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EFFECTS OF OPTIMIZATION OF NUTRITIONAL REQUIREMENTS AND INFLUENCE OF ENVIRONMENTAL PARAMETERS ON BIOSURFACTANTS DERIVED FROM MARINE BACTERIA

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ABSTRACT: In the present study, biosurfactant-producing microorganisms such as KM1 (*Bacillus licheniformis* - Kasemedu 1), KM2 (*Bacillus subtilis* - Kasemedu 2), ER1 (*Pseudomonas aeruginosa* - Ennore site 1), ER1B (*Pseudomonas mendocina* - Ennore site 1) and ER9 (*Streptococcus lactis* - Ennore site 2) were subjected to optimization studies. The optimization study was carried out using various carbon, nitrogen sources, pH, temperature, hydrocarbons, and trace elements. The effect of dry cell biomass, biosurfactant production, emulsification activity, and surface tension were studied. Among the five isolates, KM2 and ER1 grew well in sucrose at 1% concentration. Production and activity of the biosurfactant were found to a maximum at pH 7.0, and an additional increase in pH during bacterial growth does not affect the surfactant activity till pH 8.5±0.2; however, a decrease in pH showed a significant effect on the surfactant activity. The optimum temperature was identified as 37°C for all the isolates and below and above this temperature markedly affects the biosurfactant production and activity. Observations on reduction in surfactant activity with an increase in agitation above 200 rpm, were reasoned to the reduced uptake of nutrients during agitation. After 36 hours of incubation, the biosurfactant activity of all five isolates was highly pronounced and reduces the surface tension of water to lower than 33 mN/m. The isolate KM2 reduces the surface tension still lower to 23 mN/m.

INTRODUCTION: Biosurfactant is a structurally diverse group of surface-active molecules microorganisms produce. Their ability to decrease surface and interfacial anxiety with low toxicity and excessive specificity and biodegradability, lead to an increasing interest in these microbial products as alternatives to chemical surfactants [1]. However, to this point, biosurfactants cannot compete with the chemically synthesized surfactants in the surfactant market. This may be because of their high production expenses regarding inefficient bioprocessing approach, bad strain productivity, and the need to use luxurious substrates [2, 3]. The interest in biosurfactants has been progressively increasing in recent years due to the possibility of their fermentation manufacturing and their capability applications in such areas because of environmental protection.

The uniqueness with uncommon structural variety, the possibility of value-effective *ex-situ* manufacturing, and their biodegradability are some of the homes that make biosurfactant a promising preference for environmental utility [4]. Biosurfactants are categorized particularly through their chemical composition and microbial

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beginning. The principal training of biosurfactant encompasses glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants, and particulate surfactants [5] Carbon source is very important in the production of biosurfactant. The carbon sources that have been previously used include carbohydrates, hydrocarbons, and vegetable oils. A few organisms produce biosurfactants most effective in carbohydrates, others most effective in hydrocarbons, and still others eat numerous substrates, in combination or one by one. The highest quality yields are received with hydrocarbon or carbohydrate and lipids in standard. For carbohydrates, most biosurfactant manufacturing has been achieved by using the more highly-priced natural forms of sugar. As an example, glucose, fructose, and sucrose lipids are produced by several species of *Corynebacterium*, *Nocardia* and *Brevibacterium* during growth on the corresponding sugar [4].

The nitrogen source within the medium additionally has an amazing effect on the production of biosurfactants. They may also make contributions to pH control. Natural nitrogen assets include gluten meal, yeast hydrolysates, and corn germ, while inorganic nitrogen sources encompass ammonium nitrate, ammonium sulphate, and so on.

In many inorganic salts examined, ammonium salts and urea were preferred for biosurfactant production through *A. paraffineus*, whereas nitrate supported maximum biosurfactant production in *P. aeruginosa* [6]. For surfactin manufacturing with the aid of *B. subtilis*, ammonium nitrate becomes an advanced nitrogen source than ammonium chloride or sodium nitrate. Doubling the ammonium nitrate from 0.5% to 0.8% extended the surfactin production via a factor of 1.6 [7].

Yeast extract becomes located required for glycolipid manufacturing *via Torulopsis bombicola*, however, changed into very worst for *P. aeruginosa*. Nitrogen trouble not handiest causes over production of biosurfactants, but also changes the composition of biosurfactants produced [8, 9]. It is the absolute quantity of nitrogen and not its relative concentration that is important to give an optimum biomass yield. In contrast, the concentration of hydrophobic carbon source determines the conversion of carbon available to the biosurfactant [7]. Growth conditions and environmental factors, which include temperature, pH, agitation, and oxygen availability, also affect the manufacturing of biosurfactants. Temperature can also reason alteration within the composition of the biosurfactant produced using *Pseudomonas* sp. DSM-2874 [8]. A thermophilic *Bacillus* sp. grew and produced biosurfactant at temperatures above 400°C [9]. But, heat treatment of some biosurfactants brought on no appreciable alternative in biosurfactant houses, including the surface interest and the emulsification performance [10].

The pH of the medium performs an essential position in sophorolipid manufacturing via T. bambicola [11]. Penta and disaccharide lipid manufacturing by *Nocardia corynbacteroides* is unaffected inside the pH range of 6.5 to 8.0 [12]. Further, surface tension and CMC of a biosurfactant remained strong over a wide range of pH values, whereas emulsification had a narrower pH range [13]. A growth in agitation because of the shear impact results in the reduction of biosurfactant yield produced through *Nocardia erythropolis* [14, 15]. Then again, the yeast biosurfactant production will increase when the agitation and aeration charges improve [16]. Depending on its impact on cell pastime salts, attention is also located to affect biosurfactant production. However, a few biosurfactants have not affected salt concentrations up to 10% (w/v), although slight reductions in the CMC were detected [17].

MATERIALS AND METHODS:

Optimization of Biosurfactants Production:

Experimental Setup: To the pre-sterilized mineral medium containing (g/l) Sodium chloride - 19.45; Magnesium chloride - 8.8; Calcium chloride - 1.80; Sodium sulphate - 3.24; Sodium bicarbonate - 0.16; Potassium chloride - 0.55; Potassium bromide - 0.08; Sodium silicate - 0.004; Strontium chloride - 0.034; Boric acid - 0.022; Sodium fluorate - 0.0024; Ammonium nitrate - 0.0016; Disodium phosphate - 0.008, carbon, nitrogen sources were added and inoculated the five marine isolates such as *Bacillus licheniformis, Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas mendocina* and *Streptococcus lactis* individually

and incubated for 48 hours under shaking conditions.

Optimization of Nutritional Requirements:

Carbon: To assess the required carbon source and its concentration for maximum production of biosurfactant, to the pre-sterilized mineral medium supplemented with filter-sterilized glucose, fructose, sucrose, glycerol at 0.5, 1.0, 1.5 and 2.0% concentrations individually and inoculated the selected isolates at 1x106 CFU/ml and incubated at 37 °C at 180 rpm for 48 hours. Followed by growth, the biosurfactants activity of the cell free medium was assessed.

Nitrogen: Nitrogen in the form of beef extract, yeast extract, peptone at 0.5, 1.0, 1.5 and 2.0% concentrations supplemented along with the mineral medium and sterilized. To the sterile medium, inoculated the selected isolates at 1x106 CFU/ml and incubated at 37°C at 180 rpm for 48 hours. Followed by growth, assessed the biosurfactants activity of the cell free medium.

Role of Hydrocarbons: Selected bacterial isolates were grown in the mineral medium containing 2% of oils of Sesame, Olive, Crude, Sunflower, Peanut, Kerosene and Soybean. All the required oncentrations of hydrocarbons were provided to the pre-sterilized medium before inoculation. Growth and biosurfactants activity was measured.

Influence of Environmental Parameters:

Effect of Volume of the Production Medium: Since, the volume of the medium with reference to the volume of the culture flasks play a major role, all the selected isolates were grown in 1000 ml of Erlenmeyer flask containing different volumes 50, 100, 150, 200, 250 and 300 ml of optimized medium. Followed by inoculation and incubation at 37°C for 48 hours, the cell-free medium was subjected to surface activity measurements.

Effect of Shaking: Followed by the optimization of volume, experiments were repeated again with the additional changes in the agitation at 0, 50, 100, 180, 250 and 300 rpm and biosurfactant activity was measured.

Effect of pH: In order to optimize the effective pH for maximum production of biosurfactant, isolates were grown in media containing optimized concentrations of carbon and nitrogen with varied pH's 5.5, 6.5, 7.5, and 8.5. Growth and biosurfactants activity was measured at respective pH.

Effect of Temperature: To have optimum temperature for maximum production, cultures were grown at four different temperatures, 25, 30, 37, and 40°C, and measured the growth and biosurfactants activity.

Effect of Incubation Period: To assess the required incubation period, selected isolates were grown in the optimized media for 24, 48, 72 hours, and the growth and biosurfactants activity was measured accordingly. Production of Biosurfactants under optimized environmental conditions All the selected six isolates were cultured individually in 1000 ml Erlenmeyer flask containing optimized volume, pH, and temperature, and at optimum agitation and incubation period. The cell-free supernatant was subjected to (equal amount of ice-cold) ethanol precipitation followed by incubation. The residual pellet was obtained upon centrifugation, dissolved in minimum water, and subjected to surface tension measurements and yield. A potent isolate was chosen from the above-said experiments concerning yield and activity.

RESULT: All the samples were collected from the Chennai Coastal area, Tamilnadu, and isolated fifty morphologically distinct microbial colonies. Optimization of nutritional requirements and environmental parameters, only five isolates, namely KM1 (*Bacillus licheniformis* - Kasemedu 1), KM2 (*Bacillus subtilis* - Kasemedu 2), ER1 (*Pseudomonas aeruginosa* - Ennore site 1), ER1B (*Pseudomonas mendocina* - Ennore site 1) and ER9 (*Streptococcus lactis* - Ennore site 2) were subjected to optimization studies on varied nutrients and environmental conditions. Regarding the growth of the five isolates in the presence of different carbon sources at varied concentrations, isolates KM2 and ER1 grew well in the presence of sucrose at 1% concentration. Regarding biosurfactant activity, isolates grown in the presence of sucrose at 1% concentration reduce the surface tension of water from 71 to 35 mN/m, and few isolates even reduces the surface tension of water to 26 mN/m **Fig. 1**.

The surfactant activity of the biosurfactants obtained from the selected five marine isolates grown in the presence of beef, peptone, and yeast extract as nitrogen source at 1% concentration suggested, though the growth of the isolates showed no significant difference in the presence of beef or yeast extract, however, surfactant property showed significant variations and maximum production with high biosurfactant activity was with medium supplemented with yeast.

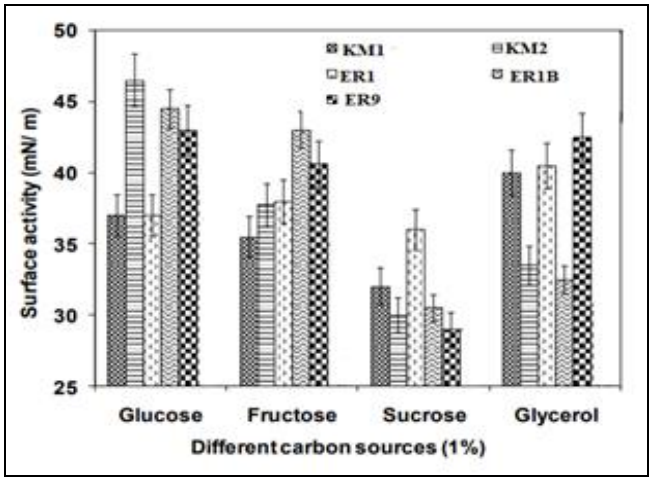

FIG. 1: EFFECT OF DIFFERENT CARBON SOURCES AT 1% CONCENTRATION ON BIOSURFACTANT ACTIVITY OF SELECTED FIVE ISOLATES. (All the values are mean ± sd of triplicate measurements).

Supplementation of peptone, though not influence growth at an appreciable level but influences the surfactant activity of the isolate KM2 and ER1 **Fig. 2**.

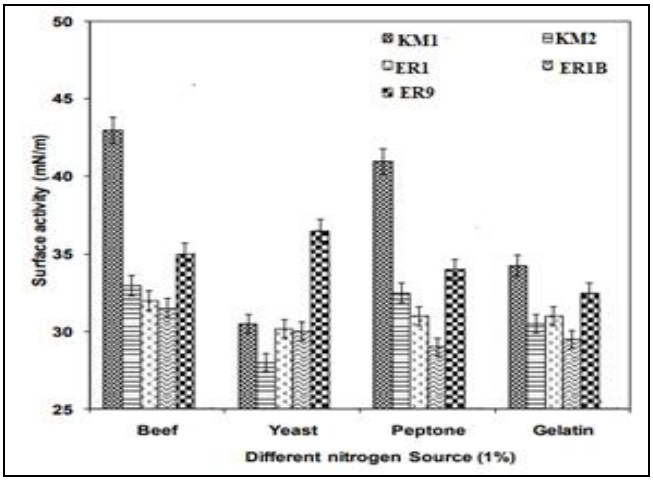

FIG. 2: EFFECT OF DIFFERENT NITROGEN SOURCE AT 1% CONCENTRATION ON BIOSURFACTANT ACTIVITY OF SELECTED FIVE ISOLATES (All the values are mean ± SD of triplicate measurements).

Further, incorporating different hydrocarbons in the form of vegetable oils and kerosene, cultures showed an appreciable emulsion after 48 hours, except for the flasks that received kerosene. Upon increasing the incubation period for more than 48 hours, the oil's phase transformation was visualized in the presence of the isolate. Results on the influence of environmental factors on biosurfactant production and activity, the optimized volume of the medium for 1000 ml capacity Erlenmeyer flasks was identified as 100ml. Regarding the agitation, 180 rpm was found to be optimum. Concerning pH, except KM2, all other four isolates showed appreciable surfactant activity (28-30 mN/m) when grown at pH 7.2 ± 0.2 **Fig. 3**. Though the chosen organisms are not requiring any hydrophobic substrates, however, they need soluble carbohydrates in the form of sucrose for the growth compared to glucose, fructose and glycerol. Similar observations were made by Rismani *et al*., for the production of biosurfactants from *Bacillus licheniformis* [17].

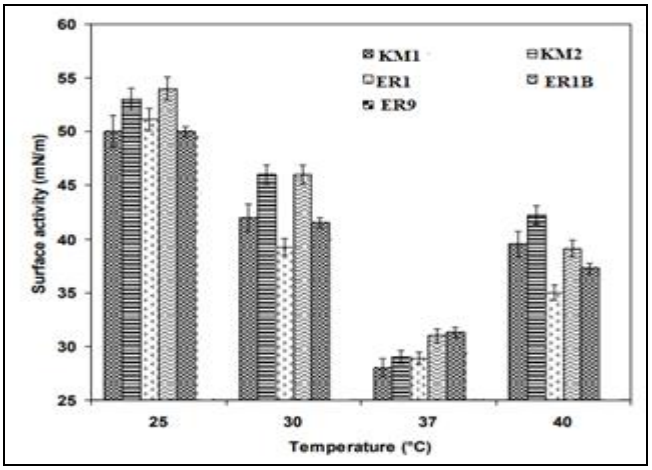

FIG. 3: EFFECT OF DIFFERENT PH RANGE BIOSURFACTANT ACTIVITY OF SELECTED FIVE ISOLATES. (All the values are mean ± SD of triplicate measurements).

With regard to nitrogen, all five isolates exhibit appreciable activity in the presence of yeast compared to beef and peptone. Mutalik *et al*., reported the production of biosurfactants in the presence of yeast extract and meat peptone from *Rhodococcus spp* [18]. Similarly, the optimum temperature was identified as 37°C for all the isolates and below and above this temperature markedly affects the biosurfactant production and activity **Fig. 4.**

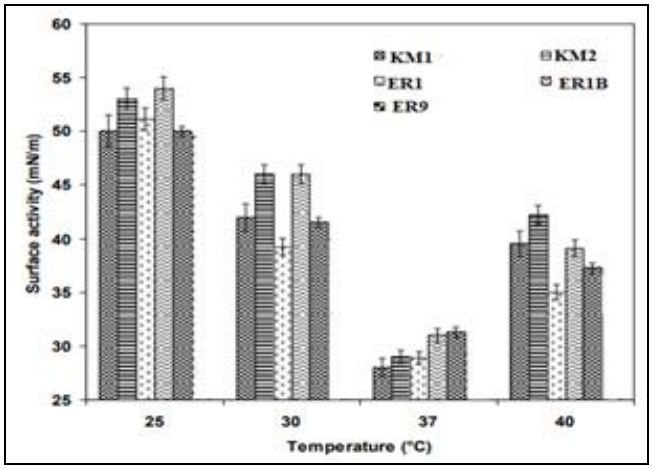

FIG. 4: EFFECT OF DIFFERENT TEMPERATURES ON BIOSURFACTANT ACTIVITY OF SELECTED FIVE ISOLATES. (All the values are mean ± SD of triplicate measurements).

Fig. 4 illustrates the isolates' growth profile in the optimized environmental conditions. All six isolates showed maximum growth within 48 hours and were stable till 72 hours of incubation. **Fig. 5** demonstrated the medium's pH profile during the growth of five isolates for 72 hours. the medium's pH increased slowly during the incubation period, and reached the final pH of 8.25 - 8.9, irrespective of the isolates.

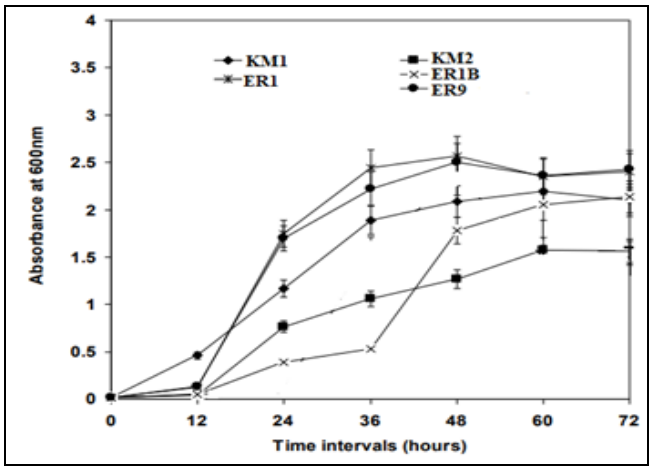

FIG. 5: GROWTH PROFILES OF FIVE MARINE ISOLATES GROWN UNDER OPTIMIZED ENVIRONMENTAL CONDITIONS (12 HOURS TIME INTERVAL). (All the values are mean ± SD of triplicate measurements).

Furthermore, in the presence of hydrocarbons, we observed complete emulsification of the applied hydrocarbons (except kerosene) within 36 hours of incubation. We suggested biosurfactants produced during the growth immediately interact with the applied hydrocarbons. Apart from carbon and nitrogen, environmental factors such as volume, aeration or agitation, incubation period, pH and temperature also play an important role in the production and activity of biosurfactants [10].

Since all the selected isolates obtained from 15 M distance from the seashore, the change in volume of the growth medium with respect to volume of flask and the aeration /agitation provided significantly impacted the product's yield and surfactant activity. The 100 ml growth medium in 1000 ml capacity flask is considered the ideal volume, and 180- 200 rpm is the optimum agitation for the maximum production and surfactant activity. Providing enough oxygen circulation in lower volume favors the microbes to grow adequately. Further, the agitation condition increases the oxygen level and promotes growth rate. However, observations on reduction in surfactant activity with an increase in agitation above 200 rpm, reasoned to the reduced uptake of nutrients during agitation.

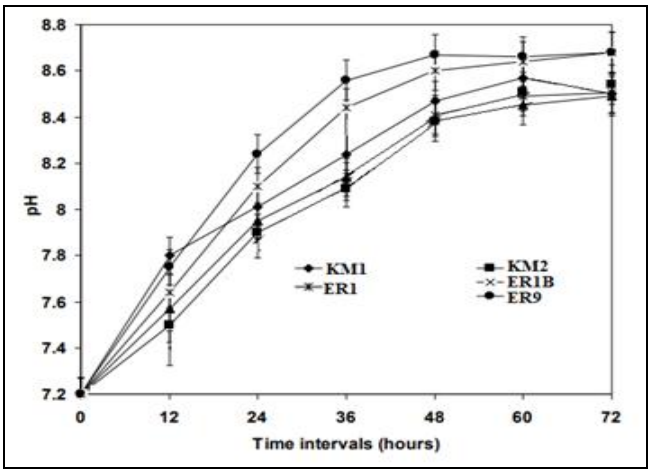

FIG. 6: PH PROFILE OF FIVE MARINE ISOLATES GROWN UNDER OPTIMIZED ENVIRONMENTAL CONDITIONS (12 HOURS TIME INTERVAL). (All the values are mean ± SD of triplicate measurements).

Concerning the incubation period, **Fig. 6** displays that after 36 hours of incubation, the biosurfactant activity of all five isolates was highly pronounced and reduced the surface tension of water to lower than 33 mN/m, and the isolate KM2 reduced the surface tension still lower to 23 mN/m. Since the biosurfactant yield is the most important part of biosurfactant production, Fig. 7 demonstrates the biosurfactant yield concerning the incubation period. A maximum yield of 27 ± 4 g/l was obtained after 48 h of incubation, and no significant difference in the yield was obtained after 48 and 72

hours of incubation. From the optimization results, KM2 exhibits appreciable surfactant activity and yield. This potent biosurfactant producer was carryover to the characterization and application studies. Syldatk *et al*. also found that an increase in agitation speed from 250 to 500 rpm decreases biosurfactant production in *R. erythropolis* [11].

Regarding the pH of the growth medium on the production of surface active agents from bacterial species, several authors reported a wide variation in production [19, 20]. Rhamnolipid production by *Pseudomonas sp* is high at pH range from 6 to 6.5 and decreased sharply above pH 7.0 (20). In the present study, the production and activity of the biosurfactant were found to be maximum at pH 7.0, and an additional increase in pH during bacterial growth does not affect the surfactant activity till pH 8.5 ± 0.2; however, a decrease in pH showed significant effect on the surfactant activity.

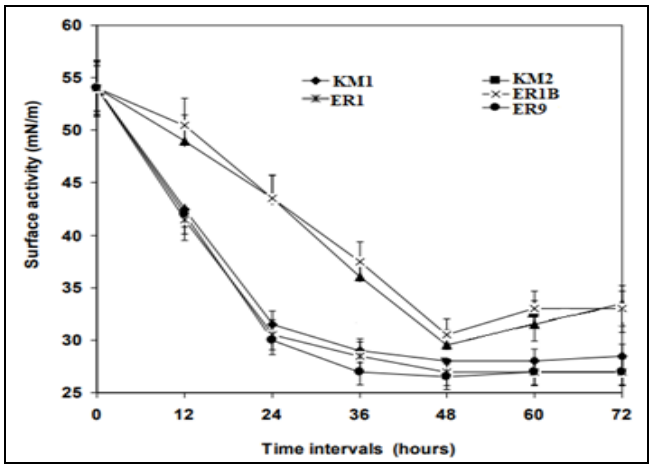

FIG. 7: BIOSURFACTANT ACTIVITY PROFILES OF FIVE MARINE ISOLATES GROWN UNDER OPTIMIZED ENVIRONMENTAL CONDITIONS (12 HOURS TIME INTERVAL). (All the values are mean ± SD of triplicate measurements).

This could be reasoned to the nature and the source of origin of the microbial species employed. The optimum temperature observed is 37°C irrespective of the isolates, and a further increase in temperature results in a decrease in growth as well as surfactant activity and suggests temperature plays a significant role in the production of biosurfactants. Syldatk *et al*. found *Pseudomonas sp* showed maximum growth and surfactant activity when grown at 37 °C [10].

CONCLUSION: The present study detailed the optimization of nutrient and environmental conditions for maximum production, characterization of the biosurfactant for its surfactant property with biologically derived surface active agents, and application of the chosen to isolate and its by-products for the management of environmental problems associated with oil contamination were analyzed. This study exemplifies the potency of the new isolate on biosurfactant production, followed by application in various environmental problems, and the role of biosurfactants on self-assembly. The future perspective of the study has also been suggested.

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REFERENCES:

1. Banat IM: The isolation of a thermophilic biosurfactant producing *Bacillus* SP. Biotechnology Letters 2019; 15: 591-594.
2. Cameotra SS and Makkar RS: Synthesis of biosurfactants in extreme conditions. Applied Microbiology and Biotechnology 2020; 50: 520-529.
3. Deleu M and Paquot M: From renewable vegetables resources to microorganisms: new trends in surfactants. Comptes Rendus Chimie 2018; 7: 641-646.
4. Hua Z, Chen J, Lun S and Wang X: 2003: Influence of biosurfactants produced by Candida antarctica on surface properties of microorganism and biodegradation of n-alkanes. Water Research 2017; 37: 4143-4150.
5. Desai JD and Banat IM: Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews 2019; 61: 47-64.
6. Desai DJ and Desai A: Production of bisurfactant. In biosurfactant: production, properties and application. in: N. Kosaric (Ed.) Surfactant Science Series. Marcel Dekker Inc., New York / Hong 2021; 65-67.
7. Mulligan CN and GB: Factors influencing the economics of biosurfactant. in: N. Kosaric (Ed.) Biosurfactant production, properties and application. Mercel Decker New York 2020; 329-371.
8. Syldatk WF: Production of biosurfactant, In: Biosurfactants and Biotechnology. in: G.N. Kosaric N. Cairns WL (Ed.). Marcel Dekker, New York 2021; 89-120.
9. Hommel R, Stiiwer O, Stuber W, Haferburg D and Kleber HP: Production of water-soluble surface-active exolipids by *Torulopsis apicola*. Applied Microbiology and Biotechnology 2019; 26: 199-205.
10. Syldatk C, Andree H, Stoffregen A, Wagner F, Stumpf B, Ernst L, Zilch H and Tacke R: 1987. Enantioselective

reduction of acetyldimethylphenylsilane by *Trigonopsis variabilis* (DSM 70714). Applied Microbiology and Biotechnology 2021; 27: 152-158.
11. Banat IM, Makkar RS and Cameotra SS: Potential commercial applications of microbial surfactants. Applied Microbiology and Biotechnology 2020; 53: 495-508.
12. Abu-Ruwaida A, Banat I, Haditirto S and Khamis A: Nutritional requirements and growth characteristics of a biosurfactant-producing *Rhodococcus bacterium*. World J of Microbiology and Biotechnology 2017; 7: 53-60.
13. Göbbert U, Lang S and Wagner F: Sophorose lipid formation by resting cells of *Torulopsis bombicola*. Biotechnology Letters 2018; 6: 225- 230.
14. Powalla M, Lang S and Wray V: Penta and disaccharide lipid formation by *Nocardia corynebacteroides* grown on alkanes. Applied Microbio and Biotech 2017; 31: 473-479.
15. Margaritis A, Zajic JE and Gerson DF: Production and surface-active properties of microbial surfactants. Biotechnology and Bioengineering 2020; 21: 1151-1162.
16. Mulligan CN, Yong RN and Gibbs BF: On the use of biosurfactants for the removal of heavy metals from oil-contaminated soil. Environmen Progress 2019; 18: 50-54.
17. Rismani E, Fooladi J and Por GHE: Biosurfactant Production in Batch Culture by a Bacillus licheniformis Isolated from The Persian Gulf. Pakistan Journal of Biological Sciences 2017; 9: 2498-2502.
18. Mutalik SRVB, Joshi RM, Desai KM and Nene SN: Use of response surface optimization for the production of biosurfactant from *Rhodococcus* spp. MTCC 2574. Bioresour Technol 2019; 99:7875-7880.
19. Göbbert U, Lang S and Wagner F: Sophorose lipid formation by resting cells of *Torulopsis bombicola*. Biotechnology Letters 2021; 6: 225- 230.
20. Guerra-Santos L, Kappeli O and Fiechter A: *Pseudomonas aeruginosa* biosurfactant production in continuous culture with glucose as carbon source. Applied and Environmental Microbiology 2020; 48: 301-305.

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