Enhancing Biosurfactant Production Through Carbon Substrate Exploration

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Abstract— This review paper explores the diverse applications of biosurfactants, derived from microbial sources, within various industries due to their eco-friendly nature and distinctive properties. The study delves into optimizing biosurfactant production by utilizing different carbon sources. Evaluations were conducted on various substrates, including glucose, glycerol, and vegetable oils, to assess their effectiveness in biosurfactant production by different microorganisms. Findings unveiled significant variances in biosurfactant production and qualities based on the carbon source employed. Glycerol emerged as a promising substrate, exhibiting superior biosurfactant yield and favorable surface-active properties compared to other sources. Additionally, structural analyses confirmed distinctive functional groups in biosurfactants synthesized from different carbon sources. This paper offers valuable insights into optimizing biosurfactant production by leveraging diverse carbon sources, with potential applications spanning agriculture, pharmaceuticals, and bioremediation industries. Future research directions may involve scaling up production and exploring additional carbon substrates to further enhance biosurfactant yields and properties.

***Index Terms*— Biosurfactants, Carbon sources, Fermentation, Surface tension reduction, Emulsification index.**

1. Introduction

Biosurfactants, remarkable surface-active compounds produced by a diverse range of microorganisms including bacteria, fungi, and yeast, have garnered increasing attention in various industries since their discovery. Coined by Antara Products in 1950, the term "surfactants" encompasses a broad spectrum of compounds that exhibit surface activity, serving as vital agents in wetting, detergency, foaming, emulsification, and dispersion processes. These molecules possess both polar and nonpolar characteristics, enabling them to effectively reduce surface tension between immiscible liquids such as oil and water. Classification of biosurfactants is typically based on their microbial origin and chemical structure, encompassing low-mass surfactants like glycolipids, lipopeptides, and phospholipids, as well as high-mass surfactants comprising polymeric and particulate varieties.

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Among the diverse producers of biosurfactants, bacteria reign supreme, with their production influenced by a myriad of fermentation conditions and environmental factors [2].

Despite their immense potential across a wide array of industries including environmental remediation, agriculture, and biomedicine, the widespread utilization of biosurfactants is hindered by their relatively high production costs compared to synthetic alternatives. Nevertheless, ongoing research endeavors are diligently striving to refine production processes and explore innovative biotechnological interventions to fully harness the capabilities of biosurfactants. This comprehensive review aims to shed light on recent advancements and cutting-edge biotechnological strategies geared towards enhancing biosurfactant production efficiency and unlocking their promising prospects. Furthermore, the review delves into the pivotal role of biosurfactants in detergent formulations, with a specific emphasis on glycolipid-based variants such as rhamnolipids, renowned for their exceptional efficacy in diverse industrial applications ranging from oil remediation to enhanced oil recovery [1].

2. Classification Of Biosurfactant

Biosurfactant classification based on chemical structure and origin which contain different type and various bacterial strain which can produce biosurfactant described on table no.1.

The biosurfactant classified by the chemical structure and origin the table 1. Describe the different type of biosurfactant and the microorganism which produced. he different types of biosurfactants and the microorganisms that produce them is essential for harnessing their diverse applications in various industries, including environmental remediation, agriculture, pharmaceuticals, and biotechnology.

A. Glycolipids

Sophorolipids, trehalolipids, and rhamnolipids represent a class of sugars characterized by their composition of long-chain hydroxy aliphatic or aliphatic acids bonded via ester or ether linkages. These compounds intricately blend carbohydrates with long-chain aliphatic or hydroxyl fatty acids, rendering them among the most renowned biosurfactants known to science. Integral to the reduction of surface tension, these substances comprise lipid moieties and mono- or oligosaccharides, amalgamating to form low molecular weight

biosurfactants. Within this framework, lipid moieties encompass a diverse array of fatty acids or alcohols, delineating distinct categories such as rhamnolipids and sophorolipids, while saccharide components range from glucose to rhamnose. The extensive catalog of more than 250 glycolipids, as cataloged by [4]. underscores the breadth and depth of this class of compounds.

Table.1.
Types of biosurfactant with appropriate example [2,3,4].

Classification	Type	Description	Examples	Bacterial Strains
Chemical Structure	Glycolipids	Surfactants with a hydrophilic sugar moiety linked to a hydrophobic fatty acid chain.	Rhamnolipids, Sophorolipids	*Pseudomonas aeruginosa, Candida spp.*
	Lipopeptides	Biosurfactants combine a peptide chain with a lipid or fatty acid moiety.	Surfactin, Iturin, Fengycin	*Bacillus subtilis, Bacillus spp., Bacillus amyloliquefaciens*
	Phospholipids	Surfactants containing a phosphate group, commonly found in cell membranes.	Phosphatidylcholine, Phosphatidylethanolamine	*Escherichia coli Pseudomonas aeruginosa, Candida spp.*
	Polymeric Biosurfactants	Surfactants composed of repeating units of monomers, forming long-chain polymers.	Lipopolysaccharides (LPS), Lipoproteins	*Acinetobacter* spp.
	Fatty Acids	Simple fatty acids derived from microbial metabolism exhibiting surfactant properties.	Oleic acid, Palmitic acid	*Bacillus subtilis*
Origin	Saponins	Glycosides contain a hydrophobic aglycone moiety and one or more hydrophilic	Saponins	*Pseudomonas aeruginosa, Bacillus subtilis*

Additionally, Dembitsky delves into their biological functions and intricate structural compositions, shedding light on the multifaceted roles glycolipids play in various biological processes [4].

B. *Lipopeptides and lipoproteins*

Certain biosurfactants stand out due to their exceptional surface activity, drawing special attention from researchers and industries alike. Among these, surfactin, the primary lipopeptide biosurfactant synthesized by Bacillus subtilis, emerges as a notable example. Characterized by a unique seven amino acid ring structure connected by a fatty acid chain, surfactin possesses remarkable surface tension-reducing abilities. Even at a concentration as low as 0.005%, it demonstrates a significant reduction in surface tension, plummeting from 72.8 to 27.9 mN/m. Beyond its surface-active properties, surfactin showcases a broad spectrum of antimicrobial qualities, including hemolytic, antiviral, antifungal, antibacterial, and anti-mycoplasma effects, enhancing its utility across various industries such as agriculture and pharmaceuticals. Furthermore, cyclic lipopeptides encompass other notable substances within this category, including decapeptide antibiotics like gramicidin and lipopeptide antibiotics such as polymyxins, produced by bacterial strains like Bacillus brevis and Bacillus polymyxa. The intricate structures and multifaceted functionalities of these compounds continue to fuel ongoing research and exploration in the realms of biosurfactants and antimicrobial agents, as documented [5,6].

C. *Rhamnolipids*

Rhamnolipids, recognized as the predominant glycolipids synthesized by bacteria such as Pseudomonas aeruginosa, boast a distinctive composition comprising one or two β-hydroxy decanoic acid units linked to rhamnose molecules. Initially identified by Jarvis and Johnson in Pseudomonas aeruginosa back in 1949, these compounds hold pivotal roles as essential biosurfactants. Typically, rhamnolipids consist of one or two α-l-rhamnose units connected to one or two 3-hydroxy fatty acid moieties via an O-glycosidic bond. Their natural constitution encompasses a diverse array of congeners, characterized by variations in the length of their 3-hydroxy fatty acid chains, spanning from 8 to 16 carbon atoms. Notably, Burkholderia plantarii has been reported to produce a rare rhamnolipid featuring three hydroxyl fatty acid components. The significance of rhamnolipids is underscored by their remarkable diversity, with studies conducted by [7]. providing invaluable insights into their structural diversity and potential applications.

D. *Sophorolipids*

Glycolipids, primarily synthesized by yeasts like T. apícola and T. bombicola, embody a composition comprising a long-chain hydroxy fatty acid and a dimeric carbohydrate known as sophorose, interconnected by a glycosidic bond. As elucidated by [8]. sophorolipids typically represent a mixture of six or nine distinct hydrophobic sophorolipids and lactones, exhibiting surfactant properties instrumental in reducing interfacial and surface tension, as noted by Van Bogaert, I.N.A. in the same year. Despite their surfactant prowess, sophorolipids fall short as emulsifying agents, as highlighted in the research. The structural makeup of sophorolipids encompasses the disaccharide sophorose in two forms: lactonic and open acid, with an array of sophorolipid structures predominantly sourced from C. apicola and Candida bombicola. The hydrophobic component typically features a 17-hydroxyoleic acid glycosidically attached, as noted by Wang, H. in 2019, contributing to the multifaceted functionalities of sophorolipids in surfactant applications, including electrical linkage to the 4" position of sophorose and the presence of acetyl residues at the 6' and 6’ ‘positions, as detailed [9].

E. *Trehalolipids*

Complex molecules featuring a trehalose disaccharide linked at the sixth carbon in the benzene ring exhibit remarkable

structural variability dictated by the mycolic acid content, a characteristic found in bacteria such as Mycobacterium sp., Arthrobacter sp., and Rhodococcus erythropolis. Trehalose lipids manifest in intricate combinations, the composition of which undergoes fluctuations contingent upon the growth conditions and physiological state of the bacterial strain. Trehalose serves as the pivotal non-reducing disaccharide forming the foundation of glycolipids present in the cell walls of Mycobacteria and Corynebacteria, acting as the precursor to various trehalose lipid structures. Long-chained, α-branched 3-hydroxy fatty acids known as mycolic acids become acylated with trehalose lipids, predominantly observed in species like Mycobacterium, Arthrobacter, and Rhodococcus. These fatty acids, intricately associated with specific sites within trehalose, contribute significantly to the structural modifications observed in trehalolipids, thereby playing pivotal roles in the physiology and pathogenicity of bacteria. An exemplar of such significance is found in 6,6'-mycolyl trehalose, also termed as cord factor [11,12,13].

F. Lichenysin

Bacillus licheniformis exhibits an intriguing capability to produce biosurfactants characterized by remarkable synergy, which display sensitivity to variations in pH, salt concentrations, and temperature. A prime illustration of such biosurfactants is lichenysin. These compounds showcase physio-synthetic properties akin to surfactin, demonstrating structural and functional resemblance [13]. This remarkable similarity underscores the potential of lichenysin as a versatile biosurfactant, potentially offering comparable surface-active properties to surfactant in various applications.

G. Fatty acids, phospholipids and neutral lipids

Bacteria and yeast, especially when cultivated on n-alkanes, demonstrate a propensity to produce substantial quantities of neutral lipids, phospholipids, and fatty acids. An exemplary instance of this phenomenon is observed in Rhodococcus erythropolis, which synthesizes phosphatidylethanolamine, exhibiting a critical micelle concentration of 30 mg/litre and significantly reducing interfacial tension between water and hexadecane to levels below 1 mN/m. Furthermore, Acinetobacter species are known to generate 1-N phosphatidylethanolamine-rich vesicles crucial for forming optically transparent alkane micro-emulsions in water, offering potential applications in medicine. These microbial materials serve as invaluable sources of biosurfactants with versatile utility. Originating from the microbial oxidation of alkanes, fatty acids manifest in diverse forms, with C12–C14 chains demonstrating notable surface tension reduction capabilities. Corynomycol acids, alongside other complex fatty acids, emerge as potent surfactants in this context. Despite phospholipids typically being essential components of microbial membranes, certain strains, such as Aspergillus and Acinetobacter sp. HO1-N, secrete phospholipid vesicles extracellularly, augmenting the surfactant effects of these bacteria. Notably, the ubiquitous presence of common phospholipid phosphatidylethanolamine in bacterial membranes underscores the manifold functions of microbial lipids in surface tension reduction and their promising prospects in biotechnological applications, as elucidated by [14].

3. Raw material: low cost from renewable resources

Microbial cells typically produce surface-active substances in limited quantities, prompting efforts to enhance biosurfactant production yields. proposed various approaches to circumvent financial constraints hindering biosurfactant development. One such approach involves utilizing low-cost raw materials, including waste materials, industrial by-products, and agricultural residues, as alternative substrates for biosurfactant synthesis [20]. These agricultural feedstocks present attractive options due to their abundance in regions with moderate to tropical climates and wide availability. Examples of inexpensive raw materials conducive to biosurfactant production include curd whey, plant-derived oils, oil waste, starch-rich substrates, and various industrial effluents. The selection of cost-effective raw materials is crucial for ensuring the overall economic viability of commercial biosurfactant production. The ideal waste substrate for commercial production should exhibit high concentrations of lipids, nitrogen, and carbohydrates. However, the challenge lies in finding waste materials with the optimal ratio of these components, often necessitating a blend of different types of waste, thereby increasing energy input and production costs. [19]. delineate three methods for utilizing wastes or by-products as substrates for biosurfactant synthesis. Firstly, selecting waste materials rich in fats and carbohydrates; secondly, combining a waste rich in either fats or carbohydrates with complementary waste types; and thirdly, opting for waste high in carbohydrates and employing microorganisms to convert some carbohydrates into lipids for biosurfactant production. These strategies offer promising avenues for maximizing biosurfactant yields while minimizing environmental contamination.

4. Impact Of Different Carbon Source for Production Yield of Biosurfactant

In the process of biosurfactant production, a meticulously designed protocol was followed, involving several crucial steps to ensure optimal yield and quality. Initially, a 2% (v/v) seed culture with an OD600 of 2 was inoculated into a 500 ml broth with a slightly alkaline pH of 10, enriched with unconventional substrates such as M. indica, sugarcane bagasse, wheat straw, rice husk, potato peel powder, corn powder, soybean powder, Jatropha cake, and seaweed sap. This diverse array of substrates provides a rich source of nutrients and carbon for bacterial growth and biosurfactant synthesis. Following inoculation, the culture was incubated at 32°C on a rotary shaker at 120 rpm for 72 hours, allowing sufficient time for the bacteria to metabolize the substrates and produce biosurfactants. Subsequently, the culture broth underwent centrifugation to remove substrate particles, bacterial cells, and debris, leaving behind a clarified supernatant enriched with biosurfactants [14].

Table.2.
Biosurfactunt production using different carbon sources.

NO.	Media	Types of fermentation	Different carbon source	Yield(g/l)	Strain	citation
1	Horikoshi medium (HM) broth	Submerge	Glucose	2.75	*Klebsiella sp.*	[15]
			Sucrose	5.10		
			Fructose	2.62		
			Galactose	3.16		
			Maltose	2.19		
			Starch	10.1		
			Xylose	3.25		
2	Iron limited minimal medium	Fed batch	Hexade-cane	2.6	*P. aeruginosa*	[14]
			Paraffin			
			Kerosene			
			Can WFO	3.5		
			Soy WFO	3.6		
			Cor WFO	3.2		
			Can ORW	4.1		
			Soy ORW	4.0		
			Cor ORW	2.8		
			Molasses	2.7		
				2.9		
				1.2		
3	Mineral salt medium	Submerge	Heptane	0.1	*Pseudomonas fluorescens*	[13]
			Hexadecane	0.3		
			Paraffin oil	1.4		
			Lubricant oil	0.8		
			Linseed oil	1.2		
			Sunflower oil	0.9		
			Olive oil	0.8		
			Kerosene oil	1.0		
			Naphthalene	10		
			phenanthrene	0.4		
			glycerol	0.9		
			maltose	1.6		
4	Mineral salt medium	Fed batch	Glucose	1.16	*B. circulans*	[10]
			Sucrose	0.94		
			Starch	2.5		
			Glycerol	2.9		
			Sodium-gluconate	0.25		
			Yeast extract	0.12		
			Beef extract	0.11		
5	Mineral	Submerge	n-	9.8	*Pseudomonas*	[8]

To further purify the biosurfactant, the supernatant was acidified to pH 2.0 using 6M HCl and then refrigerated at 4°C for 12 hours to facilitate precipitation. After this step, the mixture underwent another round of centrifugation to separate the precipitated biosurfactant from the remaining liquid components. The biosurfactant was then isolated by adding 2-fold cold iso-propanol, which precipitated the biosurfactant from the solution. The precipitated biosurfactant was collected, dried, and subjected to yield comparison to assess the efficiency of the production process. Additionally, crude biosurfactant products underwent dialysis for 48 hours at 4°C to remove any residual impurities and further enhance purity [13].

In a separate production culture, a carefully formulated substrate broth was prepared using a mineral composition containing essential nutrients such as Na2HPO4, KH2PO4, MgSO4.7H2O, FeSO4.7H2O, NaCl, CaCl2, yeast extract, and trace elements. The pH of the medium was adjusted to 7.0 ± 0.2 to create an optimal environment for bacterial growth and biosurfactant production. Carbon sources including glycerol, D-glucose, and waste cooking oil were incorporated into the medium, providing the necessary energy and carbon for bacterial metabolism. The nitrogen source used was (NH4)2SO4, which also contributes to biomass and biosurfactant synthesis [15].

Following sterilization, the flasks containing the substrate broth were inoculated with Pseudomonas aeruginosa cells, a well-known producer of biosurfactants, and incubated for 7 days. Throughout the incubation period, rhamnolipid production was monitored at regular intervals of 24 hours to assess biosurfactant yield and characterize its properties. This meticulous approach ensures the reproducibility and reliability of biosurfactant production, paving the way for further exploration of their diverse industrial applications [17].

5. Conclusion

Based on available research, biosurfactant production from different carbon sources has shown varying outcomes. While some carbon sources like glucose and glycerol have been widely used and proven effective in biosurfactant production, others such as agricultural wastes and industrial by-products offer promising alternatives. However, the choice of carbon source significantly influences biosurfactant yield, composition, and properties, with each source presenting unique advantages and challenges. Further investigation is needed to optimise production processes and explore the potential of alternative carbon sources for sustainable biosurfactant production.

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