Indian Journal of Chemical Technology
Vol. 32, January 2025, pp. 41-56
DOI: 10.56042/ijct.v32i1.13169

Wealth from waste: Multifaceted yeast bio-synthesised sophorolipids using industrial waste by-products as substrate

Srija Pal[1,2], Niloy Chatterjee[1,3], Brajadulal Chattopadhyay[4], Sriparna Datta[2] & Pubali Dhar[1,3]*

[1]Laboratory of Food Science and Technology, Food and Nutrition division, University of Calcutta, Kolkata-700 027, West Bengal, India

[2]Department of Chemical Technology, University of Calcutta, Kolkata-700 009, West Bengal, India

[3]Centre for Research in Nanoscience & Nanotechnology, University of Calcutta, Kolkata-700 098, West Bengal, India

[4]Department of Physics, Jadavpur University, Kolkata-700032, India

*E-mail: pdhomesc@caluniv.ac.in; pubalighoshdhar@yahoo.co.in

Received 15 August 2024; accepted 16 December 2024

Sophorolipid biosurfactants are known for their versatile biological and physicochemical activities. This study explores its production, characterization, and biological activities utilising, palm fatty acid distillate (PFAD), a hydrophobic substrate derived from oil refinery waste by *Starmerella bombicola, a* native sophorolipid producing yeast. The sophorolipid yield is significantly enhanced by using PFAD than that of a vegetable oil used as feedstock. The yields are 36.71 and 18.62 g/L for PFAD and refined sunflower oil, respectively. The minimum surface tension with PFADSL (palm fatty acid distillate derived sophorolipid) is 37.73 mN/m and the CMC value is 76 mg/mL. Emulsifying properties of PFADSL are found to be 54.32%, 59.45%, and 28.38% for waste frying sesame oil, refined sesame oil, and petrol, respectively. The PFADSL is characterized using TLC, FTIR, and HPLC confirming the major component to be diacetylated lactonic sophorolipids (SLs) (C18:1) consisting 15.4% acidic forms ofsophorolipids. PFADSL showed minimum inhibitory concentration values ranging between 25 - 50 µg/mL against *Proteus vulgaris, Shigella flexneri, Salmonella typhi, Vibrio cholerae, Staphylococcus aureus, Escherichia coli, Bacillus subtilis,* and *Klebsiella pneumoniae.* Minimum Bactericidal Concentrations range from 300 to 500 µg/mL. SEM images show compromised membrane integrity against both gram-negative and positive bacteria. PFADSL also confirmed its strong biofilm inhibitory and eradication capacity against tested organisms. These findings reveal a noteworthy way of bio-converting oil industrial waste into a valuable product with potent surface lowering, emulsifying, antibacterial, and antibiofilm ability which can be a gem to various fields like biomedical, agricultural, pharmaceutical, cosmetics, nanotechnology, etc.

Keywords: Anti-microbial, Bioactive, Glycolipid, Versatile, Waste-to-wealth, Yeast

Introduction

Palm Fatty Acid Distillate (PFAD) is a by-product derived from the physical refining of crude palm oil. It is primary composition includes free fatty acids (FFAs), along with minor quantities of glycerides, tocopherols, tocotrienols, sterols, and other impurities. PFAD typically contains 70-90% free fatty acids, which are primarily palmitic acid, followed by oleic acid, and smaller amounts of linoleic acid[1]. PFAD has traditionally been considered a low-value waste product in the palm oil industry. However, the increasing emphasis on sustainability and resource efficiency has led to the recognition of PFAD as a valuable feedstock for various industrial applications. PFAD is utilized in biodiesel production, the oleochemical industry for the manufacturing of soaps, detergents, and fatty acids, which are further processed into fatty alcohols and other derivatives used in personal care products, cosmetics, and lubricants[2–5]

The utilization of PFAD as a substrate for sophorolipid production by yeast strains represents a promising avenue in the field of industrial biotechnology. Sophorolipids (SLs), a major class of bio-surfactants synthesized by certain yeast and fungal species, have garnered significant consideration owing to their biodegradability, minimal toxicity, and versatile applications in industries ranging from pharmaceuticals to environmental remediation[6]. Leveraging PFAD, a palm oil refining by-product, as a feedstock for sophorolipid production offers an innovative and sustainable approach to both waste valorization and biosurfactant synthesis in current scenario.

PFAD is predominantly composed of FFAs, with palmitic and oleic acids being the major constituents.

These FFAs provide a rich carbon source that can be metabolized by certain SLs producing strains to produce SLs. The high lipid content of PFAD, coupled with the presence of minor components such as sterols and tocopherols, enhances its suitability as a substrate for microbial fermentation. Additionally, the availability and low cost of PFAD, due to its status as an industrial by-product, make it an economically attractive option for large-scale biosurfactant production.

Sophorolipid production by oleaginous yeast strains, such as *Starmerella bombicola*, involves the fermentation of fatty acids under specific conditions[7]. The process typically requires a hydrophobic carbon source, such as PFAD, along with a hydrophilic co-substrate like glucose. During fermentation, the yeast cells uptake the FFAs from PFAD and convert them into SLs through a series of enzymatic reactions, including hydroxylation, acetylation, and glycosylation. The resulting SLs can exist in lactonic or acidic forms, each with distinct physicochemical properties and potential applications[6].

Starmerella bombicola synthesizes SLs through a bioconversion process that involves the fermentation of fatty acid substrates in the presence of a carbohydrate source. The resulting sophorolipids typically exist in two primary forms: lactonic and acidic isomers. The lactonic congener is characterized by the intramolecular esterification between the carboxylic moiety of the fatty acid and the hydroxyl group of the sophorose sugar, leading to a closed-ring structure. The acidic form, on the other hand, retains the free carboxylic group and exists as an open-chain molecule. The balance between these two forms can be decided by the fermentation conditions, such as pH, temperature, and substrate concentration[6,8–11].

SLs produced by *S. bombicola* exhibit notable antimicrobial characteristics against a widespread variety of microorganisms, including fungi, yeast, and bacteria. The lactonic form, in particular, has demonstrated strong antibacterial activity, primarily by challenging the microbial cell membrane integrity, followed by cell lysis. This makes SLs promising candidates for use as natural preservatives in the food industry and as alternative antimicrobials in medical and pharmaceutical applications, especially in the face of rising antibiotic resistance[12–14]. SLs from *S. bombicola* have also been shown to hold significant antibiofilm activity. Biofilms, the structured microbial communities sheathed in a self-produced extracellular matrix, are notoriously difficult to eradicate due to their resistance to conventional antimicrobial treatments[15]. SLs can prevent biofilm formation by interfering with the initial adhesion of microbial cells to surfaces and can also disrupt established biofilms by targeting the extracellular matrix. This property is particularly valuable in medical and industrial settings, where biofilm formation on surfaces such as medical devices and pipelines poses a significant challenge[12,16,17].

Traditionally, the production of SLs has relied on glucose and various vegetable oils or oleic acid as substrates. However, the cost of these raw materials has prompted researchers to explore more sustainable and cost-effective alternatives[18]. In this context, PFAD presents a promising substrate for sophorolipid production. PFAD is rich in free fatty acids and other valuable compounds, making it a potential feedstock for microbial biosurfactant production. Despite the potential benefits, the usage of PFAD for sophorolipid production has not been extensively studied, particularly with non-conventional yeast strains. The yeast species *Starmerella bombicola* is well-known for its capability of producing high yields of SLs, but the feasibility of utilizing PFAD as a sole hydrophobic substrate in this process remains underexplored. This study aims to fill this gap by investigating the production of SLs using PFAD as the primary substrate, employing a novel strain of *Starmerella bombicola*.

The objectives of this research are twofold: first, to optimize the fermentation conditions for maximum sophorolipid yield using PFAD; and second, to characterize the produced SLs in terms of their chemical structure and potential applications. This study contributes to the growing body of knowledge on sustainable bio-processing, offering insights into the potential of PFAD as a cost-effective and ecologically friendly hydrophobic substrate for SLs production.

Experimental Section

All the microbial strains, *Starmerella bombicola* 1910, *Escherichia coli*, *Staphylococcus aureus* subsp. *Aureus* 6571, *Bacillus subtilis 3610*, *Vibrio cholera*e M010 (0139), *Shigella flexneri* 29508, *Salmonella typhimurium* OSS117, *Proteus vulgaris* 6380, and *Klebsiella pneumoniae* subsp. *pneumoniae* 418 were purchased from Microbial Type Culture Collection and Gene Bank, Chandigarh, India, and maintained in specific growth media every 30 days. The chemicals

and solvents were brought from Sigma-Aldrich, SRL Pvt. Ltd., and Hi-media, and of analytical grades. The commercial standard of SLs (1',4"-Sophorolactone 6',6"-diacetate) was obtained from Cayman Chemicals, USA. From local grocery refined sunflower oil was purchased. PFAD was collected from an oil refinery industry, Budge Budge Refineries, Budge Budge, Kolkata, West Bengal, India.

Production of sophorolipids

The media composition for SLs production was 10% glucose, 10% PFAD/ refined sunflower oil, 0.1% urea, and 1% yeast extract. Media after autoclaving was inoculated with 10% seed culture (48h grown in YPD media) and kept in a BOD shaker incubator at 28°C ± 0.5°C and 150 rpm ± 5 for consecutive 8 days. At an interval of 48h, glucose concentration, cell dry weight, pH and SLs production or yield were measured. Initially, SLs yield with different concentrations of PFAD and sunflower oil (2.5 to 20%) was measured to determine effective concentrations of hydrophobic sources.

Confirmation of biosurfactant production

Whether the PFAD can produce biosurfactant as product or not was evaluated with parafilm M test and oil displacement tests[19]. Briefly, in the parafilm M test, 25 µL of cell-free broth of PFAD production media (48 h, 120 h, and 192 h) was placed on a parafilm sheet and observed for 1 min followed by measuring the droplet diameter. Water and fresh media diameter were also measured for comparison. In the oil-displacement test, 9 cm Petri-dish was filled with 40 mL deionized water. 200 µL of vegetable oil was dropped and allowed to spread followed by placing 10 µL of cell-free broth was added at the centre of the oil phase. The displaced diameter was measured. Fresh media was used as control.

Extraction of SLs biosurfactant

Extraction of SLs from the aqueous production media was done using solvent extraction method[20]. Cell-free broth (cell removal by centrifugal force) was washed with ethyl acetate for three times, and then the solvent layer was removed under a vacuum pump followed by washing thrice with n-hexane to eliminate fatty residues. The yield was weighed gravimetrically and expressed in g/L.

Characterization

Physicochemical characterization

Surface Tension (ST) and CMC (Critical Micelle Concentration): Different concentrations of PFADSL aqueous solutions (0.5 to 1000 mg/L) were prepared to analyze their ST at ambient temperature (20 °C ± 1°C) following du Nuoy ring method with the use of DCAT 11 tensiometer (Data Physics, Germany) (21,22). Critical Micelle Concentration was calculated from the ST graph [21].

Emulsification index (%E24): The emulsifying activity of PFADSL was tested using waste frying sesame oil, refined sesame oil, and petrol (as mineral oil) [20]. Briefly, 1% PFADSL (in deionized water) was mixed well with an equal volume of oils using cyclomixer at highest speed for 15 min. Then for the next 24 h, these were kept uninterrupted followed by measuring emulsion height and total height of the liquid. %E24 was determined by the following equation:

$$\% E24 = (\text{Emulsion layer height} / \text{height of total liquid}) \times 100$$

Structural composition analysis:

Thin Layer Chromatography: After initial identification of the presence of carbohydrate moiety using the anthrone test, PFADSL components were analysed using TLC analysis with silica gel coated (Silica Gel G, 325 mesh for TLC) TLC plates and a mobile phase containing chloroform: methanol:water (65:15:2). Isolated spots were visualised under iodine vapour chamber[22]. Result was analysed by comparing it with TLC spots of SLs standard.

Fourier Transform Infrared Spectroscopy (FTIR): Using IRAffinity-1S Shimadzu spectrophotometer, FTIR spectra of PFADSL and SLs standard were recorded. ATR (Attenuated Total Reflectance) mode was implemented with 40 scans, correlated with atmospheric CO_2 and, at a wave number of 0.01 cm^{-1} and resolution accuracy of 2 cm^{-1} (Ref.22).

High Performance Liquid Chromatography (HPLC): The relative percentages of acidic and lactonic SLs in PFADSL were analysed with HPLC (Shimadzu) on the basis of standard SLs[23]. Spherisorb ODS2 Column (5 µm, 250 × 4.6 mm, 80Å) with Acetonitrile and MilliQ binary water solvent system was used for the sample run with a total 50 min run time (20% for the first 10 min, with a gradual increase to 80% up to 30 min, 100% up to 40 min, and then maintained at 100% for next 10 min) at 0.75 mL/min flow-rate. With Shimadzu SPD-20A UV/Vis Detector components were detected at 207 nm.

Antibacterial activity

Zone of Inhibition: Primarily, the antimicrobial potential of PFADSL was tested against eight

bacterial pathogenic strains (*P. vulgaris, S. flexneri, S. typhimurium, V. cholera, S. aureus, B. subtilis, E. coli, and K. pneumoniae*) with the agar well diffusion technique[24]. 25 µL of 24h grown bacterial suspensions (0.5 MacFarland standard/1.5 × 10^8 CFU mL^{-1}) were used to make spread plates on nutrient agar media. Wells (0.5 cm) were made with sterile well makers and PFADSL (in sterile PBS) in different amounts (500, 750, and 1000 µg) were loaded into it. Plates were incubated at 37 °C for 24 h. After incubation, the diameters (cm) of mortality areas were measured.

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC): For analysing MICs of PFADSL resazurine-based broth micro-dilution assay was performed[25]. Briefly, with different concentrations of PFADSL solubilised in nutrient broth 24 h grown bacterial suspensions (0.5 MacFarland standard) were added and grown at 37 °C for 24 h followed by staining with 0.015% resazurin solution. The least concentrations showing no colour change from blue to pink were marked as MICs. In positive control and negative control media with culture and only fresh media were used. MBCs were performed[26] by streaking the cultures from MICs and subsequent wells. The least concentrations giving no viable count were marked as MBCs. Tolerance levels were calculated from MICs and MBCs for each bacteria [25,27].

Scanning Electron Microscopy: To identify the mechanism of antibacterial activity of PFADSL, SEM of PFADSL treated gram positive *S. aureus* cells and gram-negative *E. coli* was performed with Zeiss EVO 18 following the previously described procedure by Shikha *et al*.[28]. Briefly, treated (300 µg/mL of PFADSL for 8h) and untreated/control (sterile PBS) cells were washed thrice with sterile PBS and fixed with 2.5% glutaraldehyde for 2 h followed by drying with alcohols (20-100% alcohol). Finally, gold sputter-coated cells were observed under SEM.

Anti-biofilm assay

Biofilm inhibition assay: Biofilm inhibitory activity of different concentrations of PFADSL (12.5 to 1000 µg/mL) against *P. vulgaris, S. flexneri, S. typhimurium, V. cholerae, S. aureus* were measured using crystal violet staining method [25]. Finally, the inhibition percentages were calculated using the following equation:

$$I\ \% = (\text{Control } OD_{570nm} - \text{Treatment } OD_{570nm}) / \text{Control } OD_{570nm} \times 100$$

The lowest concentrations with I% ≥50 were marked as MBIC (Minimum Biofilm Inhibitory Concentration) for respective bacterial strains.

Biofilm eradication assay: By the crystal violet staining technique described by Guchhait *et al*.[29] biofilm eradication activity of different PFADSL concentrations against preformed biofilms of bacteria were measured. I% (calculated from the equation of 6.1) ≥ 50 of the least PFADSL concentrations were noted as MBEC (Minimum Biofilm Eradication Concentration).

Statistical analysis

Each value was represented as mean and standard deviations of triplicates. Statistical significance for multiple comparisons of means was made with One-way ANOVA followed by Tukey test ($p<0.5$). Statistically significant different values were marked as different superscript alphabets. MS Excel and SPSS 16.0 were used for analysis.

Results and Discussion

Production of SLs biosurfactant

Refined sunflower was used for SLs production to mimic industrial SLs production where oleic acid or edible oils are used as hydrophobic feedstock enhancing total production cost[18]. Media with PFAD was tested for confirming biosurfactant production during the incubation period as it was a novel substrate and results are presented in Fig. 1, which shows a significant increase in droplet diameter and oil-cleared zones with an increase in time confirming enhanced biosurfactant synthesis.

Primarily different concentrations of both the hydrophobic substrates (PFAD and sunflower oil) were screened for evaluating optimum concentrations as per the final yield of SLs. However, Fig. 2a shows that increasing concentrations of both PFAD and sunflower oil leads to a significant increase in SLs production up to 10%, but significant deterioration was found when substrate concentrations were enhanced to 20%. These results can be simplified with the fact of possible adverse effects of lipid biodegradation products on microbial growth and subsequent decrease in biosurfactant production[30]. Furthermore, it can be seen that the use of PFAD significantly increased the SLs production as compared to sunflower oil. The SLs yield was 36.71 ± 2.622 and 18.62 ± 0.142 g/L for PFAD and sunflower oil respectively. Fig. 2b shows the different parameters during the incubation period. Glucose

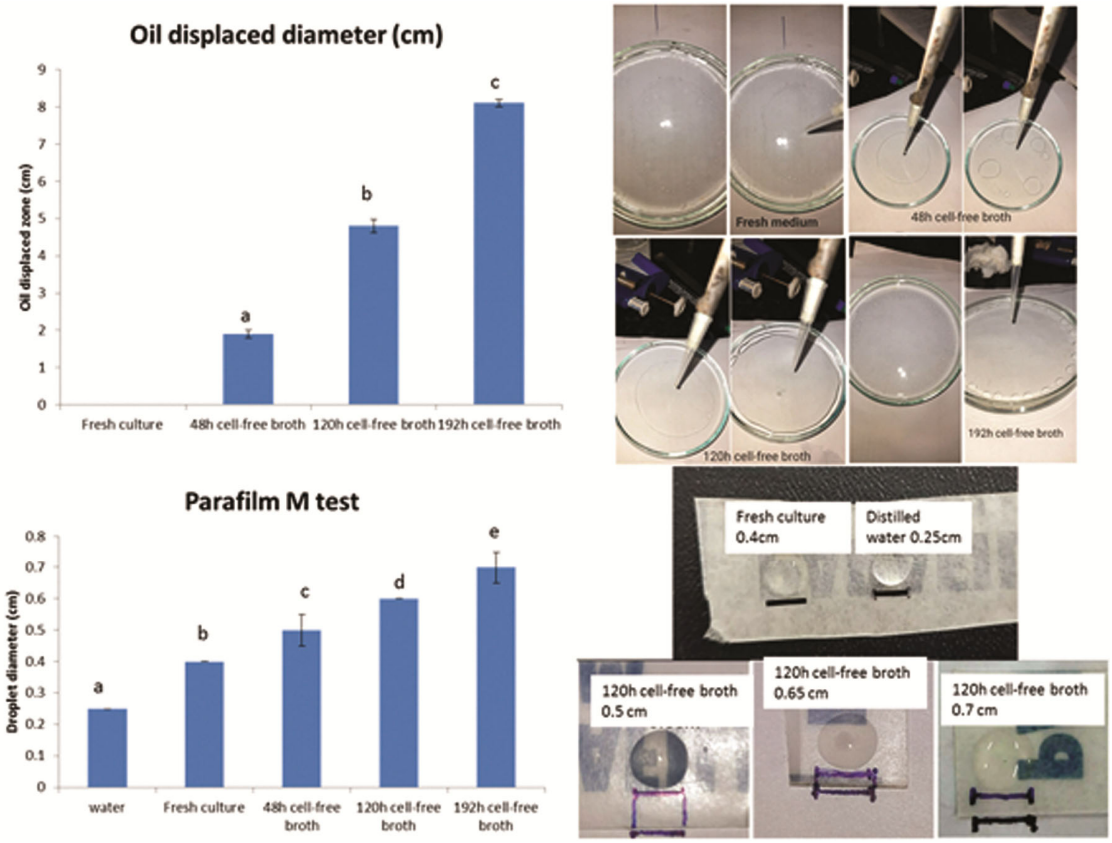

Fig. 1 — Initial screening of bio surfactant production in PFAD media during the incubation period (Different superscript alphabets above bars represent statistically significant difference at $p<0.5$)

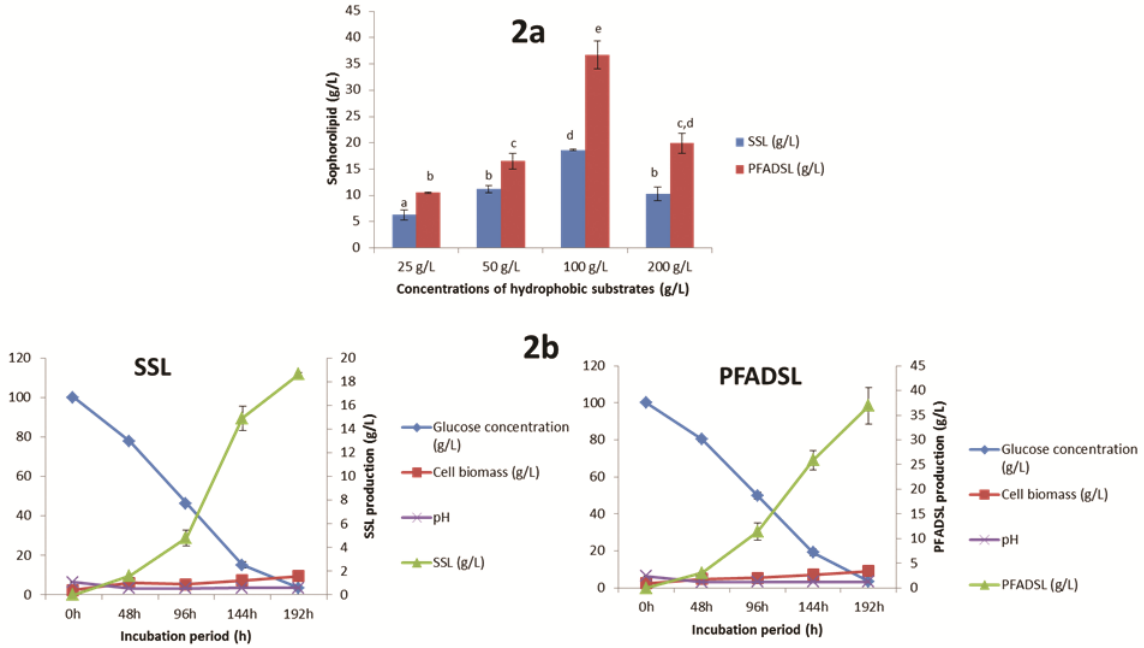

Fig. 2 — (a) Yields of SSL and PFADSL at different substrate (oil/ PFAD) concentrations. (Different alphabets above bars denote statistically significant different ($p<0.05$) values) and (b) Glucose consumption, cell biomass, pH, and yields of SSL and PFADSL during the incubation period of production medium containing 100 g/L of hydrophobic substrates

concentrations and pH were dropped while, production of PFADSL and SSL (sunflower oil derived sophorolipid), and cell biomass were increased with time. PFAD is commonly composed of a high amount of free fatty acids (65 to 94%), mainly, C16:0 and C18:1 fatty acids making this very suitable for sophorolipid production[1,6,31]. Previously sunflower acid oil, coconut oil fatty acid residues, sunflower oil soap stock, soybean dark oil, tallow fatty acid residue[32–35], etc. had been utilized for SLs production confirming satisfactory yields, though no report has been published with PFAD use till date.

As a low-cost by-product of the palm oil industry, PFAD reduces the overall production costs of SLs, making the process more economically viable. Utilizing PFAD aligns with the principles of the circular economy by converting an industrial waste product into a valuable bio-product, thereby reducing waste and promoting resource efficiency. The high fatty acid content of PFAD supports robust microbial growth and sophorolipid synthesis, potentially leading to high product yields. The production of biosurfactants from PFAD is a greener alternative to chemical surfactants, which are often derived from petrochemical sources and are less biodegradable. The ability to produce high-value products from low-cost waste materials enhances the economic viability of the process. Waste substrates are generally inexpensive and readily available, making the production of SLs more cost-effective compared to using pure or synthetic substrates. This reduction in raw material costs can lower the overall production costs of SLs, thereby making them more competitive with traditional chemical surfactants in the market. The biodegradability and low toxicity of SLs further enhance their environmental profile, making them suitable for use in eco-friendly formulations. However, the use of waste substrates can exhibit variability in their composition depending on their source and processing conditions. This variability can lead to inconsistencies in the yield and quality of SLs produced. While the laboratory-scale production of SLs using waste substrates is well-established, scaling up the process to industrial levels presents several challenges. These include the need for large-scale fermentation facilities, efficient downstream processing methods, and the management of by-products or waste generated during production.

Characterization

Physicochemical characterization

Surface Tension (ST) and CMC

Being amphiphilic, biosurfactants exhibit surface active properties with regards to reduction of surface as well as interfacial tensions and low CMC. This behaviour plays a vital role in solubilizing and emulsifying hydrophobic components and emulsion stabilization [36]. Fig. 3b shows the reduction of surface tension with increasing concentration of PFADSL. It reduced ST of water from 71.1 mN/m to 37.7 mN/m (minimum ST). The CMC was found to be 76 mg/L. The ST and CMC of SLs are critical parameters that influence their application potential in several industries. By lowering the ST of aqueous solutions, SLs enhance the wetting, spreading, and emulsification properties of the solution, making them ideal candidates for use in formulations where improved solubility and dispersion are required, such as in drug delivery systems and bioremediation processes [37–39]. The formation of micelles is essential for applications where encapsulation of hydrophobic substances is needed, such as in the targeted delivery of pharmaceuticals or the solubilization of hydrophobic pollutants in wastewater treatment. Biodegradable or food-grade emulsifiers like phospholipids, amphiphilic polysaccharides, protein isolates, etc. had been well utilized [40]. Their use has been increased for delivering nanoceuticals or other bioactive hydrophobic materials (sesame lignin rich sesame oil, clove essential oil, γ-oryzanol-rich rice bran oil etc.) via nanodelivery systems[40–43]. Sophorolipids are a potential biosurfactant in terms of such biodegradable surface active agents. Moreover, research on antimicrobial emulsifiers is in high demand[44]. Food grade antimicrobials for packaging, additives, or preservatives are in so high demand that green synthesis of different antibacterial and antifungal agents e.g., silver nanoparticles, peptides, plant products, etc. are the hot topic of research [25, 27,45–47].

Now, SLs with a low CMC are particularly advantageous because they require lower concentrations to achieve effective micellization, thus being more cost-effective and less prone to potential cytotoxicity. Moreover, the ability of SLs to form stable micelles and reduce surface tension at low concentrations underscores their utility in developing eco-friendly and sustainable alternatives to conventional synthetic surfactants. This property is especially beneficial in creating

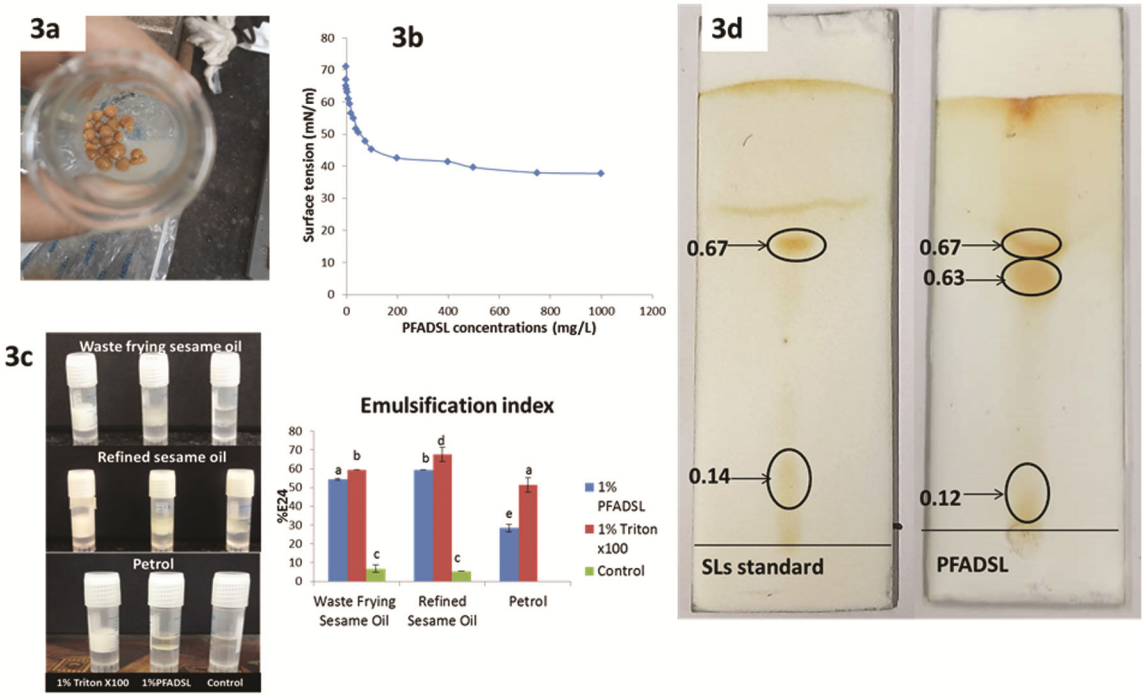

Fig. 3 — (a) PFAD derived SLs, (b) Surface Tension of PFADSL (Different superscript alphabets above bars represent statistically significant difference at $p<0.5$), (c) Emulsification index and (d) TLC plates of standard SLs and PFADSL

biodegradable cleaning agents, cosmetic products, and dispersants in oil spill remediation[48–51]. Therefore, understanding and optimizing the surface tension and CMC of SLs is crucial for maximizing their industrial applications and advancing their role as sustainable biotechnological products.

Emulsification index (%E24)

This value determines how effectively a biosurfactant increases the contact between water and oil surfaces. Here three types of hydrophobic materials were used, fresh refined sesame oil, waste fried sesame oil (after frying foods for many times), and petrol as mineral oil. Fig. 3c shows that 1% PFADSL effectively emulsified both the cooking oil and waste frying oil with %E24 values of 59.46% and 54.32% respectively which were very close to values of triton x100 (but statistically significant difference was present). %E24 of PFADSL and triton-X 100 for petrol were 28.38 and 51.35%. A higher emulsification index indicates a stronger capability of SLs to maintain stable emulsions over time, preventing the separation of phases. In the food industry, SLs with a high emulsification index can be used to create and stabilize food emulsions, such as dressings, sauces, and spreads, enhancing texture and shelf life without the need for synthetic additives. Similarly, in pharmaceuticals and cosmetics, stable emulsions are essential for formulating creams, lotions, and ointments where the active ingredients need to be uniformly dispersed within a carrier medium[52,53].

The natural origin and biodegradability of SLs make them an attractive alternative to synthetic emulsifiers, especially in products aimed at consumers seeking eco-friendly and sustainable options. In environmental applications, the emulsification properties of SLs are utilized in bioremediation, where they facilitate the breakdown and removal of hydrophobic pollutants, such as oils and hydrocarbons, from contaminated environments. By forming stable emulsions, SLs enhance the bioavailability of these pollutants to microbial degradation, thus accelerating the clean-up process. The amphiphilic nature of SLs allows them to function effectively in a variety of environments, including aqueous and non-aqueous systems. They can stabilize emulsions, solubilize hydrophobic compounds, and form micelles, which are essential properties in industries such as cosmetics, pharmaceuticals, and oil recovery. Their ability to operate under a wide range of pH and temperature

conditions further enhances their versatility as surfactants and micelle agents [52,54–57].

Structural characterization

Thin layer Chromatography

After initial confirmation of the presence of carbohydrates by anthrone, TLC spots under iodine vapour confirmed the presence of lipid moieties in PFADSL. Comparison of the separated spots of PFADSL with SLs standard and R_f value of 0.67 confirmed the presence of 1',4"-Sophorolactone 6',6"-diacetate. Fig. 3d shows different spots with respective Rf. Higher Rf values signify lactonic SLs and the lower value represents the presence of acidic forms of SLs[58].

FTIR spectra for analysing functional groups

The FTIR bands of PFADSL were compared with the SLs standard. The band stretches are represented in Fig. 4a. Table S1 (Supplementary Information) represents details of wavenumbers, transmittance%, and identified bonds. Wavenumber 3392 cm^{-1} represents O-H stretch. 2926.1 and 2854.7 cm^{-1} represented the presence of asymmetric and symmetric C-H bonds respectively. C=O bonds of esters, lactone, or acids were identified at 1744 cm^{-1} and C(=O)-O-C of lactone was seen at 1234.5 cm^{-1}. 1247 cm^{-1} represented the C(=O)-O-C of acetyl esters. The carboxylic group was denoted by 1456 cm^{-1}. C-O of carbohydrate C-O-H was revealed by 1028 cm^{-1}. Spectra of PFADSL were well matched with SLs standard as shown in Fig. 4a. The result confirmed the presence of both acidic and lactonic forms of acetylated SLs in PFADSL. These data were parallel to previously reported spectra of SLs [59–61].

HPLC analysis

The relative percentages of acidic and lactonic analogs were calculated from the HPLC chromatogram. Fig. 4b shows that both the SLs standard and PFADSL gave the highest peak at 30.9 confirming that PFADSL contains majorly 1',4"-Sophorolactone 6',6"-diacetate (C18:1). Acidic SLs had short retention time <26 minutes and lactonic ones had longer ones (>26 min, 80% acetonitrile)[23]. PFDSL and SLs standard both contained high amounts of SLs (84.6% and 85.8% lactonic SLs).

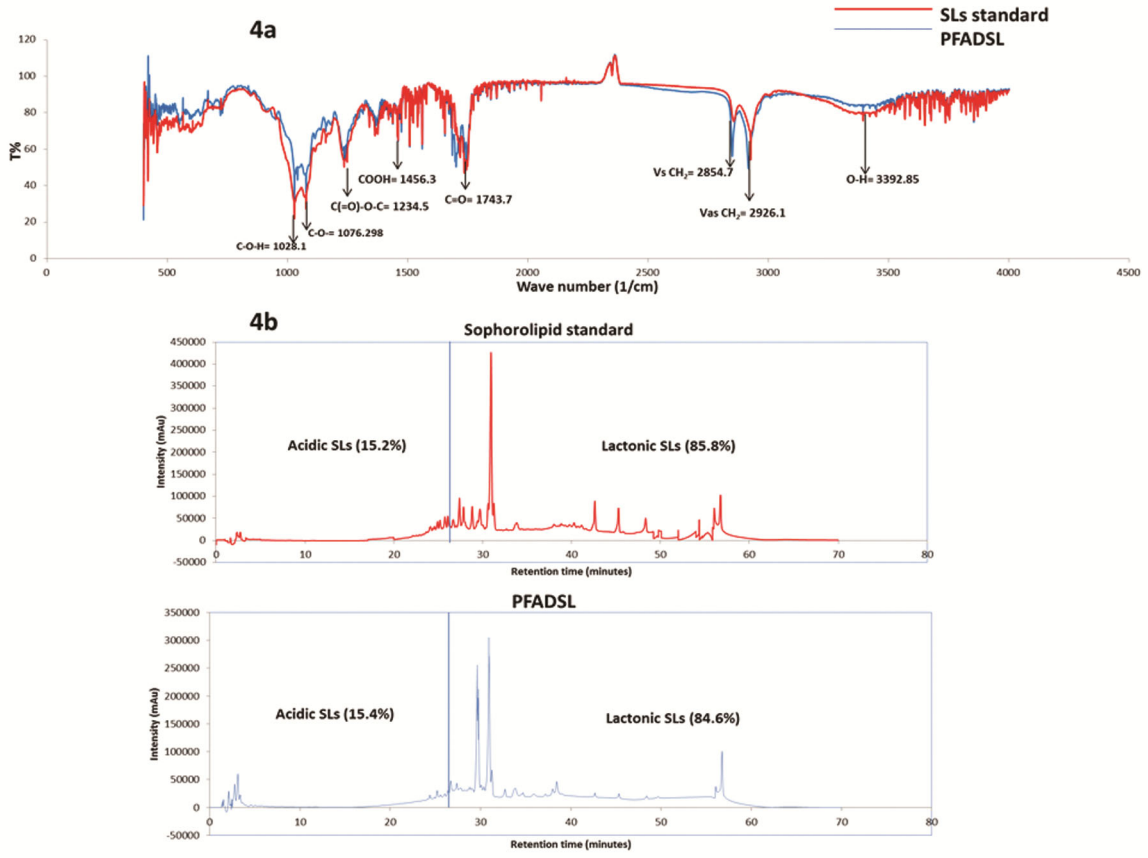

Fig. 4 — (a) FTIR spectra and (b) HPLC chromatograms of SLs standard and PFADSL

TLC offers the advantage of being cost-effective, requiring minimal sample preparation and no sophisticated equipment, making it accessible for routine analysis in research and industrial settings. While more detailed structural analyses, such as HPLC or mass spectrometry, are needed for comprehensive characterization, TLC serves as a quick and reliable screening method that informs further, more precise investigations. FTIR is a powerful tool for elucidating the functional groups present in SLs, allowing for the identification of specific molecular vibrations that correspond to different chemical bonds. By analysing the infrared absorption spectra, FTIR can differentiate between the lactonic and acidic forms of SLs, as well as detect the presence of ester, carboxyl, and hydroxyl groups. The technique is particularly valuable in confirming the structural integrity of SLs and monitoring changes that may occur during production or purification processes. FTIR, therefore, provides a rapid and non-destructive means of verifying the chemical identity and purity of SLs. HPLC is particularly useful for determining the relative concentrations of lactonic and acidic SLs, as well as identifying minor components that may influence the overall functionality of the biosurfactant. The high sensitivity and resolution of HPLC make it an indispensable tool for quality control, ensuring that sophorolipid preparations meet the specific requirements for their intended applications. The detailed characterization provided by these techniques supports the optimization of production processes, the formulation of new applications, and the advancement of SLs as sustainable and versatile biosurfactants.

Antibacterial activity

Zone of inhibition assay

Primarily the antimicrobial susceptibility of PFADSL against eight pathogenic bacterial strains was tested using agar well diffusion test. Fig. 5 shows the mortality zone diameters. The order of susceptibility based on inhibition at 1000 µg/mL was, *S. flexneri*^{a}> *K. pneumoniae*^{a}> *V. cholerae*^{a}> *P. vulgaris*^{a}>*E. coli*^{b}>*B. subtilis*^{b}>*S. aureus*^{c}> *S. typhi*^{c} (similar superscript alphabet represents no statistical significance at $p<0.05$).

MIC and MBC

Minimum inhibitory concentrations of PFADSL against *P. vulgaris* and *S. aureus* least MIC was found which was, 25 µg/mL of PFADSL. For the rest of the six bacteria, PFADSL exhibited MIC of 50 µg/mL (Fig. 6 and Table 1). Previously several studies showed an antibacterial effect on *S. aureus*. One of them reported that lactonic SLs exhibited MIC of 32 µg/mL against *S. aureus* though the ZOI was very low (0.9 cm at 2.5 mg/mL) [62]. But there is no proper data about the antimicrobial effect of any SLs against *P. vulgaris,* and *S. flexneri*. SLs from *Metschnikowia churdharensis* f.a., sp. nov. exhibited very low MICs (1 to 18 µg/mL) against pathogens like S. *aureus, V. cholerae, K. pneumoniae, L. monocytogenes, B subtilis, P. aeruginosa, B. cereus, E coli, S. enterica,* and *M. luteus* [60].

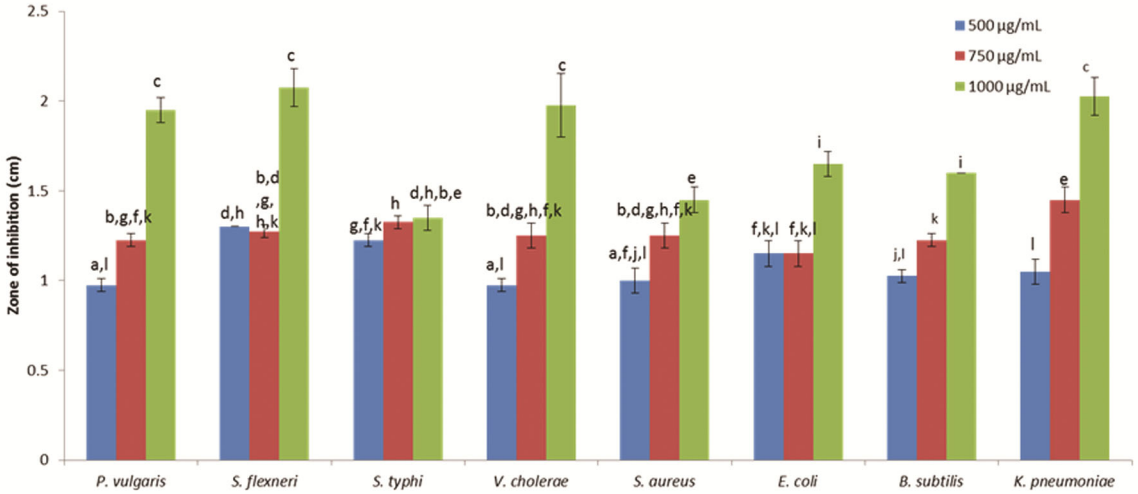

Fig. 5 — Zone of inhibition by PFADSL against bacterial pathogens. Bars with different superscript letters designate their statistically significant difference at $p<0.05$

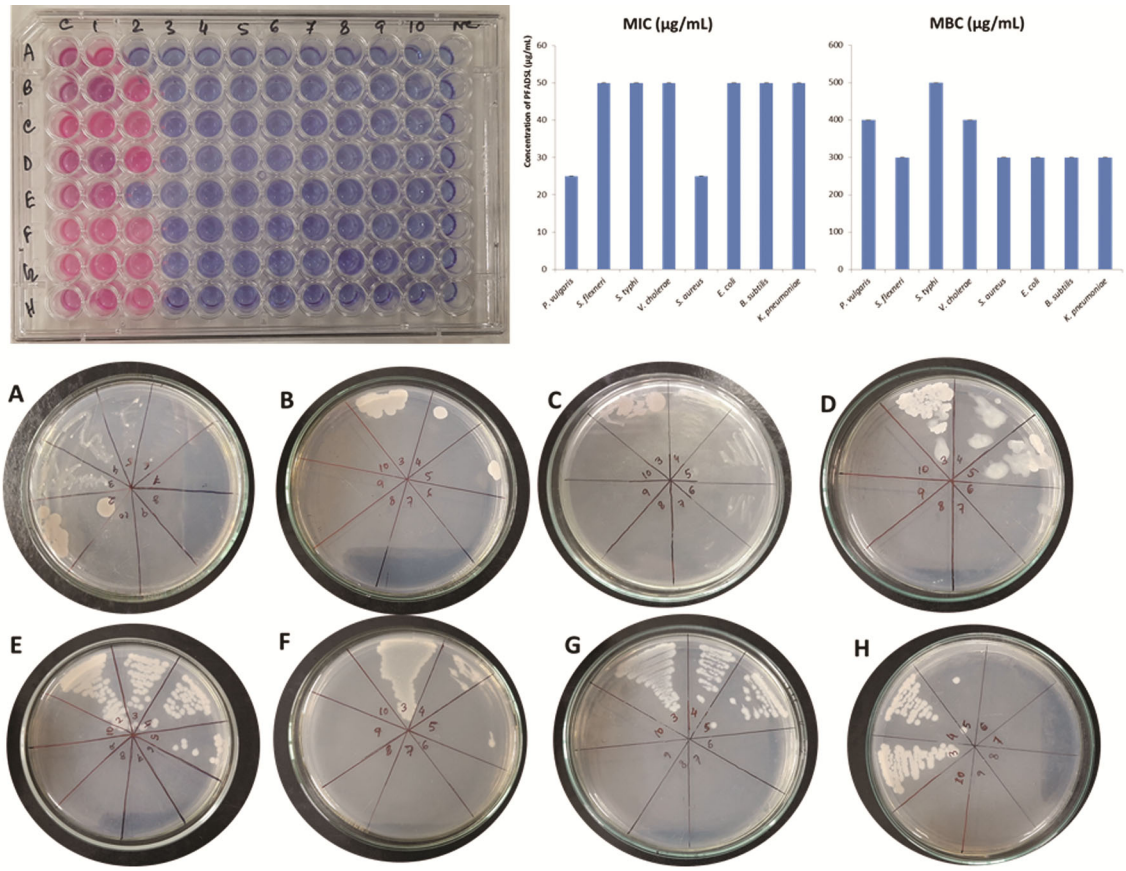

Fig. 6 — MIC and MBC of PFADSL (1-10= 12.5, 25, 50, 100, 200, 300, 400, 500, 750, and 1000 µg/mL) against, A-H= *P. vulgaris, S. flexneri, S. typhi, V. cholerae, S. aureus, E. coli, B. subtilis,* and *K. Pneumonia*

Table 1 — MIC, MBC, and tolerance values of PFADSL for bacterial pathogenic strains

Bacterial strains	Gram stain	MIC (µg/mL)	MBC (µg/mL)	Tolerance (MBC/MIC)	Inference
P. vulgaris	Gram-negative	25	400	16	Bacteriostatic
S. flexneri	Gram-negative	50	300	6	Bacteriostatic
S. typhi	Gram-negative	50	500	10	Bacteriostatic
V. cholerae	Gram-negative	50	400	8	Bacteriostatic
S. aureus	Gram-positive	25	300	12	Bacteriostatic
E. coli	Gram-negative	50	300	6	Bacteriostatic
B. subtilis	Gram-positive	50	300	6	Bacteriostatic
K. pneumoniae	Gram-negative	50	300	6	Bacteriostatic

Minimum Bactericidal concentration for *S. aureus, B. subtilis*, *E. coli,* and *K. pneumonia* wasfound to be 300 µg/mL, for *V. cholerae,* and *P. vulgaris* it was 400 µg/mL, and, for *S. typhi* MBC was 500 µg/mL. PFADSL was bacteriostatic against all the bacteria (MBC/MIC>4). All the data are represented in Fig. 6 and Table 1.

SEM for morphological characterisation:

From the SEM images represented in Fig. 7, it can be seen that control cells of both *E. coli* and *S. aureus* were intact in shape and size with no unusual changes. However, PFADSL at their MBCs caused detrimental effects on both of them. Interrupted cell walls, leakage, shrinkage, and complete membrane lysis were found in treated cells of both *S. aureus* and *E.coli*. Moreover, the cell size of the *E. coli* was found to be shorter than that of control cells. Studies have shown that membrane-targeting antibiotics can reduce volume and cell-surface area due to membrane synthesis inhibition [63]. The primary mechanism by which SLs exert their antibacterial effect is through rupturing of bacterial cell membranes. Their amphiphilic nature can insert themselves into the lipid

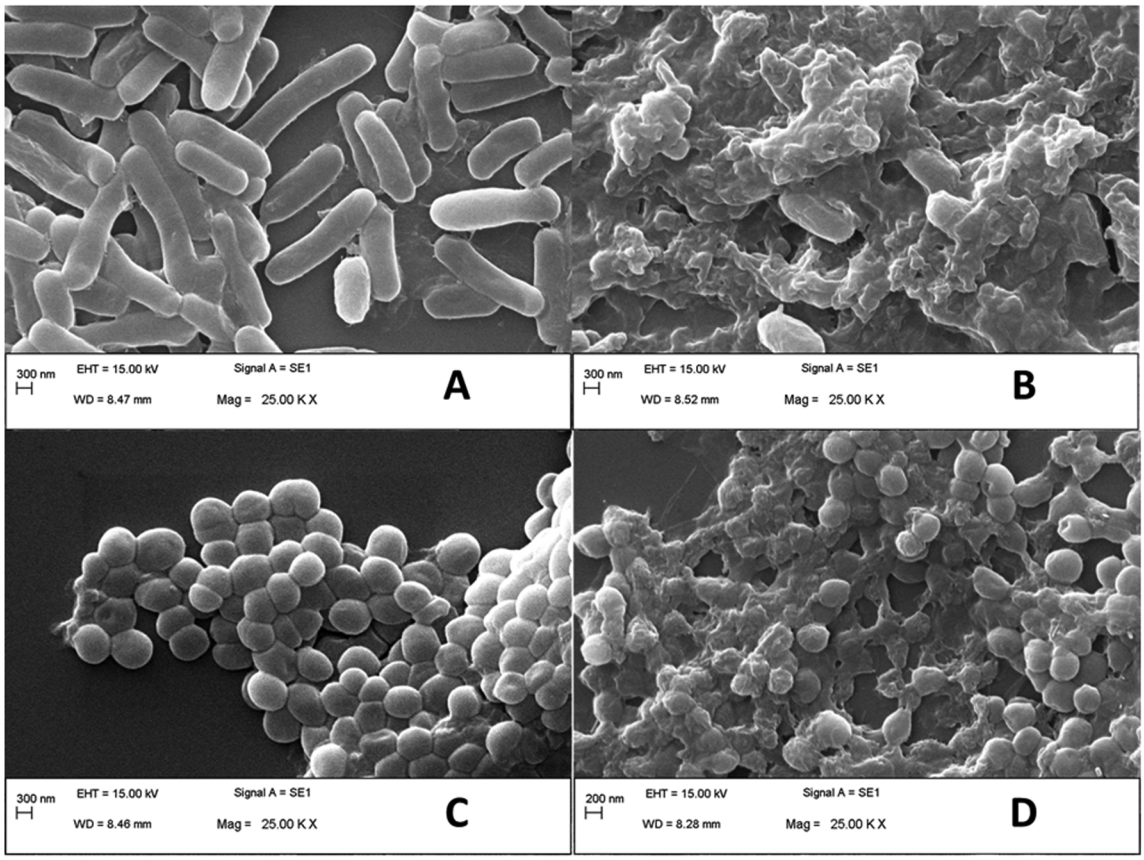

Fig. 7 — SEM images of *E. coli* and *S. aureus* control (A and C), *E. coli* and *S. aureus* (B and D) treated with PFADSL at their MBCs

bilayer of bacterial membranes, triggering increased membrane permeability. This destabilization causes the outflow of intracellular contents, loss of essential ions, and ultimately, cell lysis. The surfactant properties of SLs allow them to disrupt both Gram-positive and Gram-negative bacteria, although the specific composition of the bacterial membrane can influence susceptibility[6].

Antibiofilm activity

Biofilm inhibitory and eradication assay

Fig. 8a shows the biofilm inhibition against bacterial strains by PFADSL. PFADSL showed the lowest MBIC (I%≥50) against *P. vulgaris* and *S. aureus* (25 µg/mL). For *S. flexneri, S. typhi,* and *V. cholerae* the MBIC was found at 50 µg/mL. It was found that for all the tested organisms inhibition at MBIC was reached ~98%.

Fig. 8b shows the eradication of preformed biofilm. Treatment with PFADSL effectively eradicated preformed biofilms of all the tested bacterial strains. Biofilms were grown on polystyrene surfaces of flat bottom 96-well plates. For all five strains MBEC (I%≥50) values were the same as their MBICs. I% at their MBEC was between ~59 to ~93% (*S. typhi*[a]> *S. flexneri*[a]> *P. vulgaris*[b]> *S. aureus*[c]). Previous studies have shown that SLs either alone or in amalgamation with different antibacterial components (e.g., amphotericin-B, SDS, antimicrobial peptides, kanamycin, etc.) can effectively disrupt biofilm of different bacterial as well as fungal pathogens and combined effects reduce the SLs dosages [64–66]. SLs reduce biofilm adherence which can be utilized in biomedical fields, cosmetics, food, and agriculture industries[13,16,67]. Previous studies showed that SLs caused electrostatic repulsion and steric hindrance to disperse a biofilm component, β-glucan, and also restrict its hydration depicting a possible mechanism of its antibiofilm property[68,69].

SLs may also interfere with bacterial communication systems, such as quorum sensing, which regulate virulence factor production and biofilm formation. By inhibiting quorum sensing, SLs can prevent bacteria from coordinating the expression of genes necessary for infection, thereby reducing their pathogenicity. SLs have demonstrated the ability

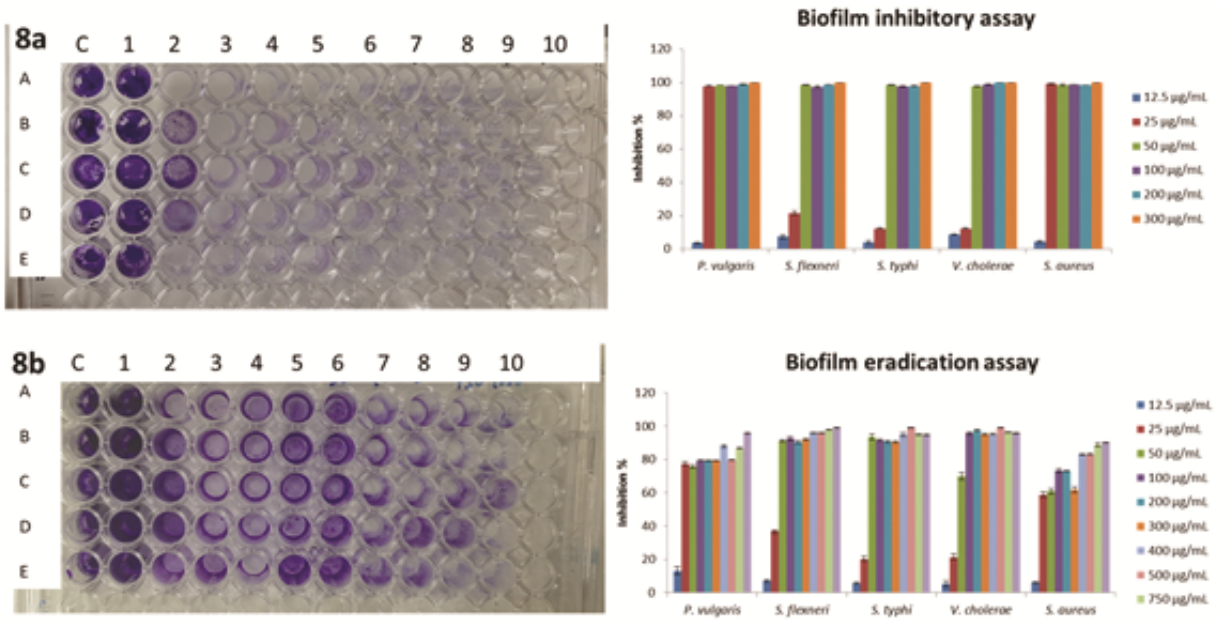

Fig. 8 — MBIC and MBEC of PFADSL (1-10= 12.5, 25, 50, 100, 200, 300, 400, 500, 750, and 1000 µg/mL) against, A-H= *P. vulgaris, S. flexneri, S. typhi, V. cholerae, S. aureus, E. coli, B. subtilis,* and *K. pneumoniae*

to inhibit the initial adhesion of bacteria to surfaces, thereby preventing biofilm formation. Additionally, SLs can penetrate and disrupt established biofilms, making the bacteria more susceptible to antimicrobial agents. This antibiofilm activity expands the potential applications of SLs in treating persistent infections associated with medical devices and chronic wounds[64,65,70,71].

One of the chief mechanisms through which sophorolipids exert their anti-biofilm effect is through the disruption of the extracellular polymeric substances (EPS) that constitute the biofilm matrix. The amphiphilic structure of sophorolipids permits them to penetrate the biofilm matrix and disrupt the cohesive forces that hold the biofilm structure together. By breaking down the EPS, sophorolipids expose the embedded microorganisms to environmental factors and antimicrobial agents, making them more susceptible to treatment [17,66]. SLs can prevent biofilm formation by inhibiting the initial adhesion of microbial cells to surfaces. This is particularly important in the early stages of biofilm development, where microbial cells attach to a substrate and begin to proliferate. SLs reduce surface tension and alter the surface properties of the substrate, making it less conducive to microbial attachment [72]. They also can detach established biofilms by destabilizing the interactions between the microbial cells and the substrate, as well as within the biofilm community itself. This detachment process involves the solubilization of hydrophobic components in the biofilm matrix and the disruption of cell-cell adhesion. In addition to their direct effects on biofilms, SLs can enhance the efficacy of conventional antimicrobial agents by increasing the permeability of biofilms. The disruption of the biofilm matrix by SLs allows antibiotics or antifungal agents to penetrate deeper into the biofilm, reaching microbial cells that would otherwise be protected [12,16,73–75].

SLs exhibit broad-spectrum antibacterial activity, effective against both Gram-positive and Gram-negative bacteria. This wide range of action is particularly beneficial in combating infections caused by multidrug-resistant pathogens, as SLs disrupt bacterial cell membranes, leading to cell lysis and death. Their mode of action, which differs from traditional antibiotics, also reduces the likelihood of developing resistance[76,77]. The versatile nature of SLs allows for their use in various industries, including healthcare, agriculture, food production, and environmental management. They can be incorporated into coatings, cleaning agents, and formulations designed to prevent bacterial contamination and biofilm formation. Their compatibility with other antimicrobial agents also enables synergistic effects, enhancing their overall

efficacy[6,13,78]. The regulatory landscape for biosurfactants like SLs is still evolving, and gaining approval for their use in specific applications, particularly in the healthcare and food industries, may be time-consuming and costly. Additionally, market acceptance of sophorolipid-based products may be hindered by competition from well-established synthetic surfactants, which are often cheaper and have a longer track record of use.

Conclusion

The present study has successfully demonstrated the bioconversion of industrial oil refinery by-product, PFAD, into valuable SLs using the yeast strain *Starmerella bombicola* MTCC 1910. The utilization of these low-cost, waste-derived substrates not only led to an enhanced production yield of SLs compared to traditional sunflower oil but also resulted in SLs with superior surface activity and emulsification properties. These findings highlight the dual advantage of using oil refinery by-products for SLs production, not only is waste effectively valorized, but also the resulting biosurfactants possess significant bioactive properties. This suggests that SLs derived from PFAD can be effectively employed in various biotechnological and medical applications, including antimicrobial formulations, biofilm control strategies, and antifungal therapies. Future research should focus on scaling up the production process and exploring the broader spectrum of biological activities of these biosurfactants, thus contributing to sustainable industrial practices and the advancement of green chemistry.

Conflict of interest

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgment

The authors would like to acknowledge the University Grants Commission of India for their financial support of this research and for awarding the fellowship to Miss Srija Pal fellowship ID 1626/(NET-DEC 2018).

Supplementary Information

Supplementary information is available on the website http://nopr.niscpr.res.in/handle/123456789.

References

1 Chang A S, Sherazi S T H, Kandhro A A, Mahesar S A, Chang F, Laghari Z H & Panhwar T, Characterization of palm fatty acid distillate of different oil processing industries of pakistan, *J Oleo Sci*, 65 (2016) 897.

2 Gapor M T, A production and utilization of palm fatty acid distillate (PFAD), *Lipid Technol*, 22 (2010) 11.

3 Handojo L, Indarto A, Shofinita D, Saadi M, Yulistia D & Hasyyati F, Calcium soap from palm fatty acid distillate for ruminant feed: Calcium oxide particles size, *MATEC Web Conf*, 268 (2019) 03001.

4 Abdul K N Z, Maniam G P, Rahim M H A & Yusoff M M, Palm fatty acid distillate as a potential source for biodiesel production-A review, *J Clean Prod*, 143 (2017) 1.

5 Estiasih T & Ahmadi K, Bioactive compounds from palm fatty acid distillate and crude palm oil, *IOP Conf Ser Earth Environ Sci*, 131 (2018) 012016.

6 Pal S, Chatterjee N, Das A K, McClements D J & Dhar P, Sophorolipids: A comprehensive review on properties and applications, *Adv Colloid Interface Sci*, 313 (2023) 102856.

7 Qazi M A, Wang Q & Dai Z, Sophorolipids bioproduction in the yeast Starmerella bombicola: Current trends and perspectives, *Bioresour Technol*, 346 (2022) 126593.

8 Ma X, Meng L, Zhang H, Zhou L, Yue J, Zhu H & Yao R, Sophorolipid biosynthesis and production from diverse hydrophilic and hydrophobic carbon substrates, *Appl Microbiol Biotechnol*, 104 (2020) 77.

9 Saerens K M J, Van Bogaert I N A & Soetaert W, Characterization of sophorolipid biosynthetic enzymes from Starmerella bombicola, *FEMS Yeast Res*, 15 (2015) 075.

10 Dierickx S, Castelein M, Remmery J, Clercq V D, Lodens S, Baccile N, Maeseneire S L D, Roelants S L K W & Soetaert W K, From bumblebee to bioeconomy: Recent developments and perspectives for sophorolipid biosynthesis, *Biotechnol Adv*, 54 (2022) 107788.

11 Van-Bogaert I N A, Zhang J & Soetaert W, Microbial synthesis of sophorolipids, *Process Biochem*, 46 (2011) 821.

12 Wang Y, Chen J & Liu X, A review on antimicrobial activity, anti-biofilm and synergistic effects of sophorolipids since their discovery, *Appl Biochem Microbiol*, 59 (2023) 580.

13 Cho W Y, Ng J F, Yap W H & Goh B H, Sophorolipids-bio-based antimicrobial formulating agents for applications in food and health, *Molecules*, 27 (2022) 5556.

14 Kim K, Yoo D, Kim Y, Lee B, Shin D & Kim E K, Characteristics of sophorolipid as an antimicrobial agent, *J Microbiol Biotechnol*, 12 (2002) 235.

15 Vestby L K, Grønseth T, Simm R & Nesse L L, Bacterial biofilm and its role in the pathogenesis of disease, *Antibiotics*, 9 (2020) 59.

16 Ceresa C, Fracchia L, Williams M, Banat I M & Díaz-De R M A, The effect of sophorolipids against microbial biofilms on medical-grade silicone, *J Biotechnol*, 309 (2020) 34.

17 Díaz-De R M A, Banat I M, Dolman B, Winterburn J & Martin P J, Sophorolipid biosurfactants: Possible uses as antibacterial and antibiofilm agent, *N Biotechnol*, 32 (2015) 720.

18 Wongsirichot P, Ingham B & Winterburn J, A review of sophorolipid production from alternative feedstocks for the

development of a localized selection strategy, *J Clean Prod*, 319 (2021) 128727.

19 Uyar E & Sağlam Ö, Isolation, screening and molecular characterization of biosurfactant producing bacteria from soil samples of auto repair shops, *Arch Microbiol*, 203 (2021) 4929.

20 Elshafie A E, Joshi S J, Al-Wahaibi Y M, Al-Bemani A S, Al-Bahry S N, Al-Maqbali D & Banat I M, Sophorolipids production by Candida bombicola ATCC 22214 and its potential application in microbial enhanced oil recovery, *Front Microbiol*, 6 (2015) 01324.

21 Sengupta D, Datta S & Biswas D, Surfactant exopolysaccharide of Ochrobactrum pseudintermedium C1 has antibacterial potential: Its bio-medical applications in vitro, *Microbiol Res*, 236 (2020) 126466.

22 Sen S, Borah S N, Bora A & Deka S, Production, characterization, and antifungal activity of a biosurfactant produced by Rhodotorula babjevae YS3, *Microb Cell Fact*, 16 (2017) 1.

23 Dubey P, Nawale L, Sarkar D, Nisal A & Prabhune A, Sophorolipid assisted tunable and rapid gelation of silk fibroin to form porous biomedical scaffolds, *RSC Adv*, 5 (2015) 33955.

24 Garg M, Priyanka & Chatterjee M, Isolation, characterization and antibacterial effect of biosurfactant from Candida parapsilosis, *Biotechnol Reports*, 18 (2018) e00251.

25 Chatterjee N, Pal S, Chakraborty A & Dhar P, Bacteria against bacteria: Green silver nanoparticle fabrication, antioxidant, anti-biofilm and antibacterial activities, *Indian J Chem Technol*, 31 (2024) 411.

26 Sahu M C, Patnaik R & Padhy R N, In vitro combinational efficacy of ceftriaxone and leaf extract of Combretum albidum G. Don against multidrug-resistant Pseudomonas aeruginosa and host-toxicity testing with lymphocytes from human cord blood, *J Acute Med*, 4 (2014) 26.

27 Das J K, Chatterjee N, Pal S, Nanda P K, Das A, Das L, Dhar P & Das A K, Effect of bamboo essential oil on the oxidative stability, microbial attributes and sensory quality of chicken meatballs, *Foods*, 12 (2023).

28 Shikha S, Chaudhuri S R & Bhattacharyya M S, Facile one pot greener synthesis of sophorolipid capped gold nanoparticles and its antimicrobial activity having special efficacy against gram negative vibrio cholerae, *Sci Rep*, 10 (2020) 1463.

29 Guchhait K C, Manna T, Barai M, Karmakar M, Nandi S K, Jana D, Dey A, Panda S, Raul P, Patra A, Bhattacharya R, Chatterjee S, Panda A K & Ghosh C, Antibiofilm and anticancer activities of unripe and ripe Azadirachta indica (neem) seed extracts, *BMC Complement Med Ther*, 22 (2022) 42.

30 Ibrahim S, Khalil K A, Zahri K N M, Gomez-Fuentes C, Convey P, Zulkharnain A, Sabri S, Alias S A, González-Rocha G & Ahmad S A, Biosurfactant production and growth kinetics studies of the waste canola oil-degrading bacterium rhodococcus erythropolis AQ5-07 from Antarctica, *Molecules*, 25 (2020) 3878.

31 Celligoi M A P C, Silveira V A I, Hipólito A, Caretta T O & Baldo C, Sophorolipids: A review on production and perspectives of application in agriculture, *Spanish J Agric Res*, 18 (2020) 1.

32 Jadhav J V, Pratap A P & Kale S B, Evaluation of sunflower oil refinery waste as feedstock for production of sophorolipid, *Process Biochem*, 78 (2019) 15.

33 Amiri F & Habibi A, Free fatty acids in sunflower oil soap stock progressed the sophorolipid production by Candida catenulata, *Can J Chem Eng*, 102 (2024) 53.

34 Felse P A, Shah V, Chan J, Rao K J & Gross R A, Sophorolipid biosynthesis by Candida bombicola from industrial fatty acid residues, *Enzyme Microb Technol*, 40 (2007) 316.

35 Kim H S, Kim Y B, Lee B S & Kim E K, Sophorolipid production by Candida bombicola ATCC 22214 from a corn-oil processing byproduct, *J Microbiol Biotechnol*, 15 (2005) 55.

36 Jahan R, Bodratti A M, Tsianou M & Alexandridis P, Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications, *Adv Colloid Interface Sci*, 275 (2020) 102061.

37 Haggag Y, Elshikh M, El-Tanani M, Bannat I M, McCarron P & Tambuwala M M, Nanoencapsulation of sophorolipids in PEGylated poly(lactide-co-glycolide) as a novel approach to target colon carcinoma in the murine model, *Drug Deliv Transl Res*, 10 (2020) 1353.

38 Kanwar R, Gradzielski M & Mehta S K, Biomimetic solid lipid nanoparticles of sophorolipids designed for antileprosy drugs, *J Phys Chem B*, 122 (2018) 6837.

39 Shah V, Dani P & Daverey A, Phytoremediation of heavy metal contaminated soil using bidens pilosa: Effect of varying concentrations of sophorolipids, *Appl Biochem Biotechnol*, 196 (2024) 2399.

40 Das T, Chatterjee N, Chakraborty A, Banerjee A, Haiti S B, Datta S, Chattopadhyay H & Dhar P, Fabrication of rice bran oil nanoemulsion and conventional emulsion with mustard protein isolate as a novel excipient: Focus on shelf-life stability, lipid digestibility and cellular bioavailability, *Food Hydrocoll Heal*, 4 (2023) 100143.

41 Chatterjee N & Dhar P, Nanoceuticals: Mystifying composites at the interface of nutrition, medicine, and technology, *Handb Nanotechnol Nutraceuticals*, (2022) 50.

42 Chakraborty A, Chatterjee N, Dey S & Dhar P, Sesame lignans as promising anti-inflammatory agent: Exploring novel therapeutic avenues with in silico and computational approach, *Indian J Chem Technol*, 30 (2023) 547.

43 Das J K, Chatterjee N, Nanda P K, Das A, Nath S, Pal S, Dhar P, Bandyopadhyay S, Verma A K, Sen A, McClements D J & Das A K, Encapsulation and delivery of clove essential oil using nanoemulsions: Impact on the physicochemical, microbial, and sensory properties of chicken meatballs, *Food Biophys*, 19 (2024) 701.

44 McClements D J, Das A K, Dhar P, Nanda P K & Chatterjee N, Nanoemulsion-based technologies for delivering natural plant-based antimicrobials in foods, *Front Sustain Food Syst*, 5 (2021) 643208.

45 Chatterjee N, Pal S & Dhar P, Green silver nanoparticles from bacteria- antioxidant, cytotoxic and antifungal activities, *Next Nanotechnol*, 6 (2024) 100089

46 Das A, Biswas S, Nanda P K, Chatterjee N, Pal S, Dhar P, Verma A K, Bhattacharya D, Koshy R & Das A K, Moringa pod derived antioxidant dietary fibre as a

quality enhancer in goat meat nuggets, *Sustain Food Technol*, 2 (2024) 232.

47 Xu K, Zhao X Y, Tan Y, Wu J, Cai Y, Zhou J & Wang X, A systematical review on antimicrobial peptides and their food applications, *Biomater Adv*, 155 (2023) 213684.

48 Karnwal A, Shrivastava S, Al-Tawaha A R M S, Kumar G, Singh R, Kumar A, Mohan A, Yogita & Malik T, Microbial biosurfactant as an alternate to chemical surfactants for application in cosmetics industries in personal and skin care products: A critical review, *Biomed Res Int*, 2023 (2023) 2375223.

49 Schippers C, Geßner K, Müller T & Scheper T, Microbial degradation of phenanthrene by addition of a sophorolipid mixture, *J Biotechnol*, 83 (2000) 189.

50 Saborimanesh N & Mulligan C N, Effect of sophorolipid biosurfactant on oil biodegradation by the natural oil-degrading bacteria on the weathered biodiesel, diesel and light crude oil, *J Bioremediation Biodegrad*, 06 (2015) 1000314.

51 Develter D W G & Lauryssen L M L, Properties and industrial applications of sophorolipids, *Eur J Lipid Sci Technol*, 112 (2010) 628.

52 Ma X, Wang T, Yu Z, Shao J, Chu J, Zhu H & Yao R, Formulation and physicochemical and biological characterization of etoposide-loaded submicron emulsions with biosurfactant of sophorolipids, *AAPS Pharm Sci Technol*, 23 (2022) 181.

53 Gaur V K, Regar R K, Dhiman N, Gautam K, Srivastava J K, Patnaik S, Kamthan M & Manickam N, Biosynthesis and characterization of sophorolipid biosurfactant by Candida spp.: Application as food emulsifier and antibacterial agent, *Bioresour Technol*, 285 (2019) 121314.

54 Elshafie A E, Joshi S J, Al-Wahaibi Y M, Al-Bemani A S, Al-Bahry S N, Al-Maqbali D & Banat I M, Sophorolipids production by Candida bombicola ATCC 22214 and its potential application in microbial enhanced oil recovery, *Front Microbiol*, 6 (2015) 1324.

55 Minucelli T, Ribeiro-Viana R M, Borsato D, Andrade G, Viviana M, Cely T, de Oliveira M R, Baldo C & Celligoi M A P C, Sophorolipids production by Candida bombicola ATCC 22214 and its potential application in soil bioremediation, *Waste Biomass Valori*, 8 (2017) 743.

56 Chandran P & Das N, Role of sophorolipid biosurfactant in degradation of diesel oil by Candida tropicalis, *Bioremediat J*, 16 (2012) 19.

57 Ahuekwe E F, Okoli B E, Stanley H O & Kinigoma B, Evaluation of hydrocarbon emulsification and heavy metal detoxification potentials of sophorolipid biosurfactants produced from waste substrates using yeast and mushroom, *Society of Petroleum Engineers - SPE African Health, Safety, Security and Environment and Social Responsibility Conference and Exhibition*, 2016 (2016) 81.

58 Sen S, Borah S N, Bora A & Deka S, Production, characterization, and antifungal activity of a biosurfactant produced by Rhodotorula babjevae YS3, *Microb Cell Fact*, 16, (2017) 95.

59 Daverey A & Pakshirajan K, Production, characterization, and properties of sophorolipids from the yeast Candida bombicola using a low-cost fermentative medium, *Appl Biochem Biotechnol*, 158 (2009) 663.

60 Kumari A, Kumari S, Prasad G S & Pinnaka A K, Production of sophorolipid biosurfactant by insect derived novel yeast metschnikowia churdharensis f.a., sp. nov., and its antifungal activity against plant and human pathogens, *Front Microbiol*, 12 (2021) 678668.

61 Bajaj V K & Annapure U S, Castor oil as secondary carbon source for production of sophorolipids using starmerella bombicola NRRLY-17069, *J Oleo Sci*, 64 (2015) 315.

62 Chen J, Zhifei L U, An Z, Ji P & Liu X, Antibacterial activities of sophorolipids and nisin and their combination against foodborne pathogen staphylococcus aureus, *Eur J Lipid Sci Technol*, 122 (2020) 1900333.

63 Cylke C, Si F & Banerjee S, Effects of antibiotics on bacterial cell morphology and their physiological origins, *Biochem Soc Trans*, 50 (2022) 1269.

64 Lydon H L, Baccile N, Callaghan B, Marchant R, Mitchell C A & Banat I M, Adjuvant antibiotic activity of acidic sophorolipids with potential for facilitating wound healing, *Antimicrob Agents Chemother*, 61 (2017) e02547.

65 Haque F, Sajid M, Cameotra S S & Battacharyya M S, Anti-biofilm activity of a sophorolipid-amphotericin B niosomal formulation against Candida albicans, *Biofouling*, 33 (2017) 768.

66 Nguyen B V G, Nagakubo T, Toyofuku M, Nomura N & Utada A S, Synergy between sophorolipid biosurfactant and SDS increases the efficiency of P. aeruginosa biofilm disruption, *Langmuir*, 36 (2020) 6411.

67 Aziz Z A A, Setapar S H M, Khatoon A & Ahmad A, The potential use of biosurfactants in cosmetics and dermatological products: Current trends and future prospects, *Biosurfactants Sustain Futur Prod Appl Environ Biomed*, (2021) 397.

68 Kaga H, Orita M, Endo K, Akamatsu M, Sakai K & Sakai H, Interaction between sophorolipids and β-glucan in aqueous solutions, *J Oleo Sci*, 73 (2024 169.

69 Kaga H, Nakamura A, Orita M, Endo K, Akamatsu M, Sakai K & Sakai H, Removal of a model biofilm by sophorolipid solutions: A QCM-D study, *J Oleo Sci*, 71 (2022) 663.

70 Anjos I, Bettencourt A F & Ribeiro I A C, Antimicrobial biosurfactants towards the inhibition of biofilm formation, *Urin Stents Curr State Futur Perspect*, (2022) 291.

71 Mendes R M, Francisco A P, Carvalho F A, Dardouri M, Costa B, Bettencourt A F, Costa J, Gonçalves L, Costa F & Ribeiro I A C, Fighting S. aureus catheter-related infections with sophorolipids: Electing an antiadhesive strategy or a release one, *Colloids Surfaces B Biointerf*, 208 (2021) 112057.

72 Valotteau C, Baccile N, Humblot V, Roelants S, Soetaert W, Stevens C V & Dufrêne Y F, Nanoscale antiadhesion properties of sophorolipid-coated surfaces against pathogenic bacteria, *Nanoscale Horizons*, 4 (2019) 975.

73 Alfian A R, Watchaputi K, Sooklim C & Soontorngun N, Production of new antimicrobial palm oil-derived sophorolipids by the yeast Starmerella riodocensis sp. nov. against Candida albicans hyphal and biofilm formation, *Microb Cell Fact*, 21 (2022) 163.

74 Valotteau C, Banat I M, Mitchell C A, Lydon H, Marchant R, Babonneau F, Claire-Marie P, Baccile N & Humblot V, Antibacterial properties of sophorolipid-

modified gold surfaces against Gram positive and Gram negative pathogens, *Colloids Surfaces B Biointerfaces*, 157 (2017) 325.

75 Valotteau C, Calers C, Casale S, Berton J, Stevens C V, Babonneau F, Claire-Marie P, Humblot V & Baccile N, Biocidal properties of a glycosylated surface: Sophorolipids on Au(111), *ACS Appl Mater Interfaces*, 7 (2015) 18086.

76 Puyol-McKenna P, Naughton P J, Dooley J S G, Ternan N G, Lemoine P & Banat I M, Microbial biosurfactants: Antimicrobial activity and potential biomedical and therapeutic exploits, *Pharmaceuticals*, 17 (2024) 138.

77 Juma A, Lemoine P, Simpson A B J, Murray J, O'Hagan B M G, Naughton P J, Dooley J G & Banat I M, Microscopic investigation of the combined use of antibiotics and biosurfactants on methicillin resistant staphylococcus aureus, *Front Microbiol*, 11 (2020) 01477.

78 Bueno-Mancebo J, Artola A, Barrena R & Rivero-Pino F, Potential role of sophorolipids in sustainable food systems, *Trends Food Sci Technol*, 143 (2024) 104265.