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Optimization of Growth Parameters for Enhancing the Production of Biosurfactants from *Pediococcus pentosaceus* S-2

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AS and NB designed the study and supervised the experiments. Author TK conducted the study, managed the literature and wrote the first manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Numerous Lactic acid bacteria (LAB) have been found to be capable of synthesizing surface-active compounds *i.e* biosurfactants. These are amphiphilic compounds produced by microorganisms on their cell surface or secreted extracellularly that have a tendency to reduce surface and interfacial tension. In the present study, different process parameters including nitrogen and carbon source, pH, temperature, aeration and agitation were optimized to maximize the production of biosurfactants from *Pediococcus pentosaceus* S-2. Xylose (1.5%) and yeast extract (1.5%) act as better carbon and nitrogen sources respectively for the production of biosurfactants. Maximum biosurfactant yield was observed at pH 6, a temperature of 35^{o} C, an agitation rate of 200 rpm and with inoculum size of 3%. The high yield of biosurfactants produced from *Pediococcus pentosaceus* S-2 by utilizing media supplemented with whey under optimized conditions.

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1. INTRODUCTION

The lactic acid bacteria (LAB) are gram-positive, non-spore-forming, cocci or rods shaped bacteria that produce lactic acid as the result of the fermentation of carbohydrates. Besides lactic acid, many LAB strains produce a significant amount of nonspecific low-molecular-weight compounds such as organic acids, hydrogen peroxide, bacteriocins, biosurfactants, diacetyl, reuterin, *etc* [1,2]. LAB has numerous applications in different industries including medicine and food industries. They are usually regarded as safe microorganisms (GRAS) according to the US Food and Drug Administration. These have received a scientific interest owing to their numerous health benefits and non-pathogenicity. Many strains of LAB have been found to be capable of synthesizing surface-active compounds i.e. biosurfactants [3]. Many LAB strains including *Enterococcus sp*, *Pediococcus sp, Lactobacillus, Bifidobacterium strains, Streptococcus thermophiles*, *Leuconostoc mesenteroides,* etc have a tendency to produce different types of biosurfactants [4,5].

Biosurfactants are amphiphilic compounds produced either on their cell surface or secreted extracellularly. These have the ability to reduce surface and interfacial tension. According to their chemical composition and microbial origin, biosurfactants can be classified into: glycolipids, lipopeptides or lipoprotein, phospholipids and fatty acids, polysaccharide-lipid complexes, polymeric surfactants and particulate type [6]. They are promising alternatives to chemical surfactants for their eco-friendly, biocompatibility, biodegradable, and non-toxic nature. Biosurfactants have enormous applications in the food, oil, biodegradation, cosmetic, agricultural, pesticide, and medicine/pharmaceutical sectors. Due to their suitable emulsifying and antiadhesive properties, biosurfactants produced by lactic acid bacteria attract interest in the food sector. Biosurfactants derived from LAB have the ability to reduce bacterial adhesion, biofilm removal and infections in a variety of clinical settings [7,8].

Therefore, biosurfactants derived from LAB act as potent candidates against some pathogenic microorganisms and inhibit pathogenic bacteria and fungi [9].

Pediococcus pentosaceus, a probiotic strain is a cocci-shaped, gram-positive, non-motile, and facultative anaerobic LAB [10]. Many strains of *Pediococcus* were shown to lack antibiotic-resistance genes [11]. Lactic acid bacteria (LAB) are important for the food industry as they are commonly used for the fermentation of food, beverages and dairy food products. *P. pentosaceus* not only enhances the flavor and preservation of food agents but also aids in the inhibition of many pathogenic bacteria. Bacteriocins as well as biosurfactants derived from *P. pentosaceus* have applications in both the food industry and intestinal health [12,13].

The aim of this study was to optimize various growth parameters for enhancing the production of biosurfactants from *Pediococcocus pentosaceus* S-2 isolated from Indian wheat-based fermented food, Seera which was reported in our previous study [14].

2. MATERIALS AND METHODS

Different culture parameters including carbon and nitrogen sources, temperature, pH, aeration, agitation, incubation time, size of inoculum and organic substrate were optimized for the maximum production of biosurfactants [15,16]. The microbial culture was diluted for inoculation to match with 1 Mac Farland standard (3.0×10^8 CFU). The emulsification index (E24) was calculated as follows.

2.1 Emulsification Index

Two ml of cell-free supernatant (CFS) and two ml of soyabean oil were added to a test tube to assess this test. The Emulsification index (E24) was calculated using the following equation after the mixture was vortexed for two minutes at 2000 rpm and then kept undisturbed at room temperature for 24 hours [17].

$$\text{Emulsification index} = \frac{\text{Height of the emulsified layer}}{\text{Total height of the solution}} \times 100$$

Emulsification activity is proportional to the Emulsification index.

2.2 Optimization of Carbon Sources

100 ml of modified MRS medium supplemented with filter sterilized carbon source (fructose, lactose, inositol, D-mannitol, xylose, sucrose,

dextrose and raffinose) at different concentrations of (0.5, 1, 1.5) % were inoculated with 1 ml of 24 hrs grown culture, followed by incubation at 37°C at 120 rpm. The biosurfactants production was examined after regular intervals of time and the emulsification index (E24) was examined.

2.3 Optimization of Nitrogen Sources

The selected isolates were added to the pre-sterilized modified MRS medium with optimized carbon source supplemented with different concentrations of nitrogen source (gelatin, urea, HM peptone, yeast, beef extract, tryptone, ammonium nitrate and sodium nitrate) in (0.5, 1,1.5) % separately and incubated at 37°C at 120 rpm in order to determine the necessary nitrogen source for maximum production of biosurfactants. After incubation, biosurfactants yield was determined after regular intervals and E24 was assessed.

2.4 Effect of pH

100 ml of modified MRS broth with optimized carbon and nitrogen source was prepared by adjusting pH with a different value (4.0 to 10.0) with 1N NaOH and 1N HCL and was sterilized. One ml of 24hrs grown culture was inoculated and incubated at 37°C with an agitation rate of 120 rpm. The biosurfactants production was determined after regular intervals and E24 was determined.

2.5 Effect of Temperature

Different flasks containing 100 mL of modified MRS broth with optimized pH and carbon and nitrogen sources were prepared and inoculated with selected strain followed by incubation at different temperatures (25-45°C). The biosurfactants production and emulsification index (E24) was determined.

2.6 Effect of Agitation

100 mL of modified MRS broth with optimized temperature, pH, carbon and nitrogen source were prepared, sterilized and inoculated with selected isolates. The growth of the selected strain was determined at different agitation rates (100, 150, 200, 250) and at static conditions. Samples were taken out after regular intervals of time for estimating the production of biosurfactants and the emulsification index was determined after 24 hrs.

2.7 Effect of Different Organic Substrate

100 ml of optimized MRS broth was taken 250 ml Erlenmeyer flask and 1% of different organic substrates were added separately in different flasks and sterilized by autoclaving. The selected strain was inoculated in the medium and incubated at optimized conditions. The biosurfactants production and emulsification index (E24) was determined.

2.8 Effect of Inoculum Size

Different sizes of inoculum (1-5%) were added in the flask containing 100 ml of optimized medium separately and incubated under optimized conditions.

3. RESULTS AND DISCUSSION

3.1 Optimization of Media

Media composition has a significant impact on the enhancement of micro-organism growth as well as on the production of metabolites. In order to increase the yield of biosurfactants from selected strains, optimization of different parameters like different carbon and nitrogen sources, temperature, pH, agitation and inoculum size are required.

3.2 Effect of Carbon Sources

The nature of the carbon substrate has a significant impact on the quality and yield of biosurfactants. Among all the carbon sources (fructose, lactose, inositol, D-mannitol, xylose, sucrose, dextrose and raffinose), it was found that xylose at 1.5% concentration produces higher biosurfactant yield i.e 1.76 g/L from *Pediococcus pentosaceus* S-2 when compared to other carbon sources. Hence, xylose was selected as the optimal carbon source for the production of biosurfactants by *Pediococcus pentosaceus* S-2.

There are very limited studies available that reported the utilization of different sugar as carbon sources for the production of biosurfactants by LAB. A study conducted by Ghasemi *et al.,* reported the utilization of MRS-Lac, date syrup and molasses act as a carbon source for enhanced production of biosurfactants derived from *Pediococcus dextrinicus* SHU1593 [18].

3.3 Optimization of Nitrogen Sources

Different nitrogen sources i.e both organic and inorganic have an impact on the growth of micro-organisms. Various nitrogen sources such as gelatin, urea, HM peptone, yeast, beef extract, tryptone, ammonium nitrate and sodium nitrate at different concentrations (0.5, 1, 1.5%) were studied for their effects on the growth of selected strains.

The production of proteins, enzymes, other metabolites and microbial growth depends on the nitrogen source present in the culture medium. In the case of *Pediococcus pentosaceus* S-2, yeast extract at a concentration of (1.5%) acts as a better nitrogen source with a biosurfactants yield of 1.82 g/L. Similar research was conducted by Agarry *et al.,* reporting that yeast extract had a high emulsification index of 60.8% and produced 2.56 g/L of biosurfactants from *Bacillus sp* [19].

3.4 Effect of pH

The strain was inoculated in optimized MRS media with different pH ranges (4-10) in order to determine the impact of pH on the biosurfactants production by *Pediococcus pentosaceus* S-2.

pH is a significant factor that influences the chemical reactions of living cells, growth and production of metabolites. Thus, a variety of pH values were assessed with the goal of choosing the optimum pH for the production of biosurfactants by selected strain. In the present study, *Pediococcus pentosaceus* S-2 was able to grow at different pH ranges and the maximum biosurfactants yield of 1.86 g/L was achieved at pH 6 with an emulsification index (E24%) of 52.7%. A study by Pato *et al.* reported pH 6.3 as the optimum pH for the growth of *Pediococcus pentosaceus.* The biosurfactants yield increased with an increase in biomass production which was maximum at optimum pH and our result was quite similar to this study [20].

3.5 Effect of Temperature

In order to determine the impact of temperature on the biosurfactants production, the strain was inoculated in optimized MRS media and incubate at different temperature (20-45^{o}C).

The strain *Pediococcus pentosaceus* S-2 was able to grow at different temperatures (20-45^{o} C). At a temperature of 35^{o} C, maximum biosurfactant yield *i.e* 1.86 g/L and emulsification index (E24%) of 64% was observed. Hence 35^{o} C was selected as the optimum temperature. Temperature influences a variety of microbial activities as well as on the yield of biosurfactants. A study conducted by Millán *et al.,* (2019) reported that higher biomass of *P. pentosaceus* was achieved at 37^{o} C which was quite similar to our study. The biosurfactants yield increased with an increase in biomass production which was maximum at optimum temperature and our result was consistent with this study [21].

3.6 Effect of Agitation

Optimized production media was inoculated with 1 ml of selected strain culture and incubated for 72 hrs at different rpm (100, 150, 200, 250 and under static conditions).

The agitation has a significant role in the transfer of oxygen from the gas phase to the aqueous phase which impacts on the synthesis of biosurfactants. In the present study, *Pediococcus pentosaceus* S-2, the higher biosurfactants yield was found to be 1.97 g/L at 200 rpm with an emulsification index (E24) of 66%. With the increase in rpm beyond that biosurfactant yield decreased. Our result was consistent with the study reported by Yaraguppi *et al.,*(2020) that the highest production of biosurfactants by *Bacillus aryabhattai* strain 4.3 g/l with E24 of 80% was achieved at 200 rpm [22]. According to Sen (1997) with an increase in agitation rate from 50 to 200 rpm, the growth rate enhanced from 0.2 to 0.72/hr and maximum biosurfactants yield was achieved [23].

3.7 Effect of Different Organic Substrate

Different organic substrates haves the influence on the production of biosurfactants.

The influence of different organic substances, both miscible and immiscible substrates including crude oil, mustard oil, pulse water, whey, rice water, soyabean oil and cheese whey were evaluated for the enhanced production of biosurfactants by *Pediococcus pentosaceus* S-2. In the present study, the yield of biosurfactants derived from *Pediococcus pentosaceus S*-2 was maximum i.e 1.99 g/L in medium supplemented with whey followed by pulse water i.e 1.71 g/L. A similar study conducted by Gudia *et al.,* reported that cheese whey was utilized by *Lactobacillus agilis* CCUG31450 for the production of biosurfactants [24]. Rodrigues *et al.,* (2006) reported that lactic acid bacteria utilized media supplemented with cheese whey for a high yield of biosurfactants production [25]. Our result was consistent with this study.

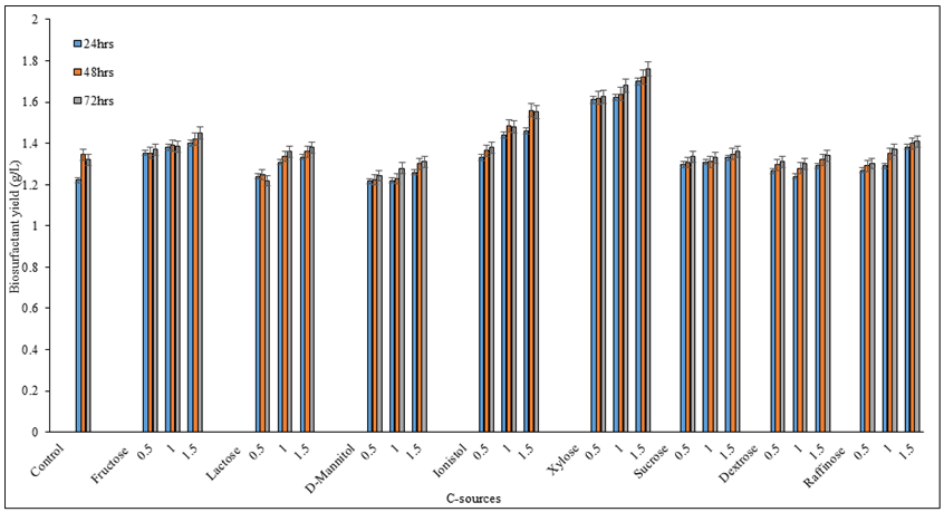

Fig. 1. Effect of carbon sources on the yield of biosurfactants derived from *Pediococcus pentosaceus* S-2

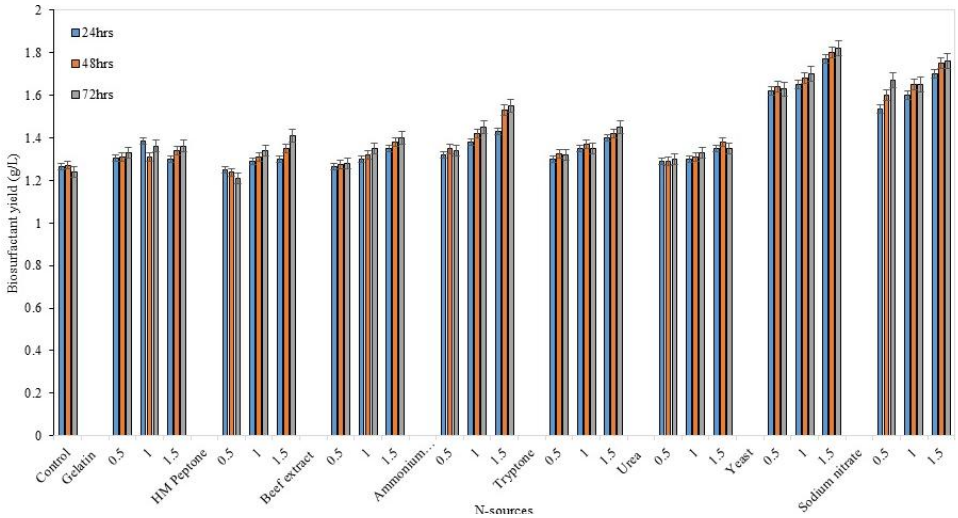

Fig. 2. Effect of nitrogen sources on the yield of biosurfactants derived from *Pediococcus pentosaceus* S-2

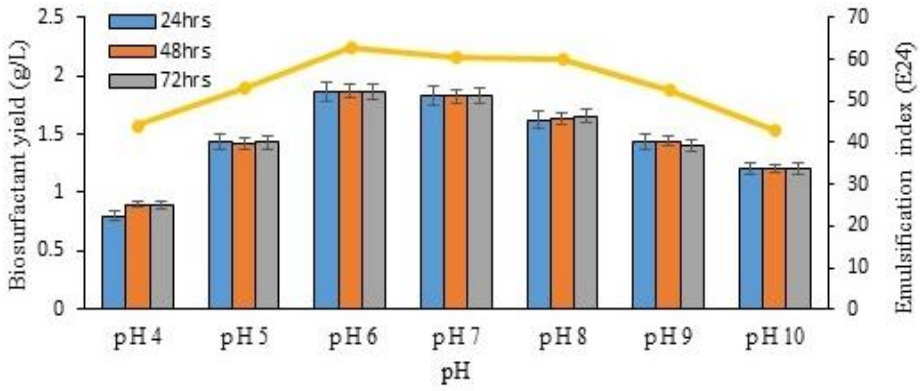

Fig. 3. Optimization of pH for *Pediococcus pentosaceus* S-2

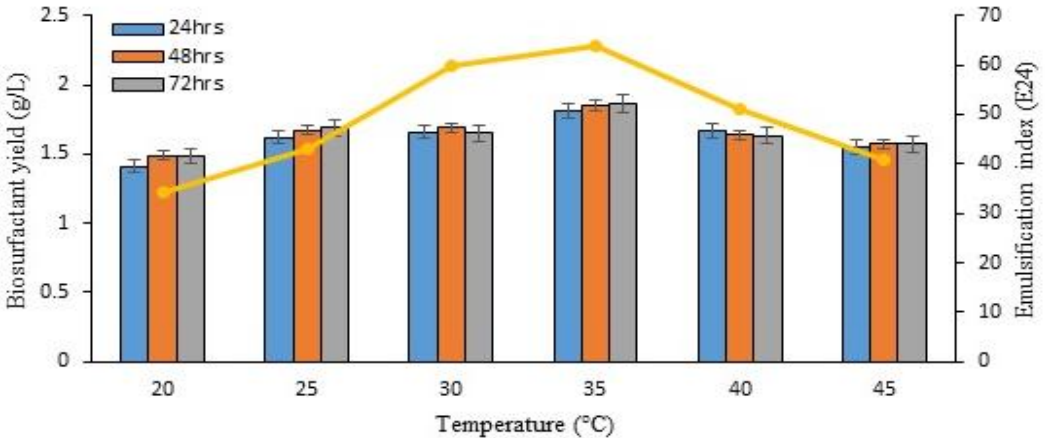

Fig. 4. Temperature optimization for *Pediococcus pentosaceus* S-2

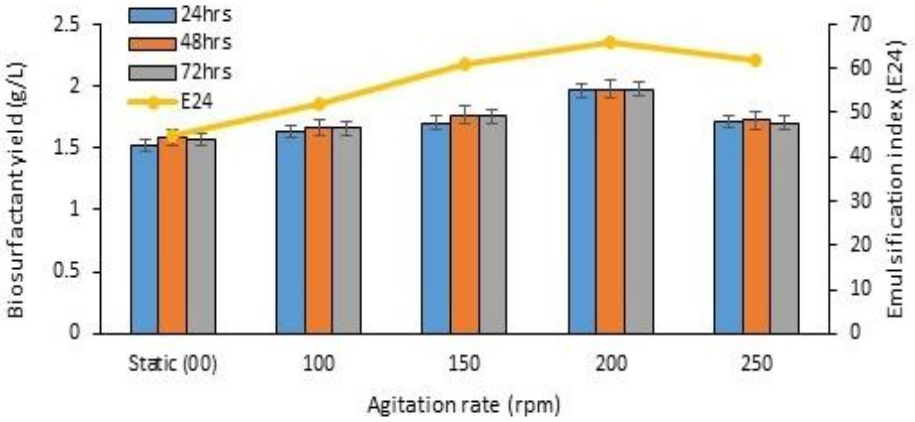

Fig. 5. Effect of different agitation rates on biosurfactants production from *Pediococcus pentosaceus* S-2

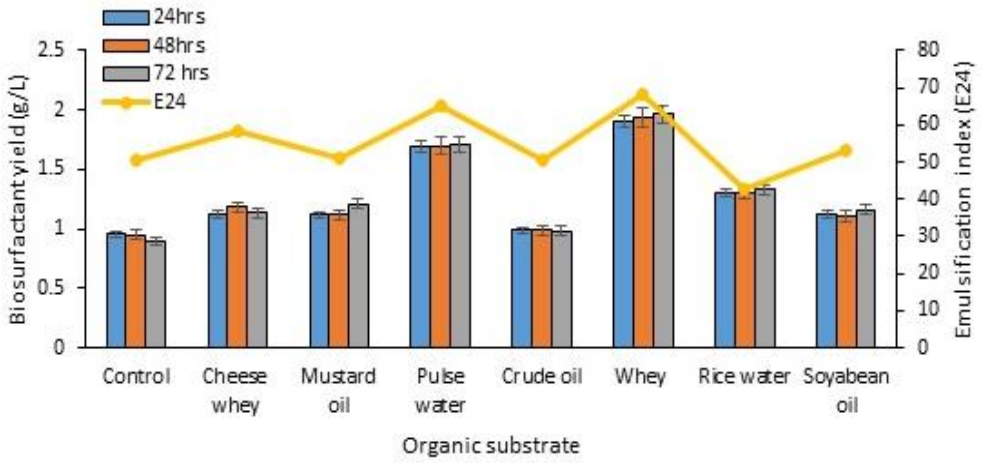

Fig. 6. Effect of different substrates on biosurfactant production from *Pediococcus pentosaceus* S-2

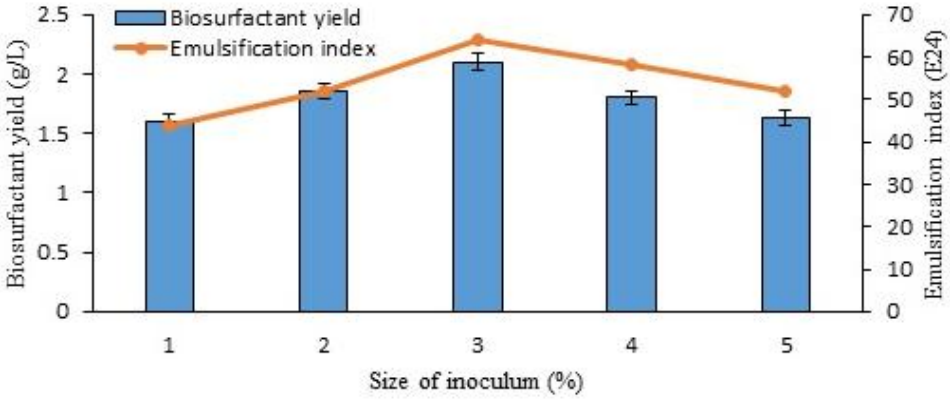

Fig. 7. Effect of inoculum size on the yield of biosurfactants derived from *Pediococcus pentosaceus* S-2

3.8 Size of Inoculum

The size of the inoculum also has an impact on the production of biosurfactants by different strains.

One of the most crucial factors in the synthesis of microbial metabolites is inoculum size. The microorganisms needed a specific number of cells in a medium for their rapid development and metabolite synthesis. Hence it's crucial to know the precise size of the inoculum to maintain a balance between the inoculum size and the volume of the media components. In the present study, the yield of biosurfactants derived from *Pediococcus pentosaceus* S-2 was increased with an increase in inoculum size and maximum yield *i.e* 2.1 g/L was achieved at inoculum size of 3% beyond that there was no further increase in biosurfactants yield occurred. A study conducted by Pato et al. (2021) reported that a 2.5% inoculum size is required for the optimum growth of *Pediococcus pentosaceus* and our result was somewhat similar to this study [26]. With the optimization of all parameters there was increase in 59.09% of total yield of biosurfactants.

4. CONCLUSION

Lactic acid bacteria produce different types of metabolites like biosurfactants, bacteriocin and other anti-microbial components. The current study aimed to explore the optimization of different parameters for the enhancement of biosurfactant yield from *Pediococcus pentosaceus* S-2. It was found that the maximum biosurfactant yield was achieved with the utilization of xylose (1.5%) and yeast extract (1.5%) as carbon and nitrogen sources respectively and at pH of 6, a temperature of 35^{o} C, an agitation rate of 200 rpm and an inoculum size of 3%.The yield of biosurfactants increased from 1.32 g/L to 2.1 g/L upon the optimization of all culture parameters. The enhancement of biosurfactants yield is very important so that it can be explored for various applications like antimicrobial, antiadhesive, antibiofilm, bioremediation *etc.*

According to many studies, biosurfactants act as better candidates for the replacement of synthetic surfactants owing to their unique properties like low toxicity, biodegradability and stability at different temperatures and pH. So, further studies will be carried out to characterize the nature of biosurfactants and to explore the applicability of biosurfactants in different sectors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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