bombicola</i> ATCC 22214	Sugar beet molasses	Sophorolipids	124
25	<i>Lactobacillus delbrueckii</i> N2	Sugarcane molasses	Glycoprotein	127
	<i>Lactobacillus cellobiosus</i> TM1			
	<i>Lactobacillus Plantarum</i> G88			
26	<i>Lactobacillus paracasei</i> subsp. <i>Tolerans</i> N2	Sugarcane molasses	Glycolipoprotein	128
27	<i>Sphingobacterium spiritivorum</i> AS43	Molasses	Lipopeptide	129
28	<i>Azotobacter vine landii</i>	Molasses		130
29	<i>Streptomyces angustmyceticus</i> CGS B11	Molasses	Lipopeptide	131
30	<i>Pseudomonas aeruginosa</i> strain CGA1	Sugar cane molasses		132
31	<i>Pseudomonas putida</i> KT2440	Sugar beet molasses		133

equipment used for this purpose are tub grinder, hammer mill, crusher and many more. The reduction in particle size eventually increases the surface area as well as pore size so that the

contact area becomes more available for the microorganisms to interact. The next step is pre-hydrolysis treatment usually carried out by using either ultrasonication or liquid

Table 5 List of microbial strains using cashew apple juice residue as carbon source for biosurfactant production

Substrate	Microorganism	Biosurfactant type	Reference
Cashew apple juice	<i>Acinetobacter calcoaceticus</i> RAG-1	Emulsan	134
	<i>Pseudomonas aeruginosa</i> ATCC10145	Rhamnolipids	135
	<i>Pseudomonas aeruginosa</i> MSIC02	Rhamnolipids	136
	<i>Bacillus subtilis</i> LAMI008	Surfactin	137
	<i>Bacillus subtilis</i> LAMI005		138
	<i>Bacillus subtilis</i> LAMI005		139
	<i>Yarrowia lipolytica</i>	Biosurfactants	140
Cashew apple bagasse	<i>Pseudomonas aeruginosa</i>	Glycolipid	141



Table 6 List of microbial strains using bagasse, pomace &amp; peels as carbon source for biosurfactant production

Substrate	Microorganism	Biosurfactant type	Reference
Banana peel	<i>H. archaeon</i> AS65	Lipopeptide	142
Orange peel	<i>Pseudomonas aeruginosa</i> MTCC 2297	Rhamnolipids	143
	<i>Bacillus licheniformis</i> KC710973	Lipopeptide	144
Carrot peel & apple peels extract	<i>Bacillus subtilis</i> I'1a	Iturin	145
	<i>Bacillus subtilis</i> KP7		
Sugarcane bagasse	<i>Corynebacterium aquaticum</i>	Biosurfactant	146
Potato peels & sugarcane bagasse	<i>Pseudomonas aeruginosa</i> AJ15	Rhamnolipids	80

ammonia.<sup>148</sup> Pre-hydrolysis stage is followed by hydrolysis step. Hydrolysis can be carried out either enzymatically or chemically and the chemical path can be of two types – alkaline hydrolysis which can be employed by using sodium hydroxide, potassium hydroxide, calcium hydroxide, ammonium hydroxide *etc.* and acid hydrolysis which can be employed using inorganic acids like sulfuric acids, hydrochloric acid *etc.* The acid solution can be concentrated or diluted a required though the use of concentrated acid solution results better yield but becomes more expensive process. In addition to this, there are biologically derived enzymes which can also be used for hydrolysis pre-treatment. Enzymes such as  $\beta$ -glucosidase was effectively applied in the hydrolysis treatment of bagasse as well as other substrates.<sup>149,150</sup> The final step is drying of the hydrolysates and then the dried substrate is incorporated in microbial media for biosurfactant production. Basically the pre-treated substrate is used as prime sugar source for the microbial growth phase and this is followed by formation of secondary metabolites resulting in production of biosurfactant.<sup>151–153</sup> A schematic representation of the pre-treatment process has been shown in Fig. 5.

## 7. Influence of factors in biosurfactant production and medium optimisation

During the production of biosurfactant, the final quantity and quality of the resulting yield depends on several internal and external factors such as different components of medium, pH, temperature, agitation, aeration, dissolved oxygen, fermentation time and so on (Fig. 6). To ensure the maximum yield and lower cost, proper medium optimization strategies should be applied. In past days classical method was applied for medium optimization which was done by changing parameters one by one keeping other parameters fixed. But this method is not only too much time consuming but also laborious and lengthy and this also does not ensure the optimum condition for the best yield. To overcome the situation, researchers used statistical optimization strategies based on RSM (response surface methodology). This method guaranteed the proper optimum condition as well as easier than previous one regarding increasing yield and decreasing production cost.<sup>154</sup>

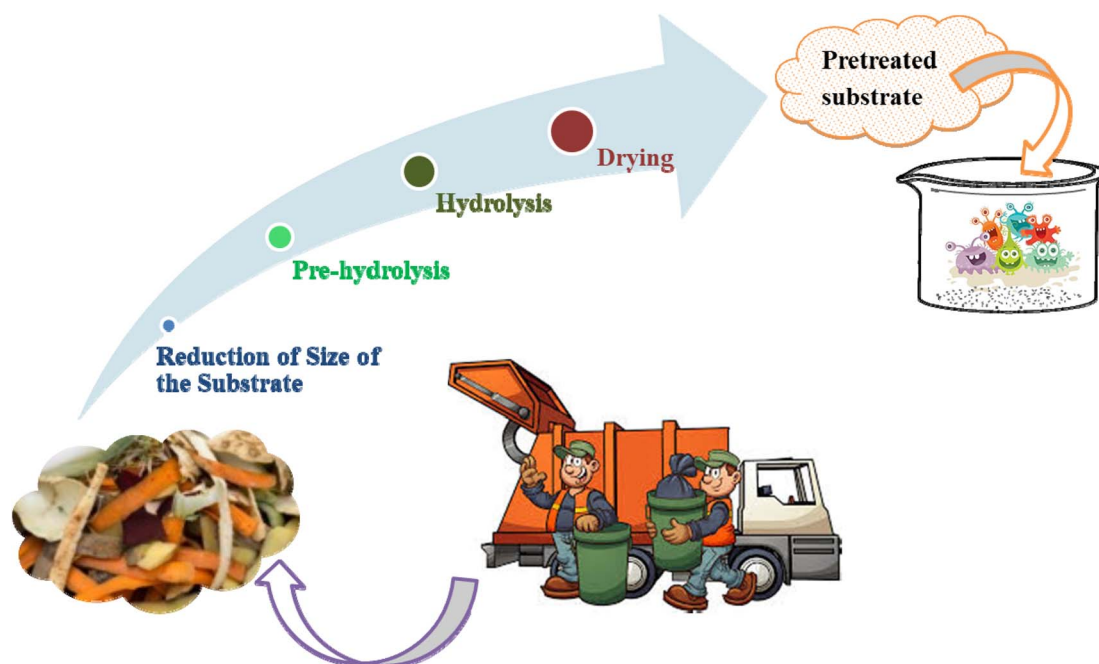


Fig. 5 Schematic representation of the pre-treatment process.





Fig. 6 List of factors influences biosurfactant production.

This method is a collection of statistical and mathematical techniques to build an empirical model to analyze the optimum condition. There are two or more factors which are involved in RSM such as dependent or output variables known as responses which are influenced by independent or input variables known as predictor. The designed experiment consists of a series of tests where input variables are made changed to analyze the response.<sup>155</sup> This method has been employed to increase the biosurfactant yield by bacterial strains *Lactococcus lactis* and *Streptococcus thermophilus*<sup>98</sup> and also in case of biosurfactant production by *Pseudomonas aeruginosa* AT 10.<sup>156</sup>

### 7.1. Influence of internal factors

The internal factors in the production of BSs are the cultural medium and its composition. The presence of different carbon sources alters the nature and types of BSs while the composition and characteristics of BSs are effected by the nature of nitrogen source and applied metal ions like iron, magnesium, sulphur, manganese *etc.*

**7.1.1. Carbon source.** Not only the composition and quality but also the amount of the produced BSs depends on the nature of the various carbon sources. We already have explained in detail about different carbon sources and their effects on BSs production. To lower the production cost many of the researcher have suggested application of agro-industrial waste as the chief carbohydrate source.<sup>157</sup> Though a huge number of studies are established using glucose, glycerol, acetates and other organic acids and *n*-alkanes which all are very costly and cannot reduce the cost of production. The investigation on production of mannosylerythritol lipids (MEL) by *C. antarctica* using *n*-alkanes as carbon sources reported that the chain

length of the alkane substrate efficiently influence the productivity of MEL; highest yield obtained from *n*-octadecane.<sup>158,159</sup>

**7.1.2. Nitrogen sources.** Nitrogen is equally important as that of carbon in the culture medium because of its role in the proteins and enzyme synthesis that are much essential for microbial growth.<sup>160</sup> Nitrogen sources like urea, peptones, ammonium salts like ammonium nitrate, ammonium sulphate,<sup>161</sup> meat and malt extract,<sup>162</sup> yeast extract, soybean flour, sodium salts like sodium glutamate, sodium nitrate and Casein acids hydrolysate (CAH)<sup>163</sup> are used for the production of BSs. The nitrogen is required during the stationary phase of cell growth.

**7.1.3. Metal ion concentration.** Metal ions are essential in biosurfactant production as these ions help production of enzyme co-factors for microbes during growth.<sup>164</sup> We in our study have found that the presence of  $MgCO_3$  helped production of rhamnolipids.<sup>18</sup> It has also been reported in other studies also. Thimon *et al.* in his surfactin production study, reported the over production of the surfactin in the presence of  $Fe^{2+}$  in the mineral salt microbial broth.<sup>165</sup>

### 7.2. Influence of external environmental factors

The growth of microbes, their metabolites and the fermentation process are greatly influenced by the pH, temperature, agitation, aeration, dissolved oxygen as well as fermentation time.<sup>166</sup>

**7.2.1. pH.** An optimum pH is always required for the growth of microbial as well as the fermentation of BSs. In an investigation it was reported that BS production by *C. antarctica*, pH range was set 4–8 where the best production was obtained at pH 8.<sup>159</sup> The decrease in BSs production without controlling pH reported in several scientific investigations thus prove its importance during the fermentation procedure.<sup>65</sup>



**7.2.2. Temperature.** One of the most significant environmental parameters in biosurfactant production is the temperature. The growth of every microbe is associated with a particular temperature range preferably 25–30 °C.<sup>161</sup> Not only the bacterial growth but also fermentation of carbohydrates needs a perfect and well-maintained temperature. Additionally temperature has impact on the metabolic process of microorganisms and physical properties of the fermentation broth.<sup>167</sup> The MEL production was reported to be the best at 25 °C.<sup>158</sup> However, if the temperature exceeds its optimum level, enzymatic activities may be hindered as well as denaturation of enzymes and other essential protein may happen. So, observing and maintaining the optimum temperature for the microbial growth and fermentation process is very important.

**7.2.3. Agitation and aeration.** Agitation is a very important factor in case of fermentation process as it effects many activities like distribution of heat, nutrients, oxygen *etc.* in the medium and influences the viscosity of the medium, cell growth of microorganisms and so on. On the other hand aeration supplies the necessary oxygen gas for the cell growth and fermentation process. Additionally it reduces the exhaust gases generated during the process.<sup>167</sup> The best production of BSs was observed by Adamczak and co-workers. They studied production of BSs by *C. antarctica*. The condition of fermentation was fixed to an air flow rate of 1vvm and amount of dissolved oxygen to 50%. The increase in air flow rate indicated decreased BSs production due to high foam formation.<sup>168</sup> Wei and his co-researchers<sup>169</sup> reported that rhamnolipid production was enhanced almost 80% as well as the rate of cell growth increased from 0.22 to 0.72 h<sup>-1</sup> by increasing the agitation rate from 50 rpm to 200 rpm. They also studied that elevated dissolved oxygen level has a positive impact over rhamnolipid production along with microbial growth.

## 8. Fermentation

### 8.1. Submerged fermentation process

Submerged fermentation process (SmF) is a method of manufacturing biomolecules in which enzymes and other reactive compounds are submerged in organic solvents like oil, alcohol or nutrient broth. SmF has been proved to be industrially applicable for bio-surfactant production due to low cost and high atom economy and additionally the process takes very short time. In SmF the purification of product is also easy. Though researchers have showed more interest in Solid State Fermentation over SmF due high scale production is easier in solid state with compared to SmF.

### 8.2. Solid-state fermentation process

Solid state fermentation process or SSF process allows microorganism to develop and raise on the damp solid substrate. SSF has been given a dedicated focus for several benefits over the submerged process. The SSF process has its increased application in the production of enzymes, antibiotics, biosides along with surfactant. The main advantages of SSF over other processes are the application of simplified equipment, chipper

carbon source, low water requirement, demand of lower energy and higher yield of products; the most efficient one is economic advantage *i.e.* the low production cost.<sup>62</sup> The best feedstocks for SSF production of biosurfactants are agro-wastes rich in carbohydrates, proteins or lipid. Rhamnolipid production by *Pseudomonas aeruginosa*. UFPEDA-614 was achieved greater yield in SSF compared to submerged fermentation.<sup>170</sup> 12 different agro-wastes have been experimented for the production of BSs by employing SSF method by the microorganism *B. subtilis*. The maximum yield was achieved in a condition where temperature was 37 °C and moisture was fixed at 88%. The fermentation time was 14 hours.<sup>171</sup>

## 9. Product recovery

After setting all the required parameters in optimum condition, the whole production procedure is incomplete until a proper economically efficient recovery and purification of the product is done *i.e.*, downstream processing. For commercialisation of biosurfactant production the recovery of product from culture medium is one of the most important parameters. There are various product recovery procedures like solvent extraction, acid precipitation, crystallisation, centrifugation and so on which are mostly reported by researchers. In addition to this, some unconventional methods are also been reported such as ultrafiltration, foam fractionation, adsorption–desorption on various media. All these methods are employed based on the characteristics of the biosurfactant such as their micelle forming ability, surface activity, and solubility *etc.* The continuous rise in demands of highly pure biosurfactants by some pharmaceuticals, foods and cosmetics industries invokes the application of these methods which able to recover highly pure biosurfactant.<sup>172</sup>

Besides that, the solvents usually applied for biosurfactant recovery such as chloroform, methanol, acetone *etc.* are toxic in nature as well as unsafe to environment. Replacing these harmful solvents researchers have used inexpensive and less harmful solvents like methyl tertiary-butyl ether (MTBE) in recent past for recovery of biosurfactants.<sup>173</sup> These types of less toxic and low-cost solvents help to reduce the recovery cost of biosurfactant production. Sometimes a single recovery procedure is not sufficient to get desired product, in that case multiple recovery techniques are employed for downstream processing.<sup>174</sup>

## 10. Market prospect

In spite of several challenges and obstacles associated to biosurfactant production, the commercialisation of biosurfactant in several sectors is being found to be growing due to its versatile features and advantages over its chemical alternatives. Basically, the rising concern among consumers regarding the use of eco-friendly and biodegradable products invokes the growth of biosurfactant market and as a result there is an increasing demand for green approach by several industries leading to high requirement for biosurfactants. Previously the global biosurfactant market was evaluated 4.20 billion USD in



Table 7 List of several biosurfactant production companies along with their country of origin<sup>a</sup>

Companies	Products	Country of origin
AGAE Technologies LLC Jeneil Biosurfactant Co. LLC Paradigm Biomedical Inc. Rhamnolipids Companies, Inc. Natsurfact	Rhamnolipids	United States
Tensio Green	Rhamnolipids Lipopeptides Trehalolipids	
Synthezyme LLC Glyco Surf Tee Gene Biotech	Sophorolipids Glycolipids Rhamnolipids Lipopeptides	United Kingdom
Ecover Groupe Soliance MG Intobio Co. Ltd Allied Carbon Solutions (ACS) Ltd Kaneka Co. Saraya Co. Ltd Fraunhofer IGB	Sophorolipids	Belgium France South Korea Japan
Henkel Evonik	Cellobiose lipids, mannosylerythritol lipids Sophorolipids, rhamnolipids, mannosylerythritol lipids Sophorolipids Rhamnolipids	Germany
BASF BASF-Cognis Kingororigin Victex	Sophorolipids, glycolipids Sophorolipids, glycolipids Glycolipids, phospholipids Rhamnolipids	China China

2017 and was expected to reach 5.52 billion USD by 2022 at a compound annual growth rate (CAGR) of 5.6% from 2017 to 2022. But, the global biosurfactant market was found to grow from USD 4.07 billion in 2022 to USD 4.39 billion in 2023 at a CAGR of 7.8% and is estimated to grow to USD 6 billion in 2027 at a CAGR of 8.1%.<sup>175</sup>

To fulfil the requirement of modern industries for eco-friendly and less expensive biosurfactants the companies belonging to the biosurfactant manufacturing sector are investing in the development of new BSs globally. Evonik Industries launched 'Rewoferm RL 100' biosurfactant to encounter the rising demand for low-emission and low-impact cleaning products in March, 2022. This biosurfactant is produced from nearby sourced feedstocks and offers efficient cleaning performance. A USA-based manufacturer, Stepan Company, took over 'Logos Technologies LLC's NatSurFact' business in December 2020 to commercialize inventive biosurfactants for building sustainable products.<sup>175</sup> The report represents the current scenario of increasing demand of biosurfactant globally. A list of several biosurfactant production companies along with their country of origin has been given in Table 7.

## 11. Future prospect and conclusions

Analysis of the global biosurfactant market basically replicates the rising demand of BS in various sectors replacing its chemical alternatives, because of its versatility and eco-friendly nature. In this present review, we have presented extensive

details regarding numerous investigations on BSs productions. The review also presents the recent advancements in this field of BSs production and processing. The use of technological support made BSs production easier and economic. Though BSs are best suit of the requirements of modern science and technology and go along with green revolution, for several reasons processing and productions of BSs are not up to the mark. During the course of our study of different literature we came to know that the reasons behind low BSs production is high cost, relatively low yield and of course time. As a result, the price of such biosurfactant is much higher than their chemical alternatives and it indicates that the commercialization of biosurfactant is still facing many challenges. So, in conclusion it must be noted that surfactant chemistry will be changed greatly if development of more economically favourable as well as eco-friendly process is emphasized for biosurfactant production from carbohydrate wastes like agro-wastes, industrial wastes *etc.* which facilitates large scale fermentation of carbohydrate substrates; hence BSs production will be a commercial success. Besides the use of cheaper carbohydrate source, proper medium optimisation through statistical methods and appropriate downstream processing is required to reduce the production cost and better biosurfactant yield. In spite of several challenges and obstacles associated to biosurfactant production, the commercialisation of biosurfactant in several sectors is being found to be growing due to its versatile features and advantages over its chemical alternatives. More improved strategies should be investigated for the sake of commercialization of biosurfactant and make it economically favourable hence



enhancing its use in various industries. If the commercially biosurfactant producing companies cooperate with utilising the waste matter for the production of biosurfactant, it will be possible to achieve the SDGs and a better environment as well as a better life on earth and water will also be achieved.

## Conflicts of interest

There are no conflicts to declare.

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