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Origin, Properties, Production and Purification of Microbial Surfactants as Molecules with Immense Commercial Potential

Microbial surfactants are produced by various sources (terrestrial and marine environments, sludges, etc.) of microorganisms. The production of biosurfactants in a culture medium is determined by the secretion of the surface active molecules, which supports both the reduction of the surface tension and the lowering of the critical micelle concentration (CMC). Biosurfactant molecules consisting of lipophilic and hydrophilic moieties have been described for various applications (physiochemical, biological, commercial and nanotechnological) due to their structural properties and their molecular weight, which is generally between 500 Da – 1500 Da. Although, biosurfactants have normally better application properties than conventional surfactants, the production of them in higher scale is limited due to certain constraints. These constrains are addressed in different studies: Kinetics, statistical design (Taguchi, factorial design, RSM etc.) and computational tool (ANN-GA) have been performed to improve the yields. Along with that, few studies also described the batch and fed batch fermentation for the better commercial level production. However, no defined commercial method is available for the purification of the biosurfactants. Few studies demonstrated the purification by gel exclusion chromatography (SEC, gel filtration chromatography) and ultrafiltration/diafiltration that have been partially established. This review article outlines the detailed reports on these.

Key words: Biosurfactants, physiochemical properties, kinetics and production, process optimization, batch and fed batch fermentation, purification technology, biological activities, nanotechnology

Herkunft, Eigenschaften, Herstellung und Reinigung von mikrobiellen Tensiden als Moleküle mit riesigem kommerziellem Potenzial. Tenside mikrobieller Herkunft werden von Mikroorganismen aus unterschiedlichen Quellen (terrestrische und marine Umwelten, Klärschlamm, etc.) erzeugt. Die Produktion von Biotensiden in einem Kulturmedium wird durch die Sekretion der oberflächenaktiven Moleküle bestimmt, was sowohl die Reduktion der Oberflächenspannung als auch die Absenkung der kritischen Mizellenkonzentration (CMC) unterstützt. Biotensidmoleküle bestehend aus liphophilen und hydrophilen Anteilen wurden aufgrund ihrer strukturellen Eigenschaften und ihres Molekulargewichts, das im allgemeinen zwischen 500 Da–1500 Da liegt, für verschiedene Anwendungen (physiochemische, biologische, kommerzielle und Nanotechnologie) beschrieben. Obwohl Biotenside in der Regel bessere Anwendungseigenschaften als konventionelle Tenside aufweisen, ist ihre Herstellung in höherem Maßstab aufgrund bestimmter Beschränkungen begrenzt. Diese Einschränkungen werden in verschiedenen Studien untersucht: Kinetik, statistisches Design (Taguchi, fraktorielle Versuchspläne, RSM etc.) und computergestützte Rechenprogamme (ANN-GA) wurden entwickelt, um die Ausbeuten zu verbessern. Es steht jedoch für die Reinigung der Biottenside kein definiertes kommerzielles Verfahren zur Verfügung. Wenige Studien zeigten, dass die Reinigung durch Gelausschlusschromatographie (SEC, Gelfiltrationschromatographie) und Ultrafiltration/Diafiltration sich teilweise bewährt hat. Dieser Übersichtsbeitrag skizziert die detaillierten Berichte, die zuvor dazu beschrieben wurden.

Stichwörter: Biotenside, Physikalisch-chemische Eigenschaften, Kinetik, Herstellungsverfahren, Verfahrensoptimierung, Batchfermentation, Fed-Batch-Fermentation (Zulauffermentation), Aufreinigungstechnologie, Biologische Aktivität, Nanotechnologie

1 Introduction

Microbial surfactants are amphiphilic surface active molecules produced by various microorganisms. The structure of these molecules are basically composed of saturated, unsaturated or fatty acids (hydrophobic tail portion) and comprised of amino acids or peptides or polysaccharides (hydrophilic head moieties) that helps to partition preferentially at the interface between two liquid phases [1–3]. Depending on the chemical nature of the two distinct moieties, microbial surfactants are broadly classified into different diverse groups such as glycolipids, lipopeptides, lipoproteins, fatty acids, neutral lipids, phospholipids, particulate and polymeric biosurfactants [3–5]. Microbial surfactants are potentially more applicable in industrial processes than other surfactants because of their desirable properties such as microbial enhanced oil recovery (MEOR) and of their use as emulsification agents in pharmaceutical, food, dye, cosmetic, and agrochemical industries [5–10]. The contribution of these molecules in environmental applications is significant [11, 12], which describe the biodegradation of hydrocarbon contaminants and the removal of heavy metals [13–15]. The potential use of biosurfactants in medical fields have also rapidly increased by the therapeutic properties of biosurfactants such as antiviral, antitumor and antimicrobial agents [16–20]. Due to menace of drug resistance against pathogenic microorganisms, these microbial surface active molecules may find potential applications as drug candidates for new age chemotherapy and may occupy an important place in biopharmaceutical industries [19, 20].

However, production and kinetics of these molecules have not been discussed extensively. The objective of this review

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is to help the scientific community for exploring the biosurfactants kinetics, production achievements, purification and its applications.

Most of the biosurfactants and their producers are reported from terrestrial and very few are from marine environments (Table 1).

Glycolipids are biosurfactants composed of carbohydrates and long chain aliphatic acids with low-molecular weight. The family comprised of rhamnolipids, sophorolipids, trehalolipids, helps in solubilizing the hydrocarbon contaminant into the fermentation broth during the fermentation, which can be utilized as substrate by producer microorganisms (Lead and Cadmium [13]) and hydrocarbons (tetradecane, aliphatic and aromatic) [11, 12, 29–50]. Recently, a new form of biosurfactant has been identified based on its structural properties and named as glycopeptide or glycolipopeptide which belongs to glycolipid family. The partially characterized glycopeptide was derived from lactic acid bacterium, *Lactobacillus pentosus* and showed a good emulsification activity [48, 119].

On other hand, lipopeptide from marine isolate *Azetobacter chroococum* displayed excellent physiochemical and biodegradation properties [38]. Similarly, a marine bacterium utilized the polyaromatic hydrocarbons (PAHs) as substrate for biosurfactant production [11] and also exhibit heavy metals (lead and cadmium) remediations [13]. The exhibition of good emulsification property and oil dispersion activity (Table 3) of this molecule suggests that it can be used as emulsifier in commercial industrial applications [38–40].

Lipopeptide type of biosurfactants gained attention, because of its unique therapeutic [16–43] and environmental applications [44–50]. Several strains of *Bacillus* sp. have been reported to be major producers of lipopeptides (described in Table 1) such as surfactin, lichenysin, fengycin, bacillomycin and iturin [46–54]. The presence of multimodular enzyme complexes known as non-ribosomal peptide synthetases (NRPs), conserved naturally in various *Bacillus* sp., that are responsible for the secretion of lipopeptides [7, 18, 62–66]. The first biologically active lipopeptide surfactin, was derived from *Bacillus subtilis* [67]. Surfactin, a cyclic lipopeptide, consists of a heptapeptide and β-hydroxy fatty acid with acyl chain length ranging from 12–18 carbons [4–7]. Various forms of surfactin are identified in the molecular mass range of (*m/z*) 900–1095 Da [68, 69]. Fengycin, another biologically active lipopeptide having an antifungal property which is also composed of β-hydroxy fatty acid chain attached with a smaller peptide part comprising of 10 amino acids [71–74]. Two variants of fengycin are identified and reported as fengycin A and fengycin B [71–73]. The basic difference among these isoforms is the presence of either valine or alanine at the sixth position of the lactone ring [70]. The lipopeptide molecules are detected in their protonated form or as Na^+ or K^+ adducts by MALDI-ToF mass spectrometry in the m/z range of 1400–1550 Da [70–74].

2 Physiochemical Properties

The physiochemical properties of biosurfactants are defined by ionic strength and low critical micelle concentration (CMC). Biosurfactants are comprised with apolar and polar moieties. The polar moieties will define the ionic strength of the biosurfactant [47–50]. These surfactants are classified into anionic, cationic, zwitterionic and non-ionic; based on the presence of ions in hydrophilic head portion of the surfactant [39–44]. Most of the biosurfactants are categorized into anionic and non-ionic which can be detected by conventional methods [2–7]. Glycolipid and gylcolipopeptide have been identified as anionic and the lipopeptides are classified

Origin	Producer Microorganisms	Isolation site	Type of Biosurfactant	References
Terrestrial	*Bacillus subtilis* C-1	Petroleum sludge	Lipopeptides	5
	Bacillus sp.	Hydrocarbon contaminated sites	Biosurfactants, (ND)*	21
	Bacillus subtilis DSM 3256	Terrestrial origin	Biosurfactants (ND)*	39
	Bacillus amyloliquefaciens FZB42	Environmental isolate	Lipopeptides	54
	Bacillus subtilis MZ-7			55
	Bacillus megaterium	Soil	Lipopeptides	46
	Bacillus thuringiensis CMB26			54
	Bacillus coagulans			64
	Bacillus licheniformis HSN221	Oil field	Biosurfactant (ND)*	62
	Bacillus licheniformis JF-2	Fermented food	Lipopeptide	51, 65
Marine	*Bacillus* sp.	Marine source	Lipopeptide	21, 23
	Bacillus circulans	Anadaman Nicobar Island, India		25–28
	Pseudomanas sp.	Islands of Xiamen, Taiwan		35
	Bacillus pumilus	Marine sponge *Iricna* sp.		36
	Brevibacillus laterosporus	Papua New Guinea		37
	Halomonas sp.	Ross Sea, Antarctica	Glycolipid	31
	Nocardiodes sp.	Antarctic soil		32
	Bacterial strain MM1	Sea-Water		33
	Pantoea sp.	Frazier Islands		34

*ND: Not determined

Table 1 Types of biosurfactant derived from different microorganisms and their origin

as non-ionic biosurfactants [2–7, 58]. The higher ionic force of the biosurfactant will be responsible for forming a stable emulsion which helps in microbial enhanced oil recovery MEOR by the adverse activity in higher salt tolerance [10, 51, 52].

The molecular dynamics of the biosurfactants are ionic strength, density, viscosities and surface tension of the solution and interaction with the solute which have been described recently [41–45]. The characteristic ionic strength curvature will be mathematically determined by the hydrophilic-lipophilic deviation HLD equation [51–54]. The hydrophilic and liphophilic balance value (the range is from 0–20) is one of the critical physiochemical parameters commenced by density, viscosity, conductivity of the biosurfactants. The lower the HLB value defines the liphophilic (water in oil emulsion) nature whereas the higher the value defines an oil in water emulsion [54, 55]. Briefly, the study described about the interaction and intermolecular dimension of commercially available synthetic surfactant (CPC, CPB, CTAB) versus EPC biosurfactant in aqueous oil mixture which described the EPC biosurfactant is the best emulsifier to use [56].

In addition to this CMC of the standard surfactin (Sigma, USA) was found to be 13.0 mg L^{-1} [57]. The lower CMC value indicates that a lower amount of surfactant is required to achieve the minimum surface tension and hence, the higher the potential and purity are [27, 58]. The CMC of biosurfactant is the minimum amount required for the onset of the process of micellization. At this and at higher concentrations, the surface tension reaches a minimum value and will form the macromolecular structure resulting in bigger micelles, vesicles and the formation of lamellae [54–57]. Biosurfactants have been reported to have wide ranges of low CMC values (9.0 –15.0 mg L^{-1}) [59–76] to very high CMC values (40.0–150.0 mg L^{-1}) [28, 58]. The properties of CMC possess several advantages over those of the chemically derived surfactants.

Different studies have been described the implication of biosurfactant stability at different pH, salinity and temperatures [8–10]. These parameters will define the exact solubility and surface activity parameters of biosurfactants which can be used for biodegradation studies [77–78]. For example, a biosurfactant from *Bacillus subtilis* exhibits an excellent stability at higher temperature (100 °C) and a wide pH range (3.0–11.0) [8]. Lipopeptide obtained from *Bacillus subtilis* C9 displayed a stability from pH 5.0–9.0, when incubated at 100 °C and also the stability was up to 1.0 M for NaCl and 10.0 mM for $CaCl_2$ [9, 10]. Recently, a synergistic effect of salt concentration, pH and temperature was studied on biosurfactant solubility using the Box–Behnken response surface methodology. The critical parameters were identified as pH (3.0–8.0 range) and salinity (NaCl, 1.0–5.0%) which influence the surface active properties whereas the temperature showed negligible effect within the tested parameters (5–56 °C) found good emulsification of gasoline/water emulsion [78].

For nano application, a biosurfactant has the capability to conjugate with nano-SiO_2 which helps to enhance the surface activity in the solution. On other hand, the author described the complex study of oil spreading nano-SiO_2 conjugated with biosurfactant that helps in forming the stable structure on surface activity evident from TEM analysis and in reducing the interfacial tension [79]. These above described properties cause the biosurfactants to be potential candidats for environmental applications as compared to chemically synthetic surfactant which can affect on the microbial activities [1–10, 76–79].

3 Production of Microbial Surfactants – Media, Kinetics and Fermentation processes

The secretion or production of these biosurfactants are dependend on critical limiting substrates; mainly of carbon and nitrogen source (see Table 2) [1–4]. The importance of the nutritional requirements for biosurfactant production has been discussed below.

3.1 Medium

3.1.1 Effect of carbon and nitrogen sources on biosurfactant production

The carbon source helps to attain higher cell concentrations which improved the biosurfactant yield by 23.0% [83, 84], (Table 2). Different forms of carbon sources are treated for the production such as the agro-industrial product/by-products, glucose or starch as carbon source for the production of biosurfactant (yield by CMC 17.0 mg L^{-1}) from *Bacillus coagulans* [75]. The best carbon sources for *Bacillus subtilis* S499 were glucose, fructose and sucrose which help to secrete the surfactin with the maximum yield of 110.0 mg L^{-1} [85]. The biosurfactant from probiotic bacteria also showed a better production improvement by utilizing a carbon source with the yield of 1.40 g L^{-1} [86, 87]. Most of the microorganisms also produced biosurfactant by utilizing the hydrocarbon substrates as carbon sources. For example, the polyaromatic hydrocarbon anthracene was utilized as carbon source by the marine strain of *Bacillus circulans* [11].

The choice of inexpensive raw materials is available nowadays possible which can be used as carbon source, especially agro-industrial waste having a higher carbon content such as corn steep liquor, brewery industries, food industries, oil industries [45–80]. By using these inexpensive raw materials, the process costs can be controlled for the market needs. The effect of carbon sources such as *n*-hexadecane, olive oil and glucose on the biosurfactant production from *Pseudomonas fluorescens* has been studied extensively. The study showed that a better production was observed in *n*-hexadecane and olive oil acting as carbon source as compared to glucose [88]. *Candida antarctica* KCTC 7804, yielded a maximum biosurfactant concentration of 41.0 g L^{-1} achieved by feeding glycerol and olive oil during the initial and exponential phase of feeding respectively [89]. When vegetable oil was used as precursor during the fermentation, a maximum biosurfactant concentration of 31.0 g L^{-1} from *Candida antarctica* KCTC 7804 was obtained [89]. The addition of a carbon source in the precursor formed during the fermentation process will also induce the biosurfactant production [22]. The addition of vegetable oil carbohydrates into the culture medium of *Torulopsis* sp. resulted in an increased biosurfactant yield of about 70.0 g L^{-1} [90].

In cells, biosurfactant production varied as intra or extracellular functionality during the fermentation process which depends particularly on the choice of the carbon source [80]. Marine bacteria are known to have the potential to degrade lipophilic compounds, and they enhance their bioavailability [88]. The growth of the microorganism on lipophilic compounds is accompanied by a cell surface modification, which helps to secrete the biosurfactant production [81].

Nitrogen source is also identified as critical component, the best nitrogen source for biosurfactant production from *Bacillus subtilis* S499 was found to be L-amino acids (L-glutamic acid, L-valine, L-lysine and β-alanine) [91]. Surfactin produced by *Bacillus subtilis* ATCC 21332 in batch culture production was highly influenced by the nitrogen metabolisms [84]. The nitrogen and C/N ratio were used as nutri-

Microorganisms	Operations	Source of induction for BS production	Kinetics determination	Type of BS	Maxima production achieved	References
Pseudomonas aeruginosa EBN-8 mutant	Shake flask	n-Paraffin,Hexadecane, kerosene oil	$Y_{P/S}$ (0.72 g g^{-1}); $Y_{P/X}$ (3.15 g g^{-1}) values are better for n-paraffin	Biosurfactant (ND)	4.10 and 6.30 g L^{-1}	14
Bacterial strain MM1	Shake flask	Glucose	ND	Glycolpid	1.70 g L^{1}	33
Pseudomonas aeruginosa A41	Shake flask	Olive oil, Palm oil and coconut oil	ND	Rhamnolipid	6.58 g L^{-1}, 2.91 g L^{-1} and 2.93 g L^{-1}	37
Bacillus circulans MTCC 8281	Shake Flask	GMSM verses MMM	μ_{xmax} (0.22 h^{-1}), $Y_{P/S}$ (0.08 gg^{-1}); $Y_{P/X}$ (0.44 mg g^{-1}); $Y_{x/s}$ (0.19 g g^{-1}) and μ_{xmax} (0.41 h^{-1}), $Y_{P/S}$ (0.13 g g^{-1}); $Y_{P/X}$ (0.55 g g^{-1}); $Y_{x/s}$ (0.24 g g^{-1})	Biosurfactant	2.58 g L^{-1} and 4.71 g L^{-1}	99
Bacillus subtilis ATCCC 21332	Shake flask	Candy producer company waste	ND	Biosurfactant (ND)	2.20 g L^{-1}	101
Bacillus sp. LB5a	Shake flask	Cassava industry effluent	ND	Biosurfactant (ND)	3.0 g L^{-1}	102
Bacillus sp. LB5a	Shake flask	Glucose, Whey from cheese Industry, Molasses and cassava flour waste water from Agro Industrial waste	ND	Biosurfactant (ND)	2.0 g L^{-1}	110
Bacillus licheniformis 86	Shake flask	Glucose	$Y_{P/S}$ (0.95 g g^{-1}); $Y_{P/X}$ (0.27 g g^{-1})	Surfactant (BL86)	0.02 g L^{-1} purified surfactin	111
Bacillus sp	Shake flask	Glucose	$Y_{P/S}$ (0.08 g g^{-1}); $Y_{P/X}$ (0.48 g g^{-1})	Biosurfactant (ND)	2.42 g L^{-1}	112
Bacillus subtilis DSM 3256	2 L Virtis Omni- culture fermenter	Glucose	ND	Surfactin	1.10 g L^{-1}	49
Bacillus licheniformis JF-2	5 L fermenter	Glucose	ND	Biosurfactant (ND)	13.0 μg ml^{-1}	76
Bacillus subtilis ATCC 21332	Bioflo II system, New Brunswick (NJ, USA).	Nitrogen limitation; ammonium nitrate	Nitrogen (N) limited, Oxygen (O) depleted $Y_{P/x}$ (0.075 g g^{-1}); Ammonium-Limited oxygen depleted, $Y_{P/x}$ (0.012 g g^{-1});Carbon-Limited oxygen depleted, $Y_{P/x}$ (0.0069 g g^{-1}); aerobic carbon limited, $Y_{P/x}$ (0.0068 g g^{-1}), aerobic nitrogen limited, $Y_{P/x}$ (0.021 g g^{-1})	Surfactin	31.20 mg L^{-1}	91
Bacillus subtilis C9	3.0 L fermenter (MDL model, B. E. Marubishi, Japan)	NH_4HCO_3, O_2 sufficient fermentation	$Y_{P/S}$ (0.175 g g^{-1}); $Y_{P/X}$ (1.27 g g^{-1}); Q_p (0.097 g $l^{-1}h^{-1}$)	Lipopeptides	13.50 g L^{-1}	93
Bacillus circulans MTCC 8281	KLF-2000 3.7 L fermenter (BioEngineering,Wald, Switzerland)	Glucose	LP model, $\alpha = 979$ g $g^{-1}$of dry cells; K_La 0.08 S^{-1}; $Y_{P/S}$ (0.17 g g^{-1}); $Y_{P/X}$ (0.88 g g^{-1}); $Y_{x/s}$ (0.18 g g^{-1})	Lipopeptides	4.61 g L^{-1}	96
Bacillus subitlis E8	KLF-2000 3.7 L fermenter (BioEngineering,Wald, Switzerland)	Soluble starch	LP model, $\alpha = 894$ mg/g of dry cells	Surfactin	5.89 g L^{-1}	98
Bacillus subtilis LAMI005	4 L Marconi (ND)	Glucose	μ_{xmax} (0.10 h^{-1}), $Y_{P/S}$ (0.11 g g^{-1}); $Y_{P/X}$ (0.23 g g^{-1}); $Y_{x/s}$ (0.49 g g^{-1}), Q_s (2.21 g $g^{-1}h^{-1}$); Q_p (0.16 g g^{-1} h^{-1})	Biosurfactant (ND)	263.64 mg L^{-1}	104

* ND = Not determined; Y = Yield coefficients

Table 2 Fermentation process operation and its key components involved in maxima production output of biosurfactants (BS) and kinetics parameters of the producer microorganisms

Microorganisms	Operations	Source of induction for BS production	Kinetics determination	Type of BS	Maxima production achieved	References
Bacillus circulans MTCC8281	KLF-2000 3.7 L fermenter (BioEngineering Switzerland)	Glucose	ND		6.98 g L^{-1}	113
Bacillus subtilis ATCC 21332	28 L/12 L (New Brunswick Scientific, NJ, USA) and 5-l bioreactor (BioFlo 110 New Brunswick Scientific, UK)	Metal ions (Ca. 5 mg/200 ml; $FeSO_4$, $MnSO_4$) Fed batch; Glucose	ND	Surfactin	0.80 g L^{-1} and 583 mg L^{-1}	1, 114
Pseudomonas aeruginosa DS 10-129	7.5 L Bioflow 110 fermenter (New Brunchwich Scientific, NJ, USA)	Lab Lemco Powder	ND	Rhamnolipid	22.0 mg mL^{-1}	115
Lactobacillus lactis 53 and *Streptococcuccus thermophilis*	1 L Bioreactor	Molasses	μ_{xmax} (0.257 h^{-1}), $Y_{P/S}$ (0.12 g g^{-1}); $Y_{P/X}$ (281 mg g^{-1}); $Y_{x/s}$ (0.43 g g^{-1}) and μ_{xmax} (0.294 h^{-1}), $Y_{P/S}$ (0.12 g g^{-1}); $Y_{P/X}$ (272 g g^{-1}); $Y_{x/s}$ (0.49 g g^{-1})	Biosurfactant	1.735 g L^{-1} and 1.40 g L^{-1}	116
Bacillus subtilis ATCC 21332	conventional 5-L jar fermentor (Firstek Scientific, Taipei, Taiwan)	Activated carbon	$K_L a$ 0.0132 S^{-1}	Surfactin	6.45 g L^{-1}	117
Lactobacillus pentosus CECT-4023 T (ATCC-8041)	2 L Applikon fermenter	Yeast extract and corn steep liquor	Pseudo-first and second order kinetics	Biosurfactant ND	11.97 mg L^{-1}	122
Pseudomonas aeruginosa YPJ-80	2.5 L Jar fermenter with out baffle (Korea fermenter company, Inchon, Korea)	Glucose and $MgSo_4$, pH stat fed batch mode	ND	Rhamnolipid	4.40 g L^{-1}	164
Candida sp. SY16	5-L Jar	Soybean oil as carbon source	$Y_{P/S}$ (0.45 g g^{-1}); Q_p (0.48 g l^{-1} h^{-1})	Mannosylerythritol lipid (MEL-SY16)	23.0 g L^{-1}	181

* ND = Not determined; Y = Yield coefficients

Table 2 (continued)

tional source for the production of rhamnose from *Pseudomonas aeruginosa* [92]. The yield of 13.50 g L^{-1} of lipopeptide biosurfactant was observed when ammonium bicarbonate (NH_4HCO_3) was used as nitrogen source [93]. The exhaustion of nitrogen source creates stress conditions which allow for high cell densities and this results in a better biosurfactant yield during the fermentation process [81]. The regulatory of gene involved in nitrogen metabolism known as sigma factor RpoN (σ^{54}) which expresses more under nitrogen limiting condition helps in improving the biosurfactant production [82].

The C/N ratio is the critical factor and this was investigated by using substrates as frying oil, as carbon source and urea as nitrogen source. A wide range of the C/N ratio (10 : 1; 70 : 1) were investigated and it was found that the 30 : 1 ratio gave a better biomass and biosurfactant yield (2.80 g L^{-1}) [94]. Similarly, the rhamnolipid type of biosurfactant (rhamnose) was found at the ratio of C(glycerol)/N(sodium nitrate) of 60 : 1, which caused to increase the yield to 3.16 g L^1 [95].

3.2 Kinetics of biosurfactant production

Biosurfactant production can be characterized as growth-associated, pseudo growth associated, non-growth associated and mixed growth associated [19, 100, 101]. The characteristic kinetic nature of the microbial production can be represented by various factors (Table 2) however, the Luedeking-Piret (L-P) model, helps to define the production manner. Thus the equation 1, for L-P model is given below,

$$Q_p = \alpha\mu + \beta \quad (1)$$

Q_p: specific product rate formation; μ: specific growth rate and α, β: empirical constants.

3.2.1 Growth associated production

The specific product formation (Q_p) rate is directly proportional to the specific growth rate (μ). L-P model for this type of production is given below in equation (2)

$$Q_p = \alpha\mu \quad (2)$$

Most of the biosurfactant productions are reported to be growth associated, which shows the relationship between the growth, substrate utilization and biosurfactant concentration. The production of lipopeptides form *B. licheniformis* JF-2 [61] and *Bacillus subitlis* LB5a [101, 102] are great examples for a growth associated biosurfactant production.

3.2.2 Mixed growth associated production

The product formation takes places during the exponential and stationary phase. In this case the L-P model has the both empirical constant values which are given by equation (3) below,

$$q_p = \alpha\mu + \beta \quad (3)$$

Biosurfactant production can also found to be pseudo metabolite during the growth of the microorganisms. For example, *Bacillus subtilis* produced surfactin in pseudo metabolic phase [60, 83].

3.2.3 Non-growth associated production

A non-growth associated product formation occurs at the stationary phase when the growth rate is zero. The L-P model for this type of production is given in the equation (4)

$$q_p = \beta \quad (4)$$

The biosurfactant production which occurs in the stationary phase will be considered as a non-growth associated production. Biosurfactants from *Pseudomonas* sp. displayed the non-growth associated production [103]. Mostly, the glycolipid production from various microorganism and yeasts is found to be non-growth associated [104, 105]. In advance, the kinetics of the cell bound biosurfactant produced by *Lactobacillus pentosus* were studied by a mathematical model of pseudo first and second order kinetics for the extraction process. The new dimension of this study describes the pseudo-second order equation for fitting more accurately in relationship with temperature and biosurfactant [122].

Despite their versatile advantages and diverse potential applications, there is a limitation in the production at a commercial level. By defining the kinetics derivations (Table 2), the yield improvement issues are addressed by few statistical and computational tools as well as fermenter process studies as described below.

3.3 Fermentation processes: Batch and Fed-batch/semi-continuous operation

Mostly biosurfactant productions have been carried out by submerged fermentation [69–104] and rarely by solid state [107, 108]. The mode of fermentation, biosurfactant yield obtained from the different sources of microorganisms have been tabulated (Table 2).

Fed batch approach for surfactin production from *Bacillus subtilis* ATCC 21332 was enhanced by feeding a nitrogen source thus yielded 439.0 mg L^{-1} [91]. By feeding the nitrogen source for the glycolipid biosurfactant production from *Candida* sp. SY16 yielded 37.0 g L^{-1}, whereas the residual oil of soybeans acting as carbon source on fed batch operation, yielded 95.0 g L^{-1} surfactin [118]. The pH fed batch mode was performed for the production of rhamnolipid from *Pseudomonas aeruginosa*, glucose was used as limiting substrate which enhanced the yield from 1.68 g L^{-1} to 4.40 g L^{-1} [117].

A new approach has been described recently for the lipopeptides production from *Bacillus circulans* MTCC 8281. For two different processes such as unsteady state fed batch operations (higher and lower flow rates) it was demonstrated how in this both processes a carbon source acted as limiting substrate. The better yield of 6.21 g L^{-1} was obtained from the lower flow rate process as compared to higher flow rate, where the last one yielded 5.83 g L^{-1} [119]. Recently, an inexpensive fed batch operation was performed by inducing the foam formation and fractionation. The stability and binding efficiency of the produced lipopeptides were investigated with different metal ions with respective to pH conditions [120].

A significant improvement in the marine biosurfactant production from (3.30 ± 0.10) g L^{-1} to (4.20 ± 0.10) g L^{-1} was attained by feeding Fe^{2+} trace element during the early exponential stage, which is approximately 27 % [121].

The foam formation becomes the main bottleneck in the submerged fermentation process for the biosurfactant production. Controlling the foam formation or foam collected though air exhaust pipe will get affected during the recovery

process. To overcome this issue, an anaerobic way of the fermentation process was studied for *Bacillus licheniformis* JF-2 [76], which was not explored further. However, the anaerobic way of fermentation processes is recently explored. A surfactin production from a foam-free anaerobic fermentation way was demonstrated in a 2.50 L fermenter (Minifors, HT Infors, Bottmingen, Switzerland). Nitrogen gas was spraged into the fermenter for the surfactin production from *Bacillus subtilis* DSM10^T. The obtained yield was 0.087 g L^{-1}. The kinetic parameters of this anaerobic conditions are: $Y_{P/X}$ (g g^{-1}) = 0.278; $Y_{X/S}$ (g g^{-1}) = 0.120; $Y_{P/S}$ (g g^{-1}) = 0.033 which shows moderate comparable results with the aerobic fermentation, described in the report [124].

The recent approach shows the fed batch process operation and its critical variables optimization using ANN-GA model. Various feeding concentrations of asparagine (Asn), glutamic acid (Glu) and proline (Pro) during the fed-batch fermentation process were studied for yielding an improvement of iturin A. The optimum yield of (13 364.50 ± 271.30) U mL^{-1} was when using the ANN-GA model [141]. The pH stat fed batch culture was demonstrated for rhamnolipid production from *Psedudomonas aeruginosa*. The maximum yield was 4.40 g L^{-1} [164]. In continuous mode reactor (CSTR) operation, maintaining the constant volume is very difficult due to foam formation and the foam itself a product. However, the attempt has been done and described various strains using CSTR [124].

3.4 Bioprocess optimization: Statistical and ANN modeling based methodologies

A prime approach applied for obtaining increased yields in the fermentative production is by medium and process optimization. The most effective statistical methods used for bioprocess modeling and optimization for yield enhancements are single-variable at a time experiments [60, 83], Plackett-Burman Design [112, 123], Taguchi Experimental Design [126], Fractional Factorial Design [86, 127, 128], Response Surface Methodology [126–130]. Similarly, a new approach has also been made on computational tools, artificial neural network modeling and genetic algorithm [134–136].

3.4.1 Single variable at a time experiments

The critical components of the medium that influence the production processes are identified by the single-factor-at-a-time optimization strategy. The experiments (n-1), n-single variable, will be designed by leaving out one of the components present in the medium, keeping all other constant. Effect of different critical components and their ranges, which influence the production can be identified by these experiments. This experimental strategy was first adopted for the optimization of the surfactin production from *Bacillus subtilis* [60, 83, 112]. A similar kind of study was reported by several authors for the biosurfactant production [129–133].

3.4.2 Plackett-Burman design

The Plackett-Burman design is a well-established and widely used tool for screening the medium components [104]. Several nutrient components and trace elements are reported to affect the biosurfactant production. Two-level fractional factorial design and Plackett-Burman design, can screen up to *n*-variables with *n*+1 experiment, while the multifactor design will be difficult because more experiments are needed. The experimental designs help to screen the critical variables with a lesser number of experiments. This design was adopted to screen nearly 14 nutritional components and their effects on the biosurfactant production from *Bacillus licheniformis* were described previously [118]. Among the 14 nutritional components, the following 8 components $CaCl_2$, H_3PO_4, H_3BO_3, $CuSO_4$, $ZnSO_4$, $FeSO_4$, $CoCl_2$ and Na-EDTA, were found to be more important in biosurfactant production. Using Box-Behnken design the critical components and their concentration were found to be: H_3PO_4 (1.0 ml L^{-1}), $CaCl_2$ (0.27 mg L^{-1}), H_3BO_3 (0.25 mg L^{-1}), Na-EDTA (30.0 mg L^{-1}) [118]. On the other hand, eleven nutrients were screened for the biosurfactant production from marine *Bacillus sp*. and from these five components were found to be important for the production Glucose, NH_4NO_3, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, KH_2PO_4, $FeSO_4 \cdot 7H_2O$ [112]. Iturin A and surfactin, two variants were derived from *Bacillus subtilis* S499, ten active variables were optimized by two successive ways by this design (12 and 16 experiments design) [165].

3.4.3 Taguchi experimental design

Applying the Taguchi experimental design, a standard orthogonal array of L_9, L_{18}, L_{27} and L_{36} ($N^{(n+1)}$) is used to examine the experimental design. The analysis of the design is performed using a statistical method; the analysis of variance (ANOVA). The Taguchi experimental design is a positive option for the optimization of the biotechnological processes however, the model is very complex. The critical influence of trace elements such as Mg^{2+}, K^+, Mn^{2+} and Fe^{2+} on the surfactin production from *Bacillus subtilis* ATCC 21332 was explored by using this design. The critical parameters were found to be Mg^{2+} and K^+ which have been optimized for the enhanced surfactin yield of 3.34 g L^{-1} [116].

3.4.4 Fractional factorial design

Factorial design can be used for screening the media components and their significant factors. The interactions between the variables will be determined by *R*-n factors. Fractional designs are expressed using the notation I^{k-p}, where "I" is the number of levels of each investigated factor, "k" is the number of investigated factors, and "p" describes the size of the fraction of the full factorial. The use of this model in biosurfactant research is scarce. The optimization of biosurfactant production from probiotic bacteria [92] and the biosurfactant production from *Yarrowia lipolytica* have been described earlier [128]. The optimization of biosurfactant from *Yarrowia lipolytica* have been measured by an indirect way of defining the EI value (81.30 %) and the surface tension value (19.50 mN m^{-1}). The effects of aeration, agitation, and the carbon and nitrogen sources were also studied using this method [128].

3.4.5 Response surface methodology

Response surface methodology (RSM) consists of a group of empirical techniques that explores the relationship between the independent variables and output values. This method is employed with multiple regression analysis by using quantitative data obtained from the properly designed experiments to solve multivariate equations [137]. Thus the performance measure is called the response and the input variables are sometimes called independent variables. In terms of the coded variables the response function is given in equation (5)

$$Y = f(X_1, X_2, \ldots\ldots, X_k) \quad (5)$$

f, is the true response which will be a function of first or second order polynomial quadratic model. The second order polynomial function is widely used in the response surface methodology for several reasons like a very flexible method of least square which can be used for this purpose and it works well in solving response surface problems. The empirical equation of the second order polynomial function is given in equation 6.

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j \chi_j + \sum_{j=1}^{k} \beta_{jj} \chi_j^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} \chi_i \chi_j \quad (6)$$

RSM has been used to optimize the critical process variables for enhancing the biosurfactant production. The design matrix for the critical medium components such as glucose ($C_6H_{12}O_6$), ammonium nitrate (NH_4NO_3), manganese sulphate ($MnSO_4$) and iron sulphate ($FeSO_4$) were generated using a 2^4 full factorial central composite design and the optimization was performed using a Monte-Carlo algorithm for the enhanced biosurfactant production (CMC^{-1} 45.50 mol L^{-1}, indirect yield measurement of surfactin) from *Bacillus subtilis* [83]. Medium components such as waste free fatty acid as carbon source, sodium nitrate ($NaNO_3$), phosphate and $FeSO_4$ were considered as critical components, influencing the biosurfactant production. The design matrix for these components was generated using a 2^4 full factorial central composite design and a optimization was performed using Essential regression software for the enhanced rhamnolipid production (12.06 g dm^{-3}) from *Pseudomonas aeruginosa* AT10 [133]. Similarly, the critical medium components were optimized for the biosurfactant production from *Pseudomonas aeruginosa* S2 [133] and the lichenysin production from *Bacillus licheniformis* R2, the critical components of NH_4NO_3, glucose, Na_2HPO_4 and $MnSO_4 \cdot 4H_2O$ were optimized [129]. On other hand, the critical medium components of sucrose (g L^{-1}), ammonium chloride(g L^{-1}), ferrous sulphate (μM), Zinc sulphate (mM) were identified and optimized to enhance the yield of novel lipopeptide (1.712 g L^{-1}) from *Bacillus subtilis* MO-01[127].

The RSM technique was also applied to study the effect of two stages of inoculum; (i) inoculum age and (ii) inoculum size. These were optimized using a Monte-Carlo algorithm for the enhanced surfactin production from *Bacillus subtilis* DSM 3256 [138]. Briefly, the first stage of inoculum size was 5.50% v/v, a 56.0 h fermentation age followed by second stage of inoculum size of 9.50% (v/v) with 4.50 h of age, helped in the higher surfactin concentration of 1.30 g L^{-1} [138].

In our recent study, the first approach has been made to optimize the critical medium components of modified marine medium (MMM) for the higher yield achievement of 3.05 g L^{-1} lipopeptide from marine origin, *Bacillus circulans* MTCC 8281 [132]. The expansion of this model is also applied to study the effect of salinity, pH, and temperature on the surface active properties of biosurfactant. The second order factorial design was applied to generate the experimental matrix whereas a Box-Behnken algorithm was used to define the optimal conditions (T = 30 °C, salinity = 3.0%, pH > 5.0 for better emulsification properties) of the biosurfactant produced from *Lactobacillus pentous* [78].

These statistical tools have also been applied for the process optimization in fermenters to optimize the critical process parameters such as stirrer speed (RPM), aeration (LPM), temperature, pH, etc. Thus, the importance to optimize the process conditions in order to maximize the product yield at very low cost is high [19, 39, 60]. For example, the process variables such as temperature, pH, aeration and agitation have critically influenced the biosurfactant production in the reactor [60]. The process variables were optimized (aeration: 0.75 vvm; agitation: 140 rpm; pH = 6.75; T = 37.4 °C) using a multi stage Monte-Carlo algorithm for the enhanced surfactin (1.10 g L^1 with relative concentration of 53.0 CMC^{-1}) production from *Bacillus subtilis* DSM 3256 [60]. The main drawback of RSM is that the optimization is confined to the quadratic non-linear model whereas biological process consisting of many complex non-linear patterns.

Other modern computational modeling and optimization tools such as artificial neural network coupled with genetic algorithm (ANN-GA) can also serve as powerful aids for bioprocess optimization to augment biosurfactant production [133–136].

3.4.6 Artificial Neural Network modeling (ANN) and Genetic algorithm (GA) optimization

ANN, a mathematical and computational tool, is a collection of interconnecting the independent process variables (input) and dependent variables (output) without any prior knowledge of the relationship between them. In 1989, Goldenberg, introduced this genetic algorithm, a globalized optimization technique which searches the global optima value of a complex objective function obtained from ANN by reproduction of the biological process such as genetics, crossover and mutation [139]. The first report on ANN-GA based optimization tools was aimed to optimize the critical medium components for the enhanced lipopeptide production from *Bacillus subtilis* MO-01 [136]. Later, the same tool was adopted for the medium optimization for the biosurfactant production from *Rhodococcus erythropolis* MTCC 2794 [131]. On other hand, four media components (sucrose, yeast extract, meat peptone, toluene) were optimized and the yield of 7.20 g L^{-1} was achieved, which increased in 3.5 fold [133]. Recently this computational approach had been adopted first time for the marine medium optimization in order to improve lipopeptide biosurfactant production from marine isolate *Bacillus circulans* [135]. Glucose, Urea, $SrCl_2$ and $MgSO_4$ were optimized using ANN-GA which enhanced the biosurfactant yield upto (4.40 ± 0.5) g L^{-1}, which is a 70.0% enhancement in the yield [135]. Same approach was applied to optimize the process parameters (pH, temperature, aeration and agitation) in a 3.7 L bioengineering fermenter [116]. By optimizing the process parameters, the yield enhanced to (6.98 ± 0.14) g L^{-1} from (4.61 ± 0.07) g L^{-1}, an overall 52.0% achievement was shown [116]. The recent study shows an ANN modelling coupled with PSO (particle swarm optimization) algorithm served as tool for optimizing the process variables of pH = 6.7, T = 33.30 °C, aeration 128.0 L h^{-1} and agitation 458.0 rpm which achieved a lipopeptide yield of (6.58 ± 0.32) g L^{-1} from *Bacillus megaterium* using food waste [140].

4 Characterization of the Biosurfactants

4.1 General analysis

The preliminary qualification and characterization of the biosurfactants have been identified by using the basic process of surface tension (ST) measurement (tends to form the vesicles which lowers the ST), interfacial tension measurement (intramolecular attractive force happens within the molecules), determination of the critical micelle concentration (CMC^{-1}, lower the CMC level lead to monomer for-

mation), the critical micelle dilution (CMD^{-1}, dilution of biosurfactant in aqueous solution), the drop-collapse method (micro plate, oil coated based test, based on the shape detection) and emulsification index measurement (E_{24} = height of emulsion layer/height total liquid layer) [27–143]. The Lipopeptide quantification using the Bradford method have also been demonstrated. In the case the lipopeptide is not soluble in the Bradford reagent, it is advised to add an equal amount of 1.0 M NaOH for the solubility, which helps to detect and quantify the lipopeptide content [145]. Similarly, glycolipids are quantified by a color based method, by anthrone or ornical test. The intensity of the color is measured at the absorbance of 625 nm to quantify the glycolipids against the standard plot (generated using rhamnose or rhamnolipid). On the other hand, the biosurfactant/bioemulsifier contains protein-lipid complexes which are generally quantified by the folin phenol method, as described previously [146]. Recently, a new simple turbidometric method has been developed to quantify the crude biosurfactant in the concentration range of 1.0 to 10.0 g L^{-1} of the turbidity ranges [144]. The presence of the total fatty acid methyl ester content can be analyzed by GC-MS by using capillary column [148] and recently with ZB-WAX column, with the m/z range of 40–400 which also helps to define the quantification of biosurfactants [147].

4.2 Specific analysis

The identification pattern and chemical nature of the biosurfactants will be determined by high performance thin layer chromatography (HPTLC) [25, 26]. However, the concentration of the biosurfactants can be easily determined by this method with a lesser amount of sample [28]. Fourier transform infrared spectroscopy (FTIR) helps to reveal the chemical bonding nature of the biosurfactant and helps also in predicting the structure in correspondence with HPLC, MALTI-ToF and NMR data. The chemical bonding nature of the lipopeptides (fengycin and surfactin), derived from marine origin were determined by C-O, C=O (stretching vibration) (1 260 cm^{-1} and 1 900 cm^{-1}), aliphatic C-H group (1 390 cm^{-1}) which corresponds to the lipid portion and the presence of NH (1 590 cm^{-1}) bond represents the peptides [28]. The spectral analysis of the biosurfactant shows the presence of peptides by N-H (IR range: 1 500 cm^{-1}), stretching and O-H of carboxylic acid along with presence of CH_2 and CH_3 group of aliphatic chains [147]. The partial characterization of the new isoform of biosurfactant, glycopeptide or glycolipopeptide and its infrared analysis has been reported recently. The infra ranges were observed for chemical structures and are described by the wave number: OH and NH stretching at 3 200–3 600 cm^{-1}, C-H (stretching) group CH_2 and CH_3 at 2 900–2 950 cm^{-1}, C=O at 1 752 cm^{-1}, 1 675 cm^{-1}, N-H bending of protein at 1 520 cm^{-1}, C-H bending vibration of CH_3 and CH_2 group, CH (Scissor) at 1 400 cm^{-1}–1 460 cm^{-1}, OH deformation vibration/CN at 1 100 cm^{-1}–1 090 cm^{-1} and C-O sugar stretching at 1 000 cm^{-1}–1 300 cm^{-1} [48, 122].

High performance liquid chromatography (HPLC) is mainly used to analyze and separate the isoforms which are present in the crude mixture of biosurfactants. This is is not possible in any other analytical method [5, 26]. A competent method has been developed for the isoform analysis and separation in HPLC with a short retention time (60.0 min run method to 20.0 min method) as described previously [27]. The functional groups present in the molecules are determined by Fourier transform infrared spectroscopy (FTIR) and by mass spectral analysis using a Mass assisted laser desorption ionization time of flight (MALDI-ToF) [5, 7, 27, 36, 52–63, 140]. However, fast atom bombardment mass spectrometry (FBAB) also revealed the presence of $[M+H^+]$ and $[M+Na^+]$ quasi molecular ions in the mixture surfactin analogs derived from marine *Bacillus pumlimus* [69]. Similarly, three isoforms of the lipopeptides surfactin, baccilomycin D and fengycin produced from *Bacillus amyloliquefaciens* strain FZB42 were identified by MALDI-ToF-MS. The isoform of surfactin was defined by the chain length of C_{13}, C_{14} and C_{15} with the molecular mass ions of $[M+Na, K]^+$, baccilomycin D with the chain length of C_{14}, C_{15} and C_{16} having the ions of $[M+H, Na, K]^+$, however, C_{17} has the mass ions of $[M+Na, K]^+$. Similarly, fengycin was identified in the presence of amino acid Ala-6-C_{15}, Ala-6-C_{16}, Ala-6-C_{17}, Val-6-C_{16}, Val-6-C_{17} with the uniform mass ions of $[M+H, Na, K]^+$ [63].

On other hand, nine different homologous of surfactin and lichenysin were identified using the electrospray ionization mass spectrophotometry (ESI-MS) coupled with a thin layer chromatography [73]. Four variants of fengycin (Fraction A to D) with C_{16} and C_{17} chain length with Na^+,H^+, K^+ adducts in protonated form [27] and two variants of surfactin (Fraction E and F) were identified by HPLC combined with MALDI-ToF, the chain length of C_{14}, C_{15} and C_{18} with the molar mass of Na^+ and K^+ ions in protonated forms. Both the fraction of E and F were identified as new variants of surfactin family [162]. The detailed structure and biological applications of surfactin have been described previously [56, 75].

5 Purification

The production of biosurfactants has been enhanced by various optimization strategies, nevertheless, still the purification remains a major challenge due to high downstream processing cost (~ 60.0 %) and purity requirements [39, 47]. To address this factor, few attempts have been made to improve the purification strategy for biosurfactants. The purification of the molecules has been employed by different ways: Biosurfactants purification with lesser quantity has been achieved by using high performance liquid chromatography (HPLC) [26, 27, 149] and purity of these molecules have been identified by critical micelle concentration values (CMC) [27]. The purified lipopetide isoforms (CMC values for isoforms A: 10.0 mg L^{-1}; B: 12.0 mg L^{-1}, C: 13.0 mg L^{-1}; D: 13.0 mg L^{-1}) obtained with a HPLC method showed a purity which is greater than 85.0 % [26]. On the other hand, the rhamnolipid purification was performed using HPLC [147]. Similarly, the biosurfactant purification has also been achieved by thin layer chromatography (TLC) and is evaluated by CMC values [26, 28, 150]. Further the purification of the biosurfactant at a commercial level has been achieved by size exclusion chromatography using Sephadex G-50 matrix. [25, 151].

Ultrafiltration is the robust method for determination of the concentration and the purification of the biosurfactant which is evident from the previous reports [57, 149, 157]. Briefly, the surfactin concentration as well as the purification has been achieved with different cutoff membranes such as XM-50 [57], Biomax 10 kDa [149], PS 30 kDa, YM 30 kDa [150, 152] and cellulose membranes [154]. The standard surfactin, having a 98.0 % purity (Sigma, USA) showed the CMC value of 13.0 mg L^{-1} whereas the CMC value of surfactin obtained from *Bacillus subtilis* was 17.0 mg L^{-1}, a purity of 70 % was achieved using an ultrafiltration process [57]. A dual gradient elution strategy has been employed. The adsorption effect of lipopeptides on four different resins

followed by step wise elution of their families (iturin, fengycin and surfactin). The eluted molecules are analyzed and confirmed by HPLC [156]. Ca_2^+ conditioned mode of diafiltration studies have been performed and reported recently [157].

6 Immense Application Possibilities of Biosurfactants

6.1 Therapeutics activities and applications

Therapeutic activities of these molecule are described based on their chemical structure [15, 17–20, 163]. The description of the biological activities of the different biosurfactant has been shown in Table 3. Briefly, the biological activities of cyclic lipopeptides (CLPs) produced by *Pseudomonas aeruginosa* showed an antagonistic activity against two pathogenic fungi with the maximum inhibition of (2.30 ± 0.6) mm, and (1.70 ± 0.6) mm [109]. The biosurfactant from *Bacillus circulans* was purified by gel filtration chromatography which delivered better antimicrobial activities against five different Gram negative and three Gram positive pathogenic bacteria as well as three fungal strains. The disc diffusion test was performed for crude and gel purified biosurfactant however, the higher activity was observed around > 21.0 mm halo diameter against Gram negative bacteria [26]. Similarly, fengycin from *Bacillus circulans* was purified by a HPLC method, and showed strong antimicrobial activities against one Gram-positive and six Gram negative bacteria with the maximum halo diameter of (17.0 ± 0.2) mm [27]. Lipopeptides from *Bacillus subtilis* natto TK-1 also displayed antimicrobial activities. The maximum zone of inhibition was observed against bacteria (18.0 mm) and fungi (48.80 mm) [158]. The Gram positive and Gram negative pathogenic bacterias were collected from different sources of human bodies and waste; such as urine, nose wound, finger wound, vaginal secretion, hemoculture, orofaringe secretion, traqueal and abdominal secretion. These samples were tested for antimicrobial activity against lipopeptide biosurfactant produced from *Bacillus subtilis* R14. The maximum inhibition of (28.20 ± 0.1) mm was observed against Gram positive bacteria [159].

On the other hand, the excellent antimicrobial activity has been shown by rhamnolipid against three Gram positive and two Gram negative bacteria with the maximum inhibition of (30.0 ± 3.0) mm [157]. A biosurfactant from *Lactobacillus paracasei* ssp. paracasei A20 showed potential antimicrobial activities against Gram positive and negative bacteria and fungi, collectively of eighteen microorganisms. The minimum bactericidal concentration was observed in the range from (71.60 ± 1.5) mm to (100.0 ± 0.0) mm against all tested microorganisms [161]. Similarly, the antifungal properties of iturin A and surfactin have been discussed in previous reports [85]. Likewise, two isoforms (Frac-

Types of Biosurfactant	Source	Active properties and applications	Detection method	References
Surfactin	*Bacillus subtilis*	Fibrinolytic/blood clotting activity	Thrombin-fibrinogen system	67
Biosurfactant (ND)	*Lactobacillus paracasei ssp. paracasei A20*	Antibacterial and antiadhesive	Micro dilution method in culture plates and staining method	161
Rhamnolipid	*Pseudomonas aeruginosa MR01*	Antibacterial activity	Serial dilution and plating method	160
Lipopeptide	*Bacillus licheniformis* M104	Antimicrobial	Agar disc diffusion method	166
C_{14} and C_{15} Surfactin	*Bacillus amyloliquefaciens* MB199	Antifungal activity	Micro well plate method	167
Surfactin	*Bacillus subtilis 573*	Antitumor activity	Cell culture based assay	170
Surfactin	*Bacillus natto* KMD 2311	Antitumor activity	Cylinder plate method	180
Lipopeptide	*Bacillus subtilis O9*	Hemolytic activity	Dilution method	171
Lipoeptide	*Bacillus subtilis* ATCC 6633	Hemolytic activity	Hemolysis assay	179
Biosurfactant (ND)	*Candida lipolytica* UCP 0988	Antimicrobial and antiadhesive	96 well plate method	172
Biosurfactant (ND)	*Lactobacilli isolate*	Antimicrobial and Antiadhesive	ND	173
Lipopeptide	*Bacillus circulan*	Antiadhesive	Antiadhesion assay	174
Biosurfactant	*Bacillus circulans*	Bioavailability and biodegradation	Test tube method	11
Biosurfactant	Marine bacterium	Heavy metal remediation	Dilution method	13
Sphorolipids (SLs)	*Candida bombicolo*	Foaming and washing test; Cytotoxicity	Ross-Miles method and Association of washing chemistry foundation test. MTT method	175
Rhamnolipid	*Pseudoxanthomonas* sp. PNK-04	Degradation of aromatic compounds	UV analysis method	176
Rhamnolipid	*Pseudomonas aeruginosa* JBR425	Arsenic removal and heavy metals	Capillary electrophoresis column experiments	177
Mannosylerythiol lipids (MELs), glycolipid	*Novel isolate Pseudozyma* sp. NII 08165	Laundry and detergent additives	Fabric wash method	178

Table 3 Immense commercially attractive properties and application of various biosurfactants in different fields

tion E and F) which belong to the surfactin family displayed an antimicrobial activity. There is no activity found from both isoforms against one of the Gram positive bacteria of *Micrococcus* and no activity was found from isoform F, against one of the Gram negative bacteria, *Klebsiella aerogenus*. The higher activity was found to be (14.0 ± 0.6) mm from the fraction E against Gram positive bacteria [161]. The C_{14} and C_{15} surfactin showed a synergistic effect on antifungal properties against *Candida albicans* SC5314 with KTC at the MIC of 12.50 μg ml^{-1} (C_{14} surfactin) and 6.25 μg ml^{-1} (C_{15} surfactin) [167].

The trehalose lipid biosurfactant (TLB) from *Rhodococcus* sp. displayed a 100% hemolysis activity which was obtained at a TLB concentration of 40.0 μM, which is well below its CMC value. Further colloid-osmotic mechanisms defined upon the addition of TLB, K^+ release the hemoglobin in advance which causes the hemolysis of human erythrocytes by a colloid–osmotic mechanism [171]. However, the first non-hemolytic properties of a lipopeptide biosurfactant derived marine *Bacillus circulans* has been studied based on blood agar plate method [26]. The anti-adhesive property of the biosurfactants has been described in several reports [155, 158, 172–174]. The adhesion of the cells along with lipopeptide was detected at the least concentration of 0.10 g L^{-1} [172].

Biosurfactant molecules are reported for anticancer activities with the preliminary strong studies [158–163]. The effect of surfacin against LoVo cancer cell lines has been studied. LoVo cells, a human colon carcinoma cells proliferation was blocked strongly by surfactin which was examined by a MTT assay. The time and dose dependent study revealed the activity of surfactin by an IC_{50} value of 26.0 μM at 48 h incubation [89]. New cyclic lipopeptides (CLPs) from *Bacillus subtilis* natto T-2 displayed a dose dependent inhibition against human leukemia K562 cells. The experiment was performed through a MTT assay followed by fluorescent staining of nuclei of K562 to detect the inhibitory effect. The accumulation of the cells in G1 phase and also the number of apoptotic cells increased with respect to the concentration of CLPs (36.50% for control and 57.60% for 32.0 μg mL^{-1} of CLPs at 24 h) [168]. Similarly, rhamnolipids from *Pseudomonas aeroginosa* B189 showed anticancer activities against human breast cancer cell line, MCF-7. Two types of rhamnolipid probably identified as rhamnolipid A (L-rhamnopyranosyl-L-rhamnopyranosyl-b-hydroxydecanoyl-b-hydroxydecanoate or Rha-Rha C_{10}-C_{10} and rhamnolipid B (L-rhamnopyrano-syl-L-rhannopyranosyl-b-hydroxydecanoyl-b-hydroxydodecanoate or Rha-Rha C_{10}-C_{12}) showed potential antiproliferative activity: The MIC was found to 6.25 μg mL^{-1} for rhamnolipid A against human breast cell line MCF-7, whereas rhamnolipid B exhibited the MIC of 50.0 μg mL^{-1} against insect cell line C6/36 [169]. However, recently a report described the first attempt for anticancer studies of the marine lipopeptides surfactin and fengycin and showed the combined potential activities against the colon cancer cell lines HCT-15 and HT-29 with the IC_{50} value of 80.0 μg mL^{-1} and 120.0 μg mL^{-1} respectively [28].

Recent work described about the anti-tumour activity of surfactin produced by *Bacillus subtilis* 573 and a glycoprotein (BioEG) produced by *Lactobacillus paracasei* subsp. paracasei A20. Two biosrufactants were tested against three cell lines, among the three two were breast cancer cell lines (T47D and MDA-MB-231) and the third served as control cell line (MC-3 T3-E1), non-tumour fibroblast cell line. The first report described the BioEG activity against the cancer cell line and found to be more potent at the concentration of 0.15 g L^{-1}, and the decrease of the cancer cell viability without affecting the normal fibroblast [170].

6.2 Commercial application

Commercial applications of these molecules are described based on their physiochemical properties (Table 3).

Sophorolipids (SLs) derived from non-pathogenic yeast *Candida bombicola* was examined for the interfacial activities. The results were extremely low-foaming properties and high detergent activity in 100 ppm hardness of water. This study was performed using commercial detergents (black-copolymer nonionic surfactant and polyoxyethylene lauryl ether (AE)) and two lipopeptide biosurfactants (surfactin and arthrofactin) were used as detergent additives.

On the other hand, biodegradability of SLs along with other surfactants are also tested according to OECD guidelines. The result declared that SLs can be readily used as quick biodegradable agents [175]. Novel sophorolipids (SLs) displayed a lower cytotoxicity activity against human epidermal keratinocytes which helped to design the cosmetic products [175], skin inflammation can be ruled out by this property.

Rhamnolipid produced from *Pseudoxanthomonas* sp. PNK-04 showed a greater solubility and degradation effect on aromatic compounds such as 2-chlorobenzoic acid, 3-cholorobenzoic acid, 4-cholorobenzoic acid, pentachlorophenol, hexachloro benzene, 1-methyl naphthalene and 2-methyl naphthalene [176]. Similarly, a rhamnolipid derived from *Pseudomonas aeruginosa* JBR425 displayed excellent properties of arsenic contamination removal from the soil by column flushing method. The oxidized Pb-Zn mine tailing samples were collected from Bathurst, Canada and subjected to arsenic and heavy metal removal. The mobilization of arsene by rhamnolipid was enhanced by the presence of other metals, which convert the arsenic ions into an aqueous phase with the correlation coefficient range from 0.8915 to 0.9214 [177]. However, the biosurfactants also exhibit the properties of heavy metal remediation by metal sorption studies: 100 ppm of lead and cadmium could completely removed at 5-fold CMC concentration level. In-depth studies were carried out to reveal the properties using FTIR, atomic adsorption spectroscopy and transmission electron microscopy [13].

The same biosurfactants showed excellent biodegradation properties against a polyaromatic hydrocarbon (PHA), anthracene. The solubility of 0.20% (w/v) anthracene and its utilization by marine bacterium during the biosurfactant production was examined by gas chromatography, high performance thin layer chromatography and FTIR [11].

A combination of three mannosylerythritol lipids (MELs) along with few unknown glycolipids derived from a novel isolate of *Pseudozyma Sp.* NII08165, removed stains (goat blood, ketchup and chocolate sauce) efficiently and can be served as potential candidate for the laundry industry. Fabric wash analysis was performed at three different conditions, with commercial detergent (Surf excel), with glycolipid and with a combination of the glycolipid and the commercial detergent. Among all the three studies, surf excel + crude biosurfactant (glycolipid) cleared all the stains in higher percentages (goat stain: ~97.0%; ketchup: ~90.0%; chocolate sauce: ~85.0%) compared to individuals. This study revealed that this type of biosurfactant can be used in laundry industries as additives [178]. However, the theory for the antifoaming and defoaming has been described by the role of emulsion and pseudo emulsion film stability. On the other hand, the defoaming property has been studied by using ultrasonic waves [184]. These theory and studies helps to design a commercial product.

6.3 Nanotechnology

The application of these molecules started breeding in nanotechnology research. The basic structures of these molecules have the advantage of nanocomposites which helps in forming nanoparticles [55, 182–183]. The lipopeptides fengycin and surfactin derived from *Bacillus subtilis* are found to be non-toxic and highly efficient diffusing mediators for carbon nanotubes, they act as biocompatible agents [183].

7 Conclusion and Perspectives

Recently, lipopeptide gained attraction in medical research fields due to its high anticancer, antifungal and antibacterial activities such as therapeutic. Comparatively, rhamnolipid, which is known for biodegradation applications, also exhibited anticancer activities. Actually lots of new research dimensions and development is going in marine biosurfactants research. Few unique properties were found from the marine biosurfactants, such as non-hemolytic activities, anticancer activities, better physiochemical activities as compared to the terrestrial derived products. Initial kinetics and production process for these molecules have been explored in few research papers which set the benchmark for the production process. Although there are bottlenecks in scale-up of the production process, is also addressed with various efficient statistical and computational models. Enough information has been provided in this paper for exploring the kinetics and production of biosurfactant at a commercial level. Advanced analytical techniques are developed for quantification and qualification studies such as the simple turbiodometric method, and shorter methods in HPLC and HPTLC. Purifications of the biosurfactants on a commercial scale are done by gel filtration chromatography. Enough detail discussions are captured in this review article by sketching the information provided by assorted researchers which helps for the new researcher communities.

The emergence of biosurfactant molecules in nanotechnology attracts markedly the researchers' attention because these molecules can be used as biocompatible agents that may help in nano-tissue engineering.

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