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Understanding Pathways, Substrates, and Media Optimization to Maximize Microbial Biosurfactant Production

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Abstract: ***The production of amphiphilic chemicals, known as biosurfactants, by different microorganisms is essential for lowering interfacial and surface tension. The pathways of biosurfactant synthesis are examined in this review, with a focus on the influence of carbon substrates on biosynthesis. It also covers the variety of substrates used in the production of biosurfactants, such as lignocellulosic wastes, agro-industrial wastes, oil processing byproducts, starch-rich wastes, industrial wastes derived from plants and animals, fish and chicken wastes, fruit and vegetable wastes, and wastes from fish and poultry operations. In addition, the optimization of media for the formation of biosurfactants is discussed, with particular attention to temperature, pH, concentration of salt, and sources of carbon and nitrogen. It is crucial to comprehend these routes and optimization techniques in order to address the economic and environmental issues related to waste management and increase the efficiency of biosurfactant production.***

Keywords: Biosurfactants; Pathways; Substrates; Production; Optimization.

I. INTRODUCTION

Biosurfactants are amphiphilic substances that are generated extracellularly or on living surfaces, primarily microbial cell surfaces. They have hydrophobic and hydrophilic moieties that lower surface tension and interfacial tension between individual molecules at the interface, respectively [1]. Microbial surfactants are a significant class of compounds that are produced by filamentous fungus, yeasts, and bacteria. These are amphiphilic compounds that accumulate at immiscible interfaces to reduce the interfacial tension and surface tension. These chemicals are also formed during the stationary phase of microbial development. They are categorised as glycolipids, lipopeptides, phospholipids, and polymeric or particulate molecules based on their chemical makeup. Long-chain fatty acids, hydroxyl fatty acids, or a-alkyl-b-hydroxyl fatty acids are the typical components of the hydrophobic moiety. In the case of the hydrophilic section, an alcohol, phosphate, carbohydrate, amino acid, or cyclic peptide may be present [2].A diverse range of microorganisms synthesise biosurfactants, a heterogeneous category of secondary metabolites having surface-active characteristics such as *Serratia rubidaea, Pseudomonas aeruginosa, Bacillus circulans, Bacillus subtilis, Starmerella bombicola, Aureobasidium thailandense, Candida lipolytica, Pleurotus djamoretc, Nocardiopsis lucentensis* [3,4,5,6,7].

II. PATHWAYS OF BIOSURFACTANTS PRODUCTION

Studies indicate that biosurfactant can be synthesized through four distinct pathways, each dependent on the availability and nature of carbon sources in microbial fermentation. These pathways are elucidated as follows:

1) *Synthesis of both carbohydrates and lipids:* In this pathway, precursor molecules for biosurfactant synthesis are derived from both carbohydrate and lipid sources. The microbial metabolism simultaneously activates pathways for carbohydrate and lipid synthesis, leading to the production of biosurfactants containing both components [8,9].
2) *Synthesis of carbohydrates with carbon source chain length-dependent lipid synthesis:* Here, carbohydrates serve as the primary carbon source, while lipid synthesis depends on the length of the carbon substrate's chain present in the medium. Short-chain carbon substrates might favor lipid synthesis over carbohydrate synthesis, leading to the production of biosurfactants enriched in lipids [10,11].
3) *Synthesis of lipids with substrate-dependent carbohydrate synthesis:* In this pathway, lipids are synthesized while carbohydrate synthesis is contingent upon the substrate utilized. The microbial metabolism primarily focuses on lipid synthesis, utilizing available substrates for lipid production while carbohydrate synthesis occurs depending on the specific substrate used [12].

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4) *Synthesis of both carbohydrates and lipids dependent on substrate:* This pathway involves the synthesis of both carbohydrates and lipids for biosurfactant production, with the synthesis of both components influenced by the characteristics of the substrate. The microbial metabolism adjusts its pathways to accommodate the available substrate, leading to the production of biosurfactants containing both carbohydrates and lipids [13, 10].

The choice of carbon source in microbial fermentation significantly impacts the production of biosurfactants by regulating precursor molecule availability and metabolic pathways. For instance, when simple carbohydrates are the primary carbon source, microbial metabolism prioritizes glycolysis to generate fatty acids, essential for lipid synthesis. Conversely, when hydrocarbons are utilized as carbon sources, microbial metabolism predominantly activates lipolytic routes and gluconeogenesis, facilitating the synthesis of fatty acids or sugars necessary for biosurfactant production [14,15,16].

In summary, the pathways of biosurfactant production are intricately linked to the metabolic processes of microbial organisms, which adapt their metabolic pathways according to the available carbon sources. This understanding underscores the importance of selecting appropriate carbon sources in microbial fermentation processes to optimize biosurfactant production [17,18,19].

III. SUBSTRATES INVOLVED IN BIOSURFACTANTS PRODUCTION

Many low-cost waste materials have been investigated as biosurfactant production substrates over the last ten years, resulting in an efficient cost-cutting approach together with the critical waste management. There is a huge potential for producing biosurfactants from a range of inexpensive, renewable industrial wastes. Food and agroindustry-related residues are prominent among them [20,21,22].

1) *Agro-industrial wastes:* Based on studies, there is a significant increase in the production of generation of agro industrial byproducts. In order to improve the sustainability of these agro-industrial operations, it is imperative to either decrease wastage or employ waste products and/or effluents in processes that might produce other valuable goods, such surfactants [23].

- *Oil processing waste & byproducts:* Vegetable oil processing results in a significant amount of waste, primarily composed of fats, oils, and other related substances. Potent pollutants, these leftovers have the potential to pollute water and soil. The limited degradability of the lipid molecules they contain may be the reason for their ibility to function as pollutants. However, research has demonstrated that using soybean oil refinery waste and olive oil mill effluents as a substrate, microbial species such as pseudomonas can synthesise rhamnolipids. Similar investigations have shown that *Candida sphaerica* may effectively create biosurfactants from groundnut oil refinery waste. Other micro-bial strains, such as *Bacillus subtilis, Starmerella bombicola, Trametes versicolor*, etc., have also been effectively used to produce biosurfactants from oil industry wastes [24,25,26].
- *Starch rich waste:* A significant volume of wastewater that is high in starch and husks from the extraction of starch from rice, wheat, cassava, potatoes, and other crops is produced. This wastewater can be utilised as a feedstock to make a variety of goods, including bio-surfactants. Potato substrate was tested as an unorthodox carbon source for surfactant synthesis using *Bacillus subtilis.* It was also utilised to turn cassava wastewater into bio-surfactants. Additionally, lipopeptides were produced by *Bacillus amyloliquefaciens* using rice straw and soybean flour as substrates [27, 28, 29].

2) *Industrial wastes from animal origin:* Large amounts of animal fat and tallow are available from the meat processing industries, and these substances are utilised to cook food. Using the yeast *C. bombicola*, researchers produced sophorolipids biosurfactants using animal fat. Growth was inadequate when fat was the only carbon source available; however, the best degree of growth was obtained when 10% glucose and 10% fat were combined, suggesting that the medium needed to have an additional carbon source [30, 31].

- *Fish waste:* Around 60% of the weight of the fish is made up of fish wastes, which include fish bones, fish skin, fish head, red flesh, and viscera that are produced during the processing of fish. Due to their high concentration of suspended particles, organic carbon, and nitrogen, those wastes have the potential to pollute the environment and even create a number of health issues. Proteins, polyunsaturated fats, and minerals, among other substances with a high organic content, can be used as nutrition and then eliminated at the same time. Their widespread use as fishmeal for animal feed has resulted in significant environmental degradation and minimal economic returns. Fish peptones isolated from fish wastes have been shown in earlier research to have the capacity to promote the proliferation of microorganisms. Compared to other waste materials, fewer attempts have been made to date to produce biosurfactants from fish wastes. Therefore, it is imperative to do more thorough research on the manufacture of biosurfactants using fish wastes as an environmentally benign substitute to fully utilise these fish wastes [32, 33, 34, 35, 36, 37].

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- *Chicken waste:* One significant environmental issue is keratinous waste from slaughterhouses, chicken processing, fur, and leather manufacturing. Proteolytic hydrolysis cannot break down keratin, which is the third most common polymer. Although the majority of research focuses on the production of keratinase enzymes, keratinolytic microorganisms can be utilised to create products with additional value. Studies indicate that by employing chicken tallow as a substrate, a new cationic biosurfactant was obtained from *Alcaligenesaquatilis* sp. The use of cationic biosurfactant was a novel strategy for the in situ bioremoval of Cr III from the soil. The effectiveness of removal was assessed, and the toxicity analysis was conducted on fenugreek, maize, and ridge gourd [38, 39, 40, 41, 42].

3) *Industrial wastes from plant origin: P. aeruginosa* (Strain GS3) was used by the researchers to produce rhamnolipid biosurfactant; the primary sources of carbon and nitrogen were molasses and corn-steep liquor. The combination of 7% (v/v) molasses and 0.5% (v/v) corn-steep liquor waste resulted in the highest biosurfactant production [69, 84]. Being a low-value byproduct of processing soybeans, soy molasses has a high potential sugar content that can be fermented, along with other growth factors that support microbial development, making it an affordable feedstock. According to research, soy molasses can be utilised in fermentation processes to create industrial compounds such poly-hydroxyalkanoates, lactic acid, butanol, sophorolipids, and biosurfactants. For the low-cost generation of sophorolipids biosurfactant by *C. bombicola*, researchers developed a soy molasses-based medium [43, 44, 45, 46].

- *Fruit & vegetable wastes:* Fruit peels and vegetable peels that are processed for their juices generate a large amount of garbage. Biosurfactant production can be facilitated by using waste materials such peels from apples, bananas, and oranges. According to studies, surface tension can be lowered by up to 17% by using cashew apple juice. One more waste product that could be used to produce biosurfactants is banana peels. *Pseudomonas aeruginosa* has been utilised to generate rhamnolipids from peels of carrot, lime, and orange varieties [47, 48, 49, 50, 51, 52].
- *Lignocellulosic wastes:* The potential of lignocellulose, a plentiful supply of organic carbon, in the synthesis of biosurfactants has been investigated. Using lignocellulosic substrates, strains of *Lactobacillus pentosus* and *Bacillus tequilensis* have been employed to generate biosurfactants. These substrates are reasonably priced and have a wide range of uses. However, pre-treatment procedures including drying, chemical/enzymatic hydrolysis, pre-hydrolysis, and particle size reduction drive up the cost of producing biosurfactants. *Cutaneotrichosporon mucoides, Lactobacillus paracasei, Starmerella bombicola*, and *C. bombicola* are among the microbe strains that consume lignocellulosic wastes [53, 54, 55, 56, 57]

IV. MEDIA OPTIMIZATION FOR BIOSURFACTANT PRODUCTION

Many investigations on media optimization, particularly for the most well known biosurfactant producers including *Pseudomonas*, *Bacillus*, and *Candida species* have been conducted within the past few years. The most widely researched factors for the generation of biosurfactants in shaking flasks and large-scale fermenters have been discovered to be the kind and quantity of carbon and nitrogen sources in the media, as well as the type and ratio of metal cations [58].

Meat extracts, yeast extracts, ammonium sulphate, ammonium nitrate, sodium nitrate, urea peptone, and malt extracts have all been used to make biosurfactants. Yeast extract is the most common source of nitrogen utilised in the synthesis of biosurfactants, but the amount used varies depending on the organism and growing medium. For the best surfactant synthesis, *P. aeruginosa* prefers nitrate, while *Arthrobacter paraffineus* prefers ammonium salts and urea as nitrogen sources [59, 60].

Since many factors can influence the growth and metabolism of microorganisms during fermentative synthesis, achieving the optimal biosurfactant yield can be challenging. The ideal combination of substrates for a certain culture medium has been the subject of numerous investigations aimed at facilitating intracellular diffusion and the production of desired compounds [61, 62, 63]. For optimal biosurfactant synthesis, the growing parameters of a selected strain of microbe must be specified. The amount of carbon and nitrogen available, the amount of lipophilic substrate, the availability of micronutrients, the size of the inoculum, temperature, pH, aeration, and agitation speed are all important variables. Even if most microorganisms that create biosurfactants do so under more restricted conditions, it's still necessary to investigate the growth phase that yields the best rate of production. Statistical methods can be used to optimise the chemical and physical parameters of the fermentation process. This allows one to study the effects of interactions between the various variables and determine the optimal culture conditions for the highest production of biosurfactants at the lowest possible cost [15, 56, 85, 94]. The parameters affecting media optimization in production are as follows:

1) *Carbon Sources:* The type of carbon sources affects & influences the synthesis of biosurfactants in terms of both quantity & quality. Studies indicate that diseal, crude oil, fructose, glucose, and sucrose are excellent sources of carbon substrate for the synthesis of biosurfactants [64].

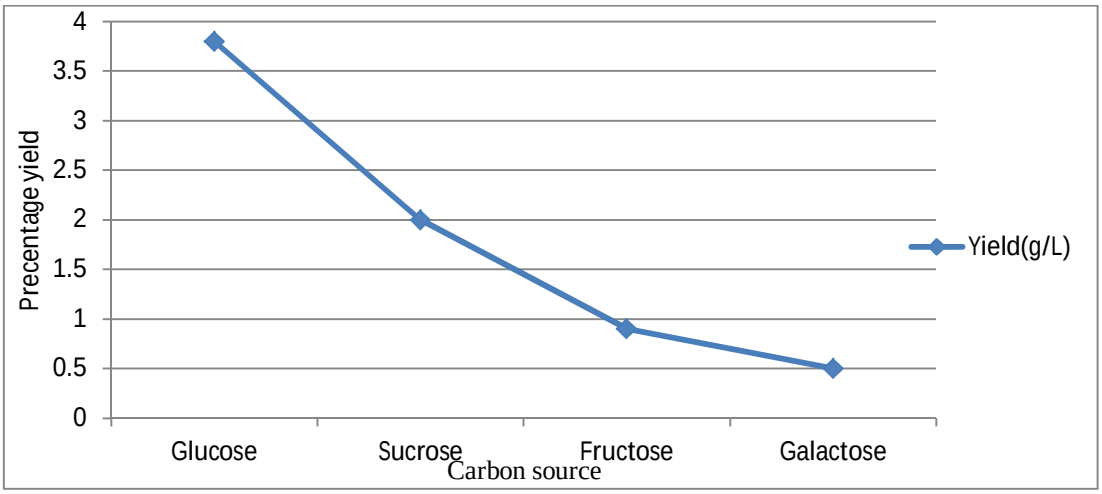


Figure 1: Yield percentage by different Carbon sources

In reviewing various research studies on biosurfactant production, it is evident that different carbon sources exhibit varying yields in biosurfactant production. Glucose consistently produces a yield of 4g/L, indicating its efficacy as a carbon source. Conversely, sucrose demonstrates a slightly lower yield of 2g/L, followed by fructose with 1g/L, and galactose with 0.5g/L. This discrepancy in yields highlights the importance of selecting an appropriate carbon source for biosurfactant production, considering factors such as cost, availability, and desired yield. Such findings underscore the need for further investigation into optimizing carbon source utilization to enhance biosurfactant yields and facilitate their industrial applications across diverse sectors. The compiled data presented in the graph serves as a valuable reference for researchers and industry professionals seeking to optimize biosurfactant production processes [65, 66, 67].

2) *Nitrogen sources:* Nitrogen is essential in the biosurfactant production medium since it is necessary for the synthesis of proteins and enzymes. Many nitrogen compounds, such as urea peptone, yeast extract, ammonium sulphate, ammonium nitrate, sodium nitrate, meat extract, and malt extracts, have been used in the production of biosurfactants. While yeast extract is the most widely used source of nitrogen for the synthesis of biosurfactants, the organism and the culture medium determine the optimal concentration for its use [68].

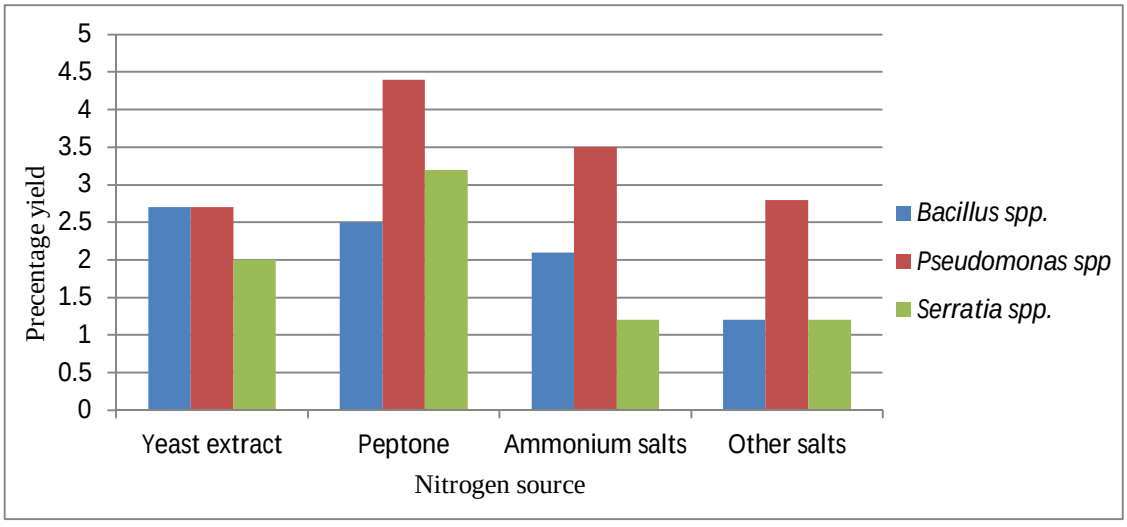


Figure 2: Yield percentage by different Nitrogen sources.

Various nitrogen sources have been investigated by different researchers to enhance the production of biosurfactants. Bacillus spp. have been found to yield 2.8g/L when utilizing yeast extract, while pseudomonas strains demonstrated varying yields with different nitrogen sources; ammonium salts yielded 3.2g/L, peptone yielded 4.3g/L, and other salts resulted in a yield of 2.9 g/L.

This data highlights the influence of nitrogen sources on biosurfactant production and provides valuable insights for optimizing production processes. A graphical representation of these findings would offer a clear comparison between the yields obtained from different nitrogen sources, aiding in the selection of the most effective substrate for biosurfactant production [69, 70, 71].

3) *Different pH:* Variation in pH have an impact on production of biosurfactant. According to different studies, pH 6.6 to 7 is ideal for the highest yield production of biosurfactant [72].

Researchers have explored various pH levels to optimize biosurfactant production, with significant variations observed in yields. At pH 3, a yield of 2.8 g/L was achieved, while increasing the pH to 4 resulted in a slight improvement to 3 g/L. The highest yields were obtained at pH 5, 6, and 7, all yielding 5 g/L, indicating an optimal pH range for biosurfactant production. However, at pH 8, the yield decreased to 4 g/L. These findings underscore the importance of pH control in biosurfactant production processes and highlight pH 5-7 as favorable conditions for maximizing yield. Graphical representation of these results would facilitate a clear comparison of the effects of pH on biosurfactant production, aiding in process optimization and industrial applications [72, 73, 74].

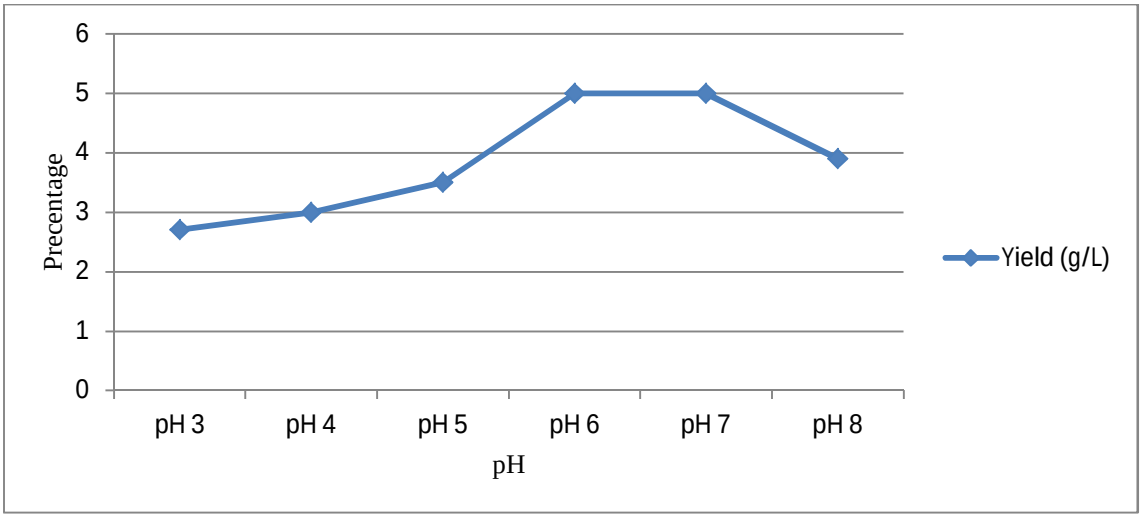


Figure 3: Yield percentage at different pH.

4) *Different temperature:* Both the quantity and quality of biosurfactant synthesis are influenced and affected by the variation in temperature. Studies suggest that the ideal temperature for efficient yield production of biosurfactant is between 40° C to 50° C [72].

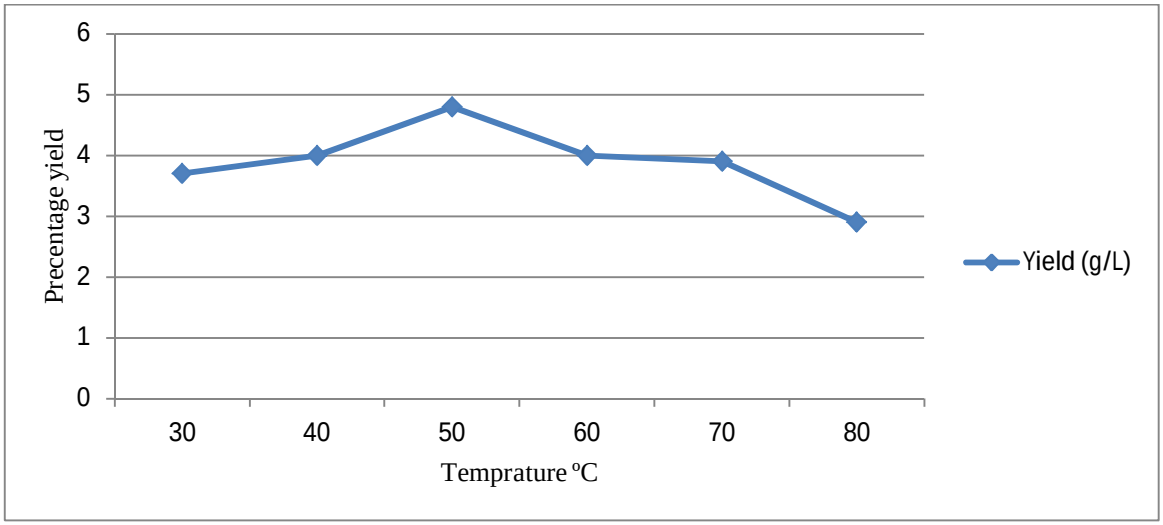


Figure 4: Yield percentage at different temperature.

Researchers have investigated various temperatures for biosurfactant production, revealing notable differences in yields. At 30°C, a yield of 3.8 g/L was achieved, while increasing the temperature to 40°C resulted in a slight improvement to 4 g/L.

The highest yield was obtained at 50°C, reaching 5.9 g/L, indicating an optimal temperature for biosurfactant production. However, yields decreased at higher temperatures, with 60°C and 70°C resulting in 4 g/L and 3.9 g/L, respectively. At 80°C, the yield decreased further to 2.9 g/L. These findings underscore the temperature sensitivity of biosurfactant production processes, with 50°C identified as the most favorable temperature for maximizing yield. A graphical representation of these results would facilitate a clear comparison of temperature effects on biosurfactant production, aiding in process optimization and industrial applications [72, 76, 77, 78].

5) *Salt Concentration:* Biosurfactant can be stable and produced at wide salinity concentration. An appropriate yield of biosurfactant is also dependent on the salinity of the production medium. According to numerous studies, 2% NaCl was optimum for sufficient yield production [79 , 80].

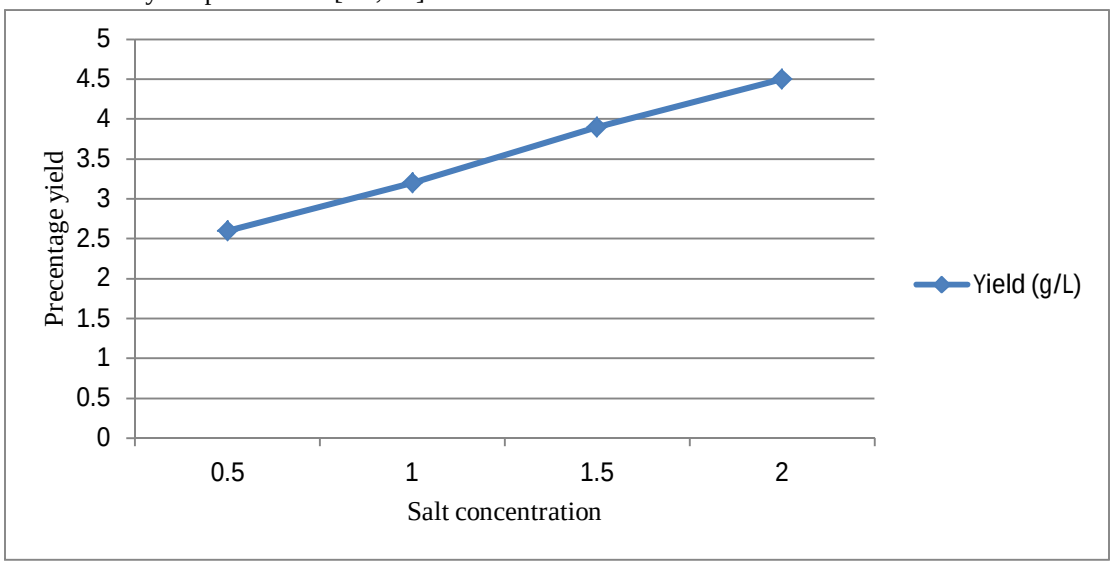


Figure 5: Yield percentage at different Salt concentration.

Various salt concentrations have been explored by researchers to optimize biosurfactant production, revealing distinct effects on yields. At a salt concentration of 2.6%, a yield of 4 g/L was achieved, indicating a positive correlation between higher salt concentrations and increased biosurfactant production. However, lowering the salt concentration to 1% resulted in a decrease in yield to 3.2 g/L. Interestingly, at 1.5% salt concentration, the yield increased to 3.9 g/L, suggesting an optimal range for salt concentration. Further increasing the salt concentration to 2% resulted in a higher yield of 4.5 g/L. These findings highlight the importance of salt concentration in biosurfactant production processes and emphasize the need for optimization within a specific range for maximum yield. Graphical representation of these results would provide a visual comparison of the effects of salt concentration on biosurfactant production, aiding in the selection of optimal conditions for industrial applications [81, 82 , 83].

V. CONCLUSION

In conclusion, the production of biosurfactants offers a promising avenue for sustainable surfactant solutions, leveraging renewable resources and diverse microorganisms. Through the examination of biosurfactant synthesis pathways and the influence of carbon substrates, it becomes evident that microbial metabolism adapts to available carbon sources, impacting biosurfactant yields and characteristics.

Furthermore, the utilization of various substrates for biosurfactant production, including agro-industrial wastes, oil processing byproducts, starch-rich wastes, industrial wastes from both plant and animal origins, fish and chicken wastes, fruit and vegetable wastes, and lignocellulosic wastes, highlights the potential for waste valorization and efficient waste management practices.

Optimization of media parameters such as temperature, pH, salt concentration, and carbon and nitrogen sources is crucial for enhancing biosurfactant production efficiency. Understanding the interplay between these factors enables researchers to tailor fermentation processes for optimal biosurfactant yields.

While challenges such as production costs, scalability, and consistency persist, ongoing research and technological advancements hold promise for addressing these issues and meeting the increasing demand for eco-friendly surfactant solutions.

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By continuing to explore diverse microbial strains and substrate sources, as well as refining media optimization techniques, the field of biosurfactant production can contribute significantly to sustainable waste management practices and environmental preservation efforts.

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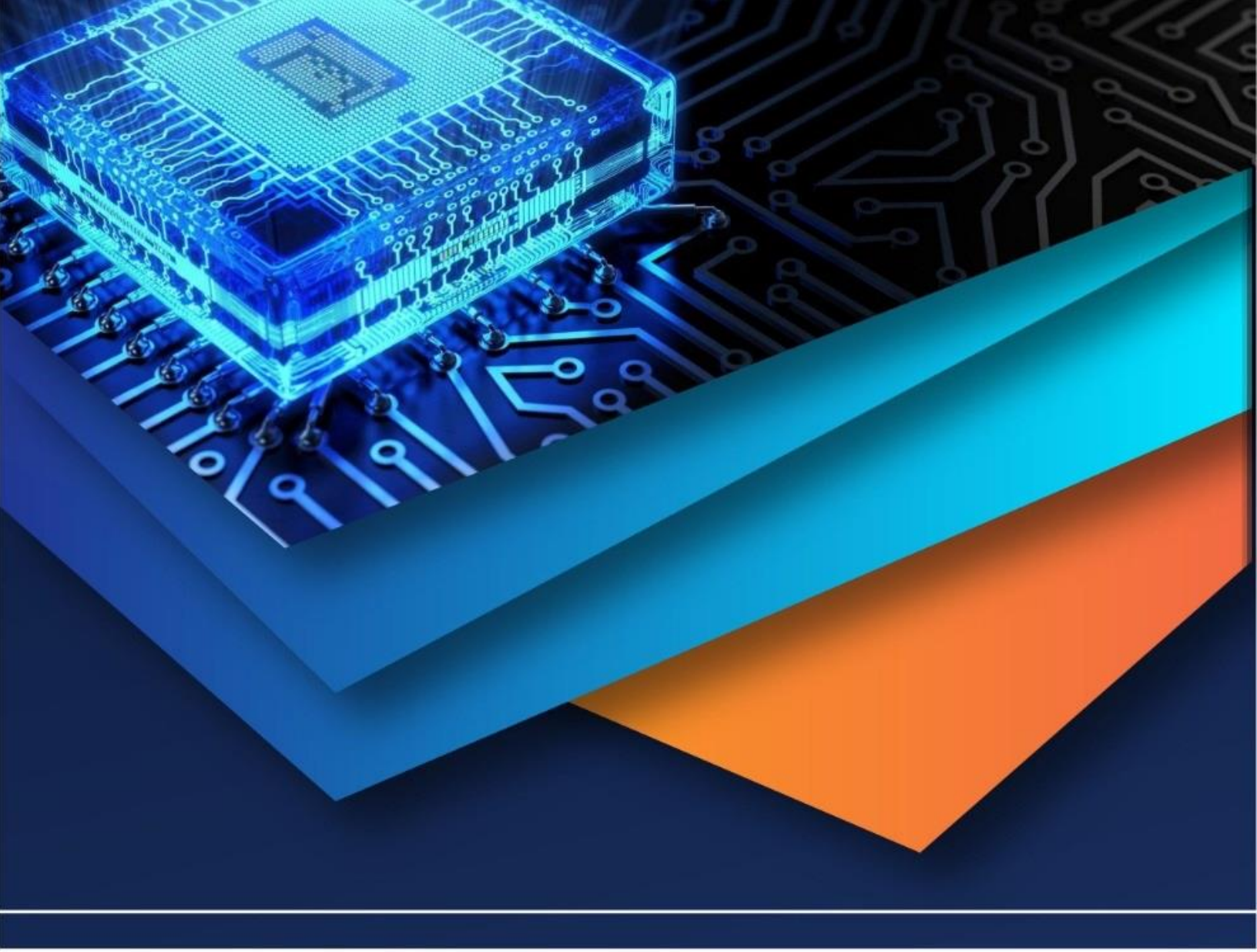
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