

Comparative Evaluation of Downstream Recovery Methods for Efficient Extraction of Sophorolipids from *Starmerella bombicola* MTCC 1910 at Pilot-Scale Fermentation

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Abstract

Sophorolipids have emerged as one of the most promising glycolipid biosurfactants for commercialization but are not widely used due to the limitations in large scale biosynthesis and economical recovery. In this work, an evaluation of sophorolipid biosynthesis and recovery on a pilot scale using *Starmerella bombicola* MTCC 1910 is presented. The fermentation process has been scaled up from 5 L lab scale bioreactor to 100 L pilot scale fermenter with optimization parameters (pH 4.5, 30°C, 1 VVM) and resulted in similar titer (109 and 110 gL⁻¹) after 192 h. The reliability of the process was tested with ten consecutive pilot scale fermentations and the resulting sophorolipids titer was more than 100 gL⁻¹ with minimal variations. The comparison of four approaches to downstream recovery – solvent extraction, acid precipitation, nanofiltration and dried broth assisted solvent extraction was carried out taking into consideration both recovery efficiency and performance of recovered biosurfactant. Conventional solvent extraction yielded maximum recovery (105.45 ± 10.0 gL⁻¹) and dried broth assisted solvent extraction had optimum compromise between recovery and biosurfactant performance. It was observed that these recovered sophorolipids had good surface activity properties, which included lowering of surface tension up to 31.12–36.10 mNm⁻¹, having an emulsifying activity index of 34–50%, and a critical micelle concentration range of 100.53–113.65 mgL⁻¹. It can be concluded from the data collected through the pilot scale fermentation study, comparative downstream processing studies, physicochemical characterization, and preliminary economic assessment that sophorolipid production is feasible at the industrial level.

1.0. Introduction

The family of glycolipid biosurfactants known as sophorolipids (SLs) is created by microbial fermentation and consists of a hydrophilic sophorose (β-1,2-linked glucose disaccharide) head connected to a fatty acid tail [1, 2]. Sophorolipids (SLs) have attracted significant research and commercial interest owing to their distinct advantages over many other biosurfactants. These advantages include high production titers, efficient biosynthesis by non-pathogenic microorganisms, and excellent surface- and interfacial tension-reducing properties. Together, these characteristics make sophorolipids one of the most promising biosurfactants for large-scale industrial production and a wide range of commercial applications[3, 4]. With a fatty acid moiety of about eighteen carbon atoms, SLs are normally produced naturally in two forms: lactonic (closed-ring) and acidic (open-ring). However, further structural alterations can be made to the SL structure to alter its physical characteristics and make it more appropriate for uses. For instance, when compared to the lactonic form, acidic SLs exhibit better surface activity, emulsification qualities, and environmental stability [5, 6]. Derivatization and post-fermentation chemical/enzymatic changes could accomplish this [7–9] as well as adjusting the feeding schedule for fed-batch fermentation and modifying the producer organisms [5].

The greatest portion of the 41.2 billion USD surfactant market, which is expected to grow to over 57.8 billion USD by 2028, is made up of SLs, a family of biosurfactants that are used in a variety of industries, including pharmaceuticals, home and personal care, and bioremediation [4, 10–15]. This size emphasizes how urgently sustainable, biodegradable substitutes for traditional surfactants are needed[16, 17]. However, when compared to conventional surfactants generated from petroleum, the market share of SL and biosurfactants in general remains incredibly small. The high production cost per unit mass of SL and biosurfactants, which restrict commercially feasible uses, is the reason for their restricted adoption [4, 18, 19]. This is in spite of the potential

benefits of biosurfactants, including their appropriateness for cutting-edge uses including antimicrobial and biomedical applications, as well as their decreased environmental effect, eco-toxicity, and biodegradability [20–22].

1.1. Development of pilot-scale sophorolipid production

Delbeke et al. used submerged fermentation of *S. bombicola*, their pilot-scale culture is the only one that is comparable to much of the literature. This demonstrates unequivocally that substantial future work is required to translate the amazing amount of progress regarding *S. bombicola* strains, medium, and fermentation at the laboratory scale toward large-scale production. A lactone esterase overexpression strain of *S. bombicola*, which generates preferentially lactonic SL at a 150-L scale using a fed-batch, was used by Delbeke et al. to carry out their fermentation. Using glucose, oleic acid, yeast extract, and ammonium sulfate, a higher SL titer of 199 gL^{-1} was attained. This is the highest level of SL titer in the literature attained at the pilot scale, and it was probably caused by the substrates used, especially oleic acid, as the SL titer dropped to 107 gL^{-1} and the SL productivity decreased from 0.9 to $0.6 \text{ gL}^{-1} \text{ h}^{-1}$ when the hydrophobic carbon source was switched to rapeseed oil [20]. Again, the switch from oleic acid to rapeseed oil coincided with SL yield reduction. One of the nitrogen sources also changed from yeast extract to corn steep liquor; however, the impact of the nitrogen source substitution is unlikely to be so significantly negative due to their previous use for high SL titer within the literature [21]. It is more likely that the decreased SL titer and productivity were caused by changes in mass transport, especially oxygen.

According to Kim et al., continual rapeseed oil feeding at a low concentration set point could probably result in higher SL production [23]. In contrast to their previous 3-L fermentation, which produced 139 gL^{-1} SL titer $0.94 \text{ gL}^{-1} \text{ h}^{-1}$ productivity, Delbeke et al. nevertheless showed a very steady SL output upon scale-up [24]. The overall SL production should be improved to less than $1 \text{ gL}^{-1} \text{ h}^{-1}$. This happened despite a peak production of $1.7 \text{ gL}^{-1} \text{ h}^{-1}$ at the 72-hour mark, which may have been brought on by SL accumulation because the SL titer was about at this midpoint. The use of semi-continuous fermentation and integrated separation, both of which have demonstrated high productivity, will surely boost pilot size production.

Another other example of submerged fermentation at a larger scale was conducted more recently by Kim et al. using *Candida batistae* [25]. Fermentation was carried out at several scales in their investigation. In this instance, SL titers of 24.1, 28.6, and 53.2 gL^{-1} were obtained using an optimized medium consisting of 50 gL^{-1} glucose, 50 gL^{-1} rapeseed oil, 5 gL^{-1} ammonium nitrate, and 5 gL^{-1} yeast extract, including shake flask, 5-L fed-batch bioreactor, and 50-L fed-batch bioreactor, respectively. Although there was a noticeable improvement in this study when scaling up, the application of a feeding regimen is responsible for the improvement. Additionally, the production of SL is smaller than that of *S. bombicola*, despite the possibility of higher added value due to the unique SL structure that contains ω -hydroxy fatty acids. According to Kim et al., *C. batistae*'s poor metabolic flow toward SL synthesis may be the cause of its low SL output. Therefore, SL production with *C. batistae* may be further improved by applying producer organism improvement techniques, such as genetic engineering to increase carbon flux toward SL synthesis [25].

Lastly, employing solid-state fermentation at 22-L and 100-L scales, the same study team as previously described Eras-Munoz et al. investigated pilot-scale SL production while evaluating the impact of organic solid

waste chemical composition and physical characteristics at lab and pilot scales [26–28]. For their 22-L and 100-L solid-state fermentations, respectively, Rodriguez et al. reported producing 0.19 and 0.14 g SL per g starting dry matter. Additionally, a notable decline in SL production was noted. Changes in aeration needs and preserving the required temperature profile during scale-up seem to be major obstacles for solid-state SL production [28]. Although a higher aeration rate would result in higher running costs, it was necessary because the organism could be adversely affected by the higher temperature. Future research should focus on improving reactor design to boost heat dissipation and air flow efficiency. In general, the literature on SL production has very little information about reactor architecture and engineering. For better reactor designs, there should be more multidisciplinary cooperation between chemical engineers and biologists/biochemical engineers. Better mass transfer and lower capital and operating costs in both solid-state and submerged SL production could result from this, which is crucial for commercial SL production.

In the present study, sophorolipid production was systematically investigated through laboratory-scale (5 L) and pilot-scale (100 L) fermentations, followed by validation of process robustness through ten consecutive pilot-scale batches. Furthermore, four downstream recovery approaches, including solvent extraction, acid precipitation, nanofiltration, and dried broth-assisted solvent extraction, were comparatively evaluated with respect to recovery efficiency and biosurfactant functionality. The recovered sophorolipids were comprehensively characterized using high-performance liquid chromatography (HPLC), surface tension measurements, emulsification index (E24), and critical micelle concentration (CMC) analyses to evaluate their composition and functional performance. Collectively, the successful scale-up of fermentation, optimization of downstream recovery, detailed physicochemical characterization, and preliminary techno-economic evaluation provide a comprehensive assessment of the industrial potential of sophorolipid production. These findings establish a practical foundation for the development of commercially viable and sustainable biosurfactant manufacturing processes, while supporting future efforts toward process optimization and industrial-scale implementation.

2.0. Material and Methods

2.1. Substrates and Chemicals

Oleic acid and commercial biosurfactant sophorolipid was obtained from M/s Godrej Industries Ltd., Mumbai. Glucose was procured from S D Fine-Chem Limited, Mumbai. All other chemicals (peptone, yeast extract, glucose, and urea) and solvents (hexane and ethyl acetate) used in this study were supplied by M/s Hi-Media, Mumbai. A commercially available sophorolipid, 1,4"-sophorolactone 6', 6"-diacetate, was procured from Sigma-Aldrich, USA. Commercial biosurfactant sophorolipid procured from Godrej Industries and sigma Aldrich is used as standard for performance testing. The culture medium was applied for sophorolipid production with the following composition: yeast extract (4 gL^{-1}), urea (1 gL^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (5 gL^{-1}), KH_2PO_4 (1 gL^{-1}), NaCl (0.1 gL^{-1}), pH 4.5. Glucose (10%) and lipid substrates (10%) were used as carbon source[29].

2.2. Microbial Strain

Starmerella bombicola is the principal microbial producer of sophorolipids and is widely recognized as one of the most efficient yeasts for industrial biosurfactant production. Despite its established commercial importance and extensive investigation in scientific research, this non-pathogenic yeast remains comparatively

less well known than many conventional industrial microorganisms. Owing to its ability to produce high titers of sophorolipids from a wide range of renewable carbon substrates, *S. bombicola* has become a preferred production host for the commercial manufacture of these glycolipid biosurfactants and continues to attract considerable interest for biotechnological and industrial applications[30]. The strain *Starmerella bombicola* MTCC 1910 used for sophorolipid production was purchased from the MTCC & Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India. This culture was regularly cultivated on YEPD agar slants where 0.3% (w/v) yeast extract, 1.0% (w/v) peptone, 2.0% (w/v) glucose, and 2.5% (w/v) agar were included. Stock cultures were grown under the suitable growth conditions, and regular sub-cultivation was done after every four weeks[29].

2.3. Development of inoculum

A loop of *Starmerella bombicola* cells taken from the slant was added into a 250 mL Erlenmeyer flask containing 50 mL YEPD broth. *Starmerella bombicola* cells were grown in a rotary shaker set at 30° C for 48 h at 210 rpm. The cells were separated by centrifugation and resuspended in sodium chloride solution (0.7%). This cell suspension was used as inoculum (2% v/v) 3.6×10^{14} CFU mL⁻¹ in a sterile production medium[29].

2.4. Estimation of Biomass

The broth was treated twice with an equal volume of ethyl acetate to estimate biomass. The ethyl acetate phase was collected to determine sophorolipid content and residual oil. The aqueous phase containing cells was centrifuged at 12,000×g for 15 min at 25°C. The resulting cell pellets were washed twice with saline water and dried at 80°C to constant weight[29].

2.5. Estimation of glucose

The glucose content was estimated from cell supernatant. Glucose consumption was calculated by the GOD/POD kit (Accurex Biomedical Pvt. Ltd., India). In this method, glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase (GOD). Hydrogen peroxide formed in this reaction oxidatively couple with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) which produce quinoneimine dye. The absorbance of the dye was measured at 505 nm and intensity of color complex was directly proportional to the concentration of glucose in the sample[29].

2.6. Estimation of Oleic acid

Oleic acid consumption was determined by evaporating the hexane extracts. Oleic acid compositions of the residual and fresh lipophilic substrate were determined by gas chromatography. The residual fatty acid content in broth was calculated based on the amount of residual lipid substrate and their fatty acid composition[29].

2.7. Production of sophorolipids

2.7.1. Flask-scale culture conditions

The shake flask study was conducted with 50 ml culture medium in 250 ml Erlenmeyer flasks along with both carbon sources. The cultures were incubated in an orbital shaker incubator (Remi CIS 24 Plus) at 30°C and 200 rpm for 8 days. The lipid source (10% w/v) used was oleic acid combined with glucose (10% w/v) as carbon sources[29].

2.7.2. Bench top fermenter scale culture conditions

The fermentations were accomplished in a BioFlo 115 bioreactor (Batch/Continuous Fermenter; New Brunswick Scientific; USA) with a capacity of 7 L. The fermenter was equipped with baffles and a 6-blade Rushton-type impeller for agitation. The yeast cells were precultured for 48 h in a flask with 50 ml of culture medium. This seed culture was used to inoculate 5 L of culture medium in the fermenter. The agitation speed was limited to 550 rpm. The temperature was kept constant at 30°C, and the aeration rate was maintained at 1 VVM. The pH was maintained at 3.5 using 5 M sodium hydroxide and 5 M sulfuric acid solution for 8 days of incubation period. During fermentation, after every 24 h, samples were withdrawn from the broth to monitor the biomass formation, residual carbon substrate, and sophorolipid concentration.

2.7.3. Pilot Scale Fermentation

The fermentations were accomplished in a 150L Fermenter manufactured by Scigenics India Pvt. Ltd. with a capacity of 150 L and working capacity of 100–120 L. The fermenter was equipped with baffles and a 6-blade Rushton-type impeller for agitation. The yeast cells were precultured for 48 h in benchtop fermenter with 2 L of culture medium. This seed culture was used to inoculate 100 L of culture medium in the fermenter. The agitation speed was limited to 550 to 1000 rpm. The temperature was kept constant at 30°C, and the aeration rate was maintained at 1 VVM. The pH was maintained at 4.5 using 5 M sodium hydroxide and 5 M sulfuric acid solution for 8 days of incubation period. During fermentation, after every 24 h, samples were withdrawn from the broth to monitor the biomass formation, residual carbon substrate, and sophorolipid concentration.

2.8. Characterization of sophorolipid

The efficiency of the recovery protocol was evaluated by measuring the permeate flux and the observed rejection coefficient for total SLs. Samples from the feed, permeate, and retentate were analyzed via HPLC-ELSD to quantify the specific recovery of both lactonic and acidic forms. An Agilent Technologies 1200 series HPLC system equipped with a Zorbax SB-C18 column (250 mm X 46 mm, 5 µm), UV detector, autosampler, and integrator was used to quantify the crude SL content. The mobile phase consists of acetonitrile and water 80:20 with a flow rate of 0.5 ml/min. The detector wavelength was 207 nm, and analysis was performed at 30°C. An Agilent 1200 HPLC system consisting of a binary pump and autosampler was used[29].

2.9. Sophorolipid recovery

2.9.1. Solvent Extraction (SL 1.0)

The culture broth obtained after harvesting the batch was used for solvent extraction study. The broth centrifuged at 12000 rpm for 20 min to remove biomass and insoluble precipitates. The cell free broth was extracted with hexane to remove residual oleic acid. 50 ml of the cell free broth was extracted with an equal volume of the solvent hexane. The extract of each stage was collected in a preweighed round-bottom flask separately. The hexane was then evaporated under vacuum, and the product was dried in a vacuum oven to remove the traces of the hexane if present. 50 ml of the cell free broth was extracted three times with an equal volume of the solvent ethyl acetate. The extract of each stage was collected in a preweighed round-bottom flask separately. The ethyl acetate were then evaporated under vacuum depending upon their boiling points, and the product was dried in a vacuum oven to remove the traces of the ethyl acetate if present. This method is used for extraction of sophorolipid at pilot scale.

2.9.2. Acid Precipitation (SL 2.0)

The culture broth obtained after harvesting the batch was used for solvent extraction study. The broth centrifuged at 12000 rpm for 20 min to remove biomass and insoluble precipitates. The culture broth obtained after harvesting the batch was used for solvent extraction study. The broth centrifuged at 12000 rpm for 20 min to remove cell mass. 2N HCl solution was used to acidify the cell free broth containing BS, until pH 2. The mixture was then incubated 24 hrs. at 60 ° C. The precipitation was collected by centrifugation at 10000 rpm, 30 ° C for 30 min. The precipitate was then dried, and after 24 hrs. pale yellow colored paste obtained.

2.9.3. Nano Filtration (SL 3.0)

The recovery of sophorolipids (SLs) was initiated by stabilizing the fermentation broth to ensure optimal membrane performance and longevity. The raw broth was first subjected to centrifugation at 12000 for 20 minutes biomass and insoluble precipitates. Following biomass removal, the pH of the fermentation supernatant was adjusted to 6.5 using 1 M NaOH. This step was essential to ensure complete deprotonation of the acidic sophorolipid (SL) congeners, thereby enhancing their ionization. Increased ionization enlarged the effective hydrodynamic diameter of the SL molecules and strengthened electrostatic repulsion between the negatively charged sophorolipids and the membrane surface, improving solute rejection while reducing membrane fouling. The pH-adjusted supernatant was subsequently clarified by passing it through a 0.22 µm polyethersulfone (PES) microfiltration membrane to remove residual proteins, microbial debris, and fine suspended particulates, producing a clean feed stream for nanofiltration. Nanofiltration was carried out using a high-pressure crossflow filtration system fitted with a thin-film composite (TFC) polyamide membrane with a molecular weight cut-off (MWCO) of 300 Da. The process was operated at a constant temperature of 25°C to maintain the structural stability of the heat-sensitive lactonic sophorolipid molecules throughout filtration. Operating conditions included a transmembrane pressure (TMP) of 1.5 MPa and a crossflow velocity of 0.5 ms⁻¹, providing effective separation while minimizing concentration polarization and membrane fouling. The system was operated in a batch concentration mode where the permeate was continuously removed and the retentate was recycled back into the feed tank until a Volume Reduction Factor (VRF) of 5 was achieved. To ensure high purity and remove residual mineral salts and glucose, the process concluded with three volumes of constant-volume diafiltration using deionized water.

2.9.4. Dried Broth and solvent extraction

The harvested broth centrifuged at 12000 rpm for 20 min to remove biomass and insoluble precipitates. The cell free broth was extracted with hexane to remove residual oleic acid. 50 ml of the cell free broth was extracted with an equal volume of the solvent hexane. The extract of each stage was collected in a preweighed round-bottom flask separately. The hexane was then evaporated under vacuum, and the product was dried in a vacuum oven to remove the traces of the hexane if present. After hexane extraction 50 ml of the cell free broth was dried at 60 ° C for 24 hrs. The dried paste material extracted three times with an equal volume of the solvent ethyl acetate. The extract of each stage was collected in a preweighed round-bottom flask separately. The ethyl acetate were then evaporated under vacuum depending upon their boiling points, and the product was dried in a vacuum oven to remove the traces of the ethyl acetate if present.

The functional integrity of the recovered sophorolipids was further evaluated by determining their critical micelle concentration (CMC) using the Wilhelmy plate method. Following concentration, the purified sophorolipid fraction was lyophilized to obtain a stable dry product suitable for storage and subsequent characterization. To ensure consistent membrane performance and process reproducibility, the nanofiltration membrane was regenerated after each filtration cycle through sequential cleaning with 0.1 N NaOH followed by deionized water. This cleaning protocol effectively restored the baseline water flux, enabling reliable operation across successive recovery batches.

3.0. Emulsifying Index (E)

The emulsifying capacity of the biosurfactant was determined by adding 2 mL of the 0.1% solution of biosurfactant into a glass test tube and 2 mL of paraffin oil. It was subsequently vortexed at high speed for 5 minutes. After allowing it to settle for 24 hours, the emulsification activity was evaluated, and the emulsification index (E₂₄) was calculated by measuring the height of the emulsion layer as a percentage of the total height of the mixture in the tube as shown in equation below

$$Emulsifying\ Index\ (E_{24}) = \frac{Height\ of\ Emulsion\ layer\ in\ tube}{Height\ of\ Total\ Solution\ in\ tube} \times 100$$

1

Surface Tension Measurement

The surface tension was measured using the Du Noüy ring method at a constant temperature of 25°C. For this test, sophorolipids were dissolved in distilled water at different concentrations. A small platinum-iridium ring was dipped into the liquid and slowly pulled out; the force required to lift the ring from the surface determines the surface tension. To ensure the accuracy and reproducibility of the surface tension measurements, the platinum ring was thoroughly cleaned by flame sterilization before each analysis to eliminate residual contaminants. The tensiometer was calibrated using ultrapure water, which has a standard surface tension of 70.0 ± 0.2 mNm⁻¹ under the measurement conditions. All samples were analyzed in triplicate, and the results are reported as the means of three independent measurements to ensure analytical reliability.

6.0. Critical Micelle Concentration (CMC)

The CMC value of the biosurfactant was taken as the concentration at which the biosurfactant started self-assembling to form micelles. To determine this value, the surface tension values were measured for varying concentrations of biosurfactant, and a graph of surface tension values vs. concentration was plotted. As the biosurfactant concentration increased, the surface tension decreased very fast because of the adsorption of the surfactants at the air-liquid interface. After a certain concentration level, further addition of biosurfactant did not affect the surface tension significantly, thereby indicating saturation of the interface and formation of micelles in the bulk solution. This breaking point of the graph was considered the CMC value.

3.0. Results

3.1. Lab Scale Fermentation (5 L)

As indicated in Fig. 3, there was an increasing trend in the production of sophorolipids during the fermentation cycle up to the point where there was a peak sophorolipid concentration of 109 gL^{-1} after 192 hours of cultivation. The fermentation process was carried out at optimum conditions at a pH level of 4.5 with a steady rate of aeration of 5 LPM (1 VVM) and the agitation rate increased gradually from 300 to 550 rpm to ensure adequate oxygen transfer for the growing biomass. There was an increasing biomass concentration up to 5.5% along with substantial utilization of the substrates where oleic acid dropped from 10% to 1.4% and glucose from 10% to 1.1%. There were fluctuations in dissolved oxygen (DO) depending on the metabolic changes in the culture with the lowest level of 15.7% attained at 48 h during the exponential phase, rising to 54.4% at the end of cultivation as a result of decreased utilization of substrates.

3.2. Pilot Scale Fermentation (100 L)

Figure 4 demonstrates that the production of sophorolipids was scaled up to 100 L reactor, where the fermentation was carried out under optimized conditions with a constant pH value of 4.5 and aeration rate of 100 LPM (1 VVM). With the aim of meeting the growing oxygen requirements related to biomass accumulation and production of sophorolipids, the speed of agitation increased from 400 to 800 rpm during the fermentation process. There was a constant increase in the production of sophorolipids, which reached its peak level at 110 gL^{-1} after 192 hours of fermentation. At the same time, there was an accumulation of biomass up to 18%, which showed the growth and metabolic activity of cells. There was efficient utilization of the substrates, since there was depletion of oleic acid and glucose from 10% to 1.5%. Minimum dissolved oxygen (DO) concentration was observed as 15.7% at 48 hours due to increased oxygen consumption because of the rapid growth of bacteria during the exponential growth phase. However, it gradually rose to 54.4% towards the end of fermentation because of reduced metabolic activities and limited substrates. The strong association between biomass, substrate and sophorolipid production reveals efficient conversion of carbon and oxygen management. In addition, the trend of production of sophorolipid and its titer was like those observed in the 5 L bioreactor. This indicates that the process is reproducible, robust and scalable to higher volumes.

Table 1

The sophorolipid yield data obtained from the 10 consecutive trail batches at pilot scale fermenter.

Batch No.	Batch Size (L)	Yield of Sophorolipid (g L⁻¹ ± 10.0)	Sophorolipid Recovered (kg100 L⁻¹ ± 1.0)	Residual Oleic acid (Kg 100 L⁻¹ ± 1.0)
1	100	95.32	9.532	1.34
2	100	100.97	10.097	1.54
3	100	93.23	9.323	2.43
4	100	98.43	9.843	1.69
5	100	97.87	9.787	2.5
6	100	108.73	10.873	1.39
7	100	104.53	10.453	1.2
8	100	93.56	9.356	2.4
9	100	110.43	11.043	2
10	100	102.76	10.276	1.9

To assess the robustness and repeatability of the developed process, 10 consecutive fermentation runs of pilot-scale were performed in a 100-L bioreactor under the same operating conditions. Production of sophorolipids was quite consistent across all batches with sophorolipid titers in the range of 95.62-110.43 gL⁻¹ and an average of around 101.4 gL⁻¹. In correspondence with the produced amount of sophorolipids, the sophorolipid yield recovered ranged from 9.56 to 11.04 kg in 100 L of fermentation broth. Residual amounts of oleic acid used in the process were low – in the range of 1.2–2.5 kg per 100 L of culture broth (on average 1.89 kg per 100 L). The low variability of the results obtained with respect to production of sophorolipids, their recovery, and substrates used provide evidence of robustness and stability of the process. The overall results obtained demonstrate the high reproducibility of the optimized fermentation process at the pilot scale, which may ensure high titers of sophorolipids.

Table 2

Sophorolipid yield comparison between Bench top scale 5 L and pilot scale 100 L fermenter scale.

Age (Hours)	5 L fermenter Sophorolipid Yield ($\text{g L}^{-1} \pm 10.0$)	100 L fermenter Sophorolipid Yield ($\text{g L}^{-1} \pm 10.0$)
0	0	0
24	11	13
48	25	22
72	37	32
96	48	49
120	69	73
144	89	92
168	94	105
192	109	110

Table 3

Comparison of Sophorolipid recovered by different methods and their properties.

Sr. No.	Sample	Sophorolipid Yield g L^{-1}	Lactonic Sophorolipid ¹ (%)	Acidic Sophorolipid ² (%)	Emulsifying Index (EI_{24}) (%)	Surface Tension of 0.1% Solution (mNm^{-1}) ¹⁾	CMC (mg L^{-1})
1	Standard Sophorolipid	----	82.41 ± 5.0	----	----	----	55.43
2	Commercial Sophorolipid	----	68.53 ± 5.0	27.64 ± 5.0	50 ± 5.0	32.63 ± 5.0	98.94
3	SL 1.0	105.45 ± 10.0	66.61 ± 5.0	28.53 ± 5.0	50 ± 5.0	31.12 ± 5.0	100.53
4	SL 2.0:	56.24 ± 10.0	38.41 ± 5.0	14.52 ± 5.0	35 ± 5.0	36.10 ± 5.0	103.68
5	SL 3.0	67.31 ± 10.0	42.16 ± 5.0	10.25 ± 5.0	34 ± 5.0	33.68 ± 5.0	105.34
6	SL 4.0	83.23 ± 10.0	46.53 ± 5.0	12.45 ± 5.0	40 ± 5.0	32.51 ± 5.0	113.65

¹: The lactonic sophorlipid yield is measured by using HPLC chromatogram attached in supplementary material

2

The acidic sophorlipid yield is measured by using HPLC chromatogram attached in supplementary material

3.3. Comparison of Sophorolipid Production in 5 L and 100 L Fermenters

A comparative assessment of sophorolipid production in 5 L and 100 L stirred-tank bioreactors demonstrated the successful scale-up of the fermentation process while maintaining high product productivity. In both systems, sophorolipid production increased steadily throughout cultivation and reached its maximum concentration after 192 h of fermentation. The 5 L bioreactor produced a final sophorolipid concentration of 109 gL^{-1} , whereas the 100 L pilot-scale bioreactor achieved a slightly higher titer of 110 gL^{-1} . Throughout the fermentation process, sophorolipid accumulation was generally higher in the 100 L bioreactor, with concentrations of 13, 22, 32, 49, 73, 92, and 105 gL^{-1} recorded at 24, 48, 72, 96, 120, 144, and 168 h, respectively, compared with 11, 25, 37, 48, 65, 89, and 94 gL^{-1} in the 5 L system.

The improved production profile observed in the 100 L bioreactor is likely associated with enhanced oxygen transfer, more efficient mixing, and favorable hydrodynamic conditions that promoted microbial growth and sophorolipid biosynthesis. Although the pilot-scale system exhibited higher productivity during the intermediate stages of fermentation, both bioreactors achieved nearly identical final product titers, indicating that the optimized fermentation strategy was successfully translated from laboratory to pilot scale without compromising process efficiency. The similarity in the final yields further demonstrates that critical operating parameters, including pH control, aeration, agitation, and substrate feeding, were effectively maintained during scale-up. Overall, these results confirm the robustness, reproducibility, and scalability of the developed fermentation process, highlighting its potential for industrial scale sophorolipid production and commercial biosurfactant manufacturing.

3.4. Sophorolipid Recovery Strategies

Four downstream recovery strategies were systematically evaluated to determine their effectiveness for sophorolipid purification and to assess their influence on product yield, composition, and surface-active properties. The conventional solvent extraction method (SL 1.0) achieved the highest sophorolipid recovery, yielding $105.45 \pm 10.0 \text{ gL}^{-1}$. The recovered product consisted predominantly of lactonic sophorolipids ($66.61 \pm 5.0\%$), with acidic sophorolipids accounting for $28.53 \pm 5.0\%$. This preparation also exhibited the most favorable functional characteristics, reducing surface tension to $31.12 \pm 5.0 \text{ mNm}^{-1}$, achieving an emulsification index (E24) of $50 \pm 5.0\%$, and displaying the lowest critical micelle concentration (CMC) of 100.53 mgL^{-1} , indicative of high surface activity.

Acid precipitation (SL 2.0) produced a substantially lower sophorolipid yield of $56.24 \pm 10.0 \text{ gL}^{-1}$ and exhibited reduced emulsification capacity ($E24 = 35 \pm 5.0\%$). Nevertheless, the recovered product retained appreciable surface-active properties, reducing surface tension to $36.10 \pm 5.0 \text{ mNm}^{-1}$ with a CMC of 103.68 mgL^{-1} . Although this method offers operational simplicity and lower processing costs, the reduced recovery suggests that a significant proportion of sophorolipids remained unrecovered during precipitation.

Nanofiltration (SL 3.0) provided an intermediate recovery of $67.31 \pm 10.0 \text{ gL}^{-1}$ and preferentially enriched the acidic sophorolipid fraction ($42.16 \pm 5.0\%$), while the lactonic fraction accounted for only $10.25 \pm 5.0\%$. The recovered biosurfactant exhibited an emulsification index of $34 \pm 5.0\%$, reduced surface tension to 33.08 ± 5.0

mNm^{-1} , and showed a CMC of 105.34 mgL^{-1} . These results indicate that nanofiltration can selectively concentrate acidic sophorolipids while maintaining acceptable surface activity, making it a promising membrane-based alternative for downstream processing.

The dried broth-assisted solvent extraction method (SL 4.0) demonstrated an effective compromise between recovery efficiency and product performance, yielding $83.23 \pm 10.0 \text{ gL}^{-1}$ of sophorolipids. The recovered product exhibited favorable biosurfactant characteristics, including an emulsification index of $40 \pm 5.0\%$, a surface tension of $32.51 \pm 5.0 \text{ mNm}^{-1}$, and a CMC of 113.65 mgL^{-1} . Although the overall recovery was lower than obtained by conventional solvent extraction, this approach produced a functionally active biosurfactant while potentially reducing solvent consumption and improving process practicality.

Overall, the comparative analysis demonstrates that the choice of downstream recovery method significantly influences sophorolipid yield, congener composition, and functional performance. Conventional solvent extraction (SL 1.0) provided the highest recovery and the best surface-active properties, whereas dried broth-assisted solvent extraction (SL 4.0) offered a favorable balance between recovery efficiency and biosurfactant functionality. These findings identify solvent-based recovery strategies as the most suitable approaches for pilot-scale sophorolipid purification and provide valuable insights for the development of economically viable and industrially scalable downstream processing protocols.

4.0. Discussion

4.1. Upstreaming Process

Sophorolipid production in the 5 L bioreactor followed the characteristic pattern of a predominantly non-growth associated secondary metabolite. During the early stages of fermentation, biomass increased gradually, reaching approximately 5.5%, while sophorolipid synthesis continued throughout the cultivation period and achieved a maximum concentration of 109 gL^{-1} after 192 h. Simultaneously, the steady depletion of glucose and oleic acid demonstrated efficient utilization of both hydrophilic and hydrophobic carbon sources, indicating effective metabolic conversion toward glycolipid biosynthesis. Dissolved oxygen (DO) dynamics closely reflected the physiological state of the culture during fermentation. The DO concentration declined to approximately 15.7% within the first 48 h, corresponding to the exponential growth phase, when cellular respiration and oxygen demand were at their highest. As fermentation progressed, DO levels gradually increased despite continued sophorolipid production, suggesting a reduction in biomass formation and respiratory activity while carbon metabolism shifted toward extracellular biosurfactant synthesis. This transition indicates that sophorolipid production became increasingly dominant during the later stages of cultivation, when cellular growth slowed but metabolic resources continued to be directed toward product formation. The observed relationship among biomass growth, substrate consumption, dissolved oxygen profile, and sophorolipid accumulation demonstrates that the optimized fermentation conditions effectively supported both microbial growth and high product productivity. Similar physiological trends have been reported for *Starmerella bombicola* and other sophorolipid-producing yeasts, where oxygen demand is greatest during exponential growth and subsequently decreases as metabolic flux shifts from biomass generation to secondary metabolite production. These findings further confirm that the optimized operating conditions

provided a favorable environment for sustained sophorolipid biosynthesis and efficient substrate conversion in the 5 L bioreactor.

The fermentation process was successfully scaled up from a 5 L laboratory bioreactor to a 100 L pilot-scale fermenter without any measurable loss in process performance or product yield. Under the optimized operating conditions, sophorolipid production reached a maximum concentration of 110 gL^{-1} after 192 h of cultivation, demonstrating excellent process reproducibility and operational robustness. Although scale-up is often accompanied by challenges such as altered hydrodynamics, reduced oxygen transfer efficiency, and heterogeneous mixing, these limitations were effectively mitigated through a controlled aeration rate of 1 VVM combined with a stepwise increase in agitation speed from 400 to 800 rpm. This operating strategy ensured adequate oxygen availability throughout the fermentation process and supported sustained microbial activity. The higher biomass concentration achieved in the 100 L bioreactor (18%) suggests that the pilot-scale system provided improved mixing efficiency and more uniform substrate distribution, thereby creating a favorable environment for microbial growth. In addition, sophorolipid concentrations during the intermediate stages of fermentation were consistently higher than those observed in the 5 L bioreactor, indicating enhanced biosynthetic productivity under pilot-scale conditions. The improved production kinetics may be attributed to more efficient oxygen transfer, superior mass transfer characteristics, and optimized hydrodynamic conditions that facilitated substrate uptake and metabolite synthesis. Importantly, despite the higher productivity observed during the intermediate stages, the final sophorolipid titers obtained in the 5 L and 100 L bioreactors were nearly identical, demonstrating that the optimized fermentation strategy was successfully translated across scales. These results confirm the robustness and scalability of the developed process and highlight its suitability for pilot-scale operation. The ability to maintain high product titers while preserving stable fermentation performance underscores the potential of this process for further scale-up and commercial production of sophorolipids.

The successful completion of ten consecutive pilot-scale fermentation batches further demonstrated the robustness and reproducibility of the developed production process. Across all batches, sophorolipid concentrations ranged from 95.62 to 110.43 gL^{-1} , with an average product titer exceeding 100 gL^{-1} , highlighting the ability of the process to consistently achieve high productivity under identical operating conditions. The relatively small variation in product yield between batches reflects stable fermentation performance, effective process control, and reliable operation of the pilot-scale system. Residual oleic acid concentrations remained consistently low, with an average of 1.89 kg per 100 L of fermentation broth, indicating efficient substrate utilization and carbon conversion throughout the cultivation process. Based on the residual substrate levels, oleic acid conversion efficiencies were estimated to exceed 80–85%, demonstrating effective metabolic utilization of the hydrophobic carbon source for sophorolipid biosynthesis. The simultaneous achievement of high product titers and low residual substrate concentrations further confirms the efficiency of the optimized fermentation strategy. From an industrial perspective, maintaining consistent performance across multiple production batches is a critical requirement for commercial manufacturing. The observed batch-to-batch reproducibility minimizes process variability, facilitates product quality assurance, and improves process predictability, all of which contribute to reduced production risk and enhanced economic feasibility. Collectively, these findings validate the scalability and operational stability of the developed fermentation process and support its suitability for large-scale sophorolipid production and future commercial implementation.

4.2. Downstream Processing

Downstream processing is widely recognized as one of the most significant cost-intensive stages in biosurfactant manufacturing, making the development of efficient recovery methods essential for improving overall process economics. To identify an effective purification strategy, four sophorolipid recovery methods were comparatively evaluated with respect to product yield, composition, and functional performance. Among the approaches investigated, conventional solvent extraction (SL 1.0) achieved the highest recovery, yielding approximately 105 gL^{-1} of sophorolipids while preserving the characteristic distribution of acidic and lactonic congeners. The high extraction efficiency is likely attributable to the strong affinity of ethyl acetate for amphiphilic glycolipid molecules, together with the preliminary removal of unreacted fatty acids by hexane extraction, which enhanced the selectivity of the recovery process. In addition to its superior recovery efficiency, solvent extraction produced sophorolipids with excellent surface-active properties. The recovered product exhibited a high emulsification index (E24) of approximately 50% and reduced the surface tension to approximately 31 mNm^{-1} , demonstrating that the extraction process effectively preserved the functional integrity of the biosurfactant molecules. These results indicate that conventional solvent extraction not only maximizes product recovery but also maintains the physicochemical characteristics required for industrial applications. Consequently, this method represents a reliable benchmark for pilot-scale sophorolipid purification and serves as an effective reference for evaluating alternative downstream processing strategies.

Acid precipitation (SL 2.0) represented a relatively easy, economical, and practical method for recovery of sophorolipids; however, its recovery efficiency was much lower compared to that of the standard solvent extraction method. The relatively low product yield could be due to the following reasons related to the process of acid precipitation to pH 2.0 such as precipitation of proteins and other impurities of the fermentation medium, poor recovery of the acid form of sophorolipid congeners, as well as the degradation or alteration of sensitive acid compounds. All together, these could be reasons for the relatively low recovery efficiency of the studied method. Despite the relatively low product yield, the recovered sample still possessed certain surface-active properties, proven by the surface tension reduction properties. At the same time, the low emulsification activity could indicate that the recovered sample was either not very pure or had a different ratio between the lactonic and acidic sophorolipid congeners. While acid precipitation has several benefits regarding ease and cost-effectiveness, its inefficiencies in terms of recovery and product quality could limit the use of this process in sophorolipid production on an industrial scale.

The SL 3.0 nanofiltration was observed to show promise as an alternative purification technique for sophorolipids based on membranes. The adjustment of the pH of the fermentation broths to 6.5 facilitated ionization of the acidic sophorolipids, making them larger in size hydrodynamically, hence facilitating their retention by the nanofiltration membrane. The use of the 300 Da thin-film composite (TFC) membrane in crossflow mode minimized the possibility of concentration polarization and fouling of the membrane while enhancing the separation of the sophorolipid molecules. Further, the use of diafiltration ensured higher purity of the product by removal of any salts, sugars, and low molecular weight impurities present in the retentate. While nanofiltration provided a more purified fraction of the sophorolipids, the yield was observed to be lower compared to solvent extraction. The yield loss is due to the adsorption of some of the sophorolipids on the membrane, losses during membrane handling, and lack of retention of the smaller lactonic sophorolipid congeners close to the molecular weight cut-off of the membrane. However, despite such limitations, there are

multiple benefits of nanofiltration that make this method a suitable solution for industrial downstream processing. Nanofiltration does not involve organic solvents, which helps decrease their consumption during the procedure and thus makes it more environmentally friendly and safer. In addition, membrane technologies can easily be implemented in continuous production processes, need less energy for operation, and allow streamlining downstream processing. Hence, although more development and optimization is needed to enhance the recovery rates, nanofiltration is a perspective technique for sophorolipid purification on an industrial scale.

Solvent extraction using dried broth (SL 4.0) was found to be an attractive technique for isolating sophorolipids since it is a good compromise between recovery efficacy and sophorolipid quality. Solvent extraction after the drying of fermentation broth could have resulted in the higher concentration of sophorolipid molecules in that area and better interactions between sophorolipids and ethyl acetate compared with acid precipitation and membrane separation. Water elimination during the drying process might have made the penetration of the solvent into dried broth easier, contributing to a better isolation of sophorolipids without water-soluble contaminants. The sophorolipids isolated via this technique possessed desirable physical and chemical properties, as evident from their ability to demonstrate low surface tensions and good emulsifying activities, suggesting that biosurfactants were successfully isolated without compromising their functional properties. Although this method yielded a smaller amount of biosurfactants as compared to the solvent extraction technique, product quality was good enough to be utilized for possible industrial applications. Another benefit of this technique is that it requires significantly lesser amounts of solvents to isolate the products as compared to the conventional solvent extraction technique. In addition to lowering the solvent requirement, this can lower costs associated with solvent recovery, minimize the environmental burden, and make the process safer. These aspects, in conjunction with acceptable recovery efficiencies and biosurfactant properties, suggest that dried broth-assisted solvent extraction can be an excellent downstream processing method for sophorolipid production on a pilot and industrial scale.

In addition, the functional characterization of the recovered sophorolipids showed that all subsequent purification techniques had been able to preserve the inherent surface-active attributes of the biosurfactant. Whether or not any of the recovery techniques were used, the surface tension values obtained for the purified sophorolipids were within the range of about 31 and 36 mNm⁻¹, which is similar to what has been observed with commercially available sophorolipids. The CMC values of about 100 mgL⁻¹ show high surface efficiency in that relatively low biosurfactant amounts were required in order to attain maximum surface tension reduction and form micelles. Relatively low CMC values are usually considered to be one of the key indicators of the performance of biosurfactants as they usually imply efficient adsorption of biosurfactant molecules at the air-liquid interface and efficient self-assembly of surfactant molecules into micelles after achieving interfacial saturation. All these results confirm that the sophorolipids collected were not impaired by the process of separation and have physical and chemical properties similar to commercial products, making them suitable for a large variety of applications. In addition, the results of the emulsification indices conducted show that there is great potential of application for sophorolipid preparations in environmental remediation, enhanced oil recovery, detergency, cosmetics, and pharmaceutical formulations.

Collectively, the present investigation establishes a scalable sophorolipid production platform capable of achieving product titers exceeding 110 gL⁻¹ at pilot scale with excellent process reproducibility. Among the

recovery techniques investigated, conventional solvent extraction currently provides the highest recovery efficiency and product quality, whereas membrane-assisted separation offers a promising environmentally sustainable alternative for future industrial implementation. The successful demonstration of ten pilot-scale batches confirms the technological readiness of the process and provides a strong foundation for subsequent techno-economic analysis, process intensification, and commercial-scale sophorolipid manufacturing.

4.3. Preliminary Techno-Economic Assessment of Pilot-Scale Sophorolipid Production

Even though there have been many advances in the area of sophorolipid research, several scientific and technological issues still need to be solved in order for these biosurfactants to gain wide acceptance commercially. The main research needs for the future are the need to close the gaps that exist between the lab scale and industrial scale. The most important issues here are: (i) testing at the pilot scale of any fermentation and downstream processing methods that seem to be promising, (ii) cooperation among microbiologists, biotechnologists, biochemical engineers, and process engineers in order to solve the biological, chemical, and hydrodynamic issues involved in the scale up process, and (iii) conducting TEA and LCA of large scale sophorolipids production.

It is important to perform techno-economic analysis (TEA) to evaluate the competitive position, economic feasibility, and sustainability of the commercial SL production processes. Though the number of sophorolipids has gained attention as eco-friendly surfactants, very few works have performed economic assessment of these processes, and that too on a smaller scale of fermentation processes. This points out the necessity of more process-specific studies on TEA for commercializing sophorolipid production technologies. From the studies that have been carried out, Martínez et al. carried out a techno-economic assessment on the production of sophorolipids through the process of solid state fermentation from lignocellulosic substrates, which include wheat straw, rice husk, and coconut fiber. They predicted that an annual production capacity of 325–414 tons/year would be achieved, at a cost of producing sophorolipid of between US\$5.1 to US\$6.9 per kg. In a second study, the same researchers did TEA on sophorolipid production from sugarcane bagasse through submerged fermentation using *Cutaneotrichosporon mucoides* UFMG-CM-Y6148, and found that a minimum selling price of around US\$19.53/kg was realized. In the similar manner, Wang et al. studied the production of sophorolipids through food waste by utilizing the yeast *Starmerella bombicola* and reported that the cost of producing the crystalline sophorolipids was US\$19.65 per kg and that of sophorolipid syrup was US\$16.45 per kg. However, it is vital to realize that besides food waste, glucose and oleic acid were used as co-substrates to produce the sophorolipids [19, 31, 32]. In summary, all these research studies have shown that there is a high level of dependence of the commercial viability of sophorolipid production on feedstocks used, fermentation techniques, efficiency of down-streaming operations, and integration. There is a need to develop highly productive fermentation techniques and efficient purification methods to achieve commercially viable production costs [33].

It should be noted that to reduce reliance on costly pure substrates, much of the TEA studies reported in literature have focused on the utilization of alternate feedstocks and biorefining. Any potential negative or inhibitory effects of these alternate feedstocks also need to be considered. For determining their impact on reducing unit costs of production and improving competitiveness of the SL market, future TEA study taking into account the benefits of new technologies especially new producer strains and fermentation process would be

necessary. It should be noted that overall cost savings in the production process of SLs would definitely require innovations both in fermentation process as well as downstream processing, which would include purification among other aspects [34].

In terms of techno-economics, the current study has some positive aspects regarding the commercial viability of SL production. These include the attainment of titers higher than 100 gL^{-1} , the scale-up process from 5 L to 100 L, consistency of the results among ten pilot-scale runs, and testing of various recovery pathways.

4.3.1. Capital Expenditure (CAPEX)

The capital cost (CAPEX) required for the production of sophorolipids is mainly a result of the capital costs required for both the upstream fermentation unit operations as well as downstream processing. The main capital assets include production fermenters, seed bioreactors, sterilization equipment, centrifugation equipment, membrane filters, solvent extraction and recovery equipment, evaporators, lyophilizers, utility equipment, pipelines, instrumentation, and process control systems. Of these capital assets, fermentation equipment forms the majority of the capital investment required, due to the complex nature of aerobic fermentation using microorganisms. From various studies conducted on techno-economics in the literature, it can be observed that fermentation equipment accounts for about 30–45% of the total installed equipment costs, while the second largest capital asset is the downstream purification system, especially when using solvent extraction. According to Martínez et al., the total CAPEX for a pilot plant for producing sophorolipids, which is capable of producing 325–414 tons annually, is approximately US\$ 6.7 million, which confirms that fermentation equipment constitutes the major component of the capital investment costs in biosurfactant manufacturing. Similar studies show that upstream processing and downstream recovery constitute the primary factors responsible for the total capital investment costs, highlighting the need for scaling up the processes of biosurfactants production and making them economically viable. The findings of the current study have significant implications in mitigating capital investment risks in industry-scale biosurfactant manufacturing. The successful scaling up of sophorolipid biosynthesis from a 5 L laboratory fermenter to a 100 L pilot-scale fermenter, along with consistency across ten consecutive pilot-scale batches, is indicative of a highly robust process.

The degree of validation on a pilot scale significantly diminishes the technical uncertainty that is normally associated with the process scale-up procedure, one of the greatest sources of CAPEX risk during biosurfactant commercialization. Thus, the ability to reproduce the process consistently and the high process stability increase the technology readiness level (TRL) of the created process and allow for a sound engineering basis for the development of demonstration and commercial plant design and cost estimation.

4.3.2. Operating Expenditure (OPEX)

The operating expenditure (OPEX) of sophorolipid synthesis is mainly dictated by the expenses incurred on the raw material, utility consumption, labor, downstream processing, and waste disposal. These include (i) carbon sources, including glucose, oleic acid, vegetable oil, or even industrial waste feedstock; (ii) utility consumption, which includes oxygen, energy, and steam; (iii) skilled labor; (iv) chemicals used in the downstream recovery process; (v) solvent usage and recovery; (vi) membrane change and cleaning agents; and (vii) waste treatment. Of all the cost factors mentioned above, the carbon source used and the downstream recovery efficiency have been identified to be the most important factors influencing sophorolipid manufacturing cost.

According to published techno-economic analyses, downstream processing accounts for one of the biggest shares of operational expenses, sometimes comprising even more than 50% of all OPEX in case multiple solvent extraction, concentration, and recycling is involved. According to the calculations of Martínez et al., annual operating expenses for an industrial-scale facility for sophorolipid production can be about US\$1.9 million, resulting in production costs of about US\$5.1–6.9 per kg of sophorolipid, depending on the substrate and the process type chosen. Their calculations revealed that downstream recovery and purification are responsible for the biggest part of operating expenses and pointed to the need for development of economically viable separation technologies. Comparative analysis of four methods of downstream processing done in the current study gives valuable information about possibilities of OPEX reduction. Among the traditional methods, conventional solvent extraction (SL 1.0) provided the best recovery of the product while maintaining its properties unchanged. But the use of the method for industrial application requires extra expenses connected with solvent procurement, solvent evaporation and recycling, extra energy costs and explosion-proof equipment for safe handling of flammable solvents.

In comparison to solvent extraction, nanofiltration (SL 3.0) provides a number of technical and environmental benefits which might enhance the economics of sophorolipid production in the long run. The membrane technique requires a minimal quantity of organic solvents to be used, resulting in reduced expenditures on their acquisition and recovery as well as low levels of emissions of volatile organic compounds. Furthermore, the nanofiltration process is easy to implement in continuous mode, results in generation of less hazardous waste and is characterized by the ability to concentrate and purify the product selectively. It should be noted that the recovery yield of the current work was somewhat lower than that of solvent extraction.

In accordance with these results, past studies related to the techno-economic aspects of sophorolipid manufacture have indicated that the use of membrane-based downstream processing is a viable method for decreasing the cost of production as well as minimizing the environmental impact of sophorolipid manufacturing. It thus becomes necessary for the proper choice of the downstream processing method to not only take into account the product yield but also factors such as solvent usage, energy demands, waste production, etc.

4.3.3. Techno-Economic Assessment (TEA)

From a TEA standpoint, several findings from this investigation positively impact process economics:

Table 4
Techno Economic Assessment of production of sophorolipid

Parameter	Observation	TEA implication
Final titer	109–110 g L ⁻¹	Reduced fermenter volume requirement
Scale-up	5 L → 100 L successful	Lower process uncertainty
Batch reproducibility	10 successful batches	Improved investment confidence
Residual substrates	Low residual oleic acid	Enhanced substrate utilization efficiency
Recovery strategies	Four methods evaluated	Opportunity for cost optimization
Product functionality	Low CMC and high E ₂₄	Access to high-value specialty markets

Conducted techno-economic sensitivity studies have repeatedly shown that the economic feasibility of sophorolipid production depends significantly on a few process parameters, namely titer of the produced substance, length of the fermentation cycle, recovery efficiency of the downstream processing, utility requirements, solvent recycling efficiency, and efficiency of oxygen transfer during scale-up. In terms of economic importance, titer of the produced substance and recovery efficiency of the downstream processing can be named as two most significant parameters because they directly influence the output capacity of the facility, the utilization of equipment, requirements for raw materials, and cost of production of each unit of product.

Techno-economic studies conducted at the industrial scale have shown that with the optimal design of biorefineries including membrane-based purification processes, it is possible to minimize the minimum selling price of biosurfactants to less than US\$20 per kilogram. In addition, simplified economic analysis has indicated positive economic performance in the production of sophorolipids, where the IRR is expected to be around 31%, while the NPV is about US\$6.4 million, and the payback period is approximately 3.2 years.

The process developed in the current study possesses certain features that would allow it to increase its economic competitiveness. The process includes high sophorolipid titer greater than 100 g L^{-1} , successful upscaling of the process from the laboratory scale to pilot scale, successful operation of the process for ten batches at pilot scale and existence of various downstream processes that can be used to recover the produced sophorolipids. All these features should allow us to improve the efficiency of equipment usage and decrease the variability of the production process while providing an opportunity to choose downstream processes based on economic considerations.

Although these results show that there is potential from an economic point of view, further process-specific techno-economic analysis needs to be carried out in order to determine the economic feasibility of the manufacturing process that was developed. This analysis involves equipment sizing, capital investment, efficiency of solvent recovery and recycling, cost of membrane replacement, utility and energy balance, labor requirements, maintenance cost, depreciation, tax, and discounted cash flow. This information can be integrated in order to estimate the minimum selling price (MSP), net present value (NPV), internal rate of return (IRR), and payback period for commercial production. This will facilitate the commercialization of the biosurfactants produced from sophorolipids.

5.0. Conclusion

The current research has been successful in developing and validating the integrated upstream and downstream bioprocess for sophorolipids pilot scale production with high yields, very good reproducibility, and functional properties of sophorolipids. The proposed optimized fermentation process was effective in obtaining the sophorolipids yield of 109 g/L and 110 g/L in 5 L and 100 L stirred-tank bioreactor, respectively, within 192 h of fermentation, which confirmed that the process could be scaled up efficiently without sacrificing the yield or performance of the process. In addition, the sophorolipids concentration exceeding 100 g/L could be obtained for ten consecutive fermentation batches of pilot scale without significant deviation between batches and high efficiency of substrate utilization. Thus, the robustness and reproducibility of the developed process could be confirmed. Comparative analysis of the effectiveness of four different downstream processes for sophorolipids recovery showed that conventional solvent extraction process allowed obtaining

the highest yield of sophorolipids and preserving the physicochemical properties and surface-active properties of the biosurfactant. In contrast, nanofiltration appeared as promising sustainable alternative that would allow solvent-free purification of sophorolipids, which would make it possible to develop sustainable and continuous downstream processes. Whatever downstream process would be used, the functional properties of purified sophorolipids were very high, which was confirmed by high efficiency in reducing surface tension, good emulsifying properties, and low CMC values. Thus, the integration of optimized fermentation, pilot scale validation, comparison of downstream processes, and physicochemical characterization proves the technical feasibility of large scale sophorolipids production.

Declarations

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval and consent to participate Consent for publication

All authors read and approved of the final manuscript. All authors accepted participation for publication.

Data availability

The data that supports the findings of this study are available on request from the corresponding author, APP. The data is not publicly available due to privacy, legal and restrictions.

Author contribution: CRediT

Conceptualization: Prof. Amit P. Pratap APP, Prof. Vivek Gaval VG; Methodology: Ninad Mhatre NM; Formal analysis and investigation: Ninad Mhatre NM, Dr. Jagruti Jadhav JJ, Deepak Sonawane DS; Writing - original draft preparation: Ninad Mhatre NM; Writing - review and editing: Prof. Amit P. Pratap APP, Prof. Vivek Gaval VG;; Funding acquisition: Rajiv Gandhi Science and Technology Commission (RGSTC), Government of Maharashtra State, India; Resources;; Supervision: Prof. Amit P. Pratap APP, Prof. Vivek Gaval VG.

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Figures



Figure 1

(a) Lab benchtop scale 5 L fermenter (b) Pilot scale 100 L fermenter

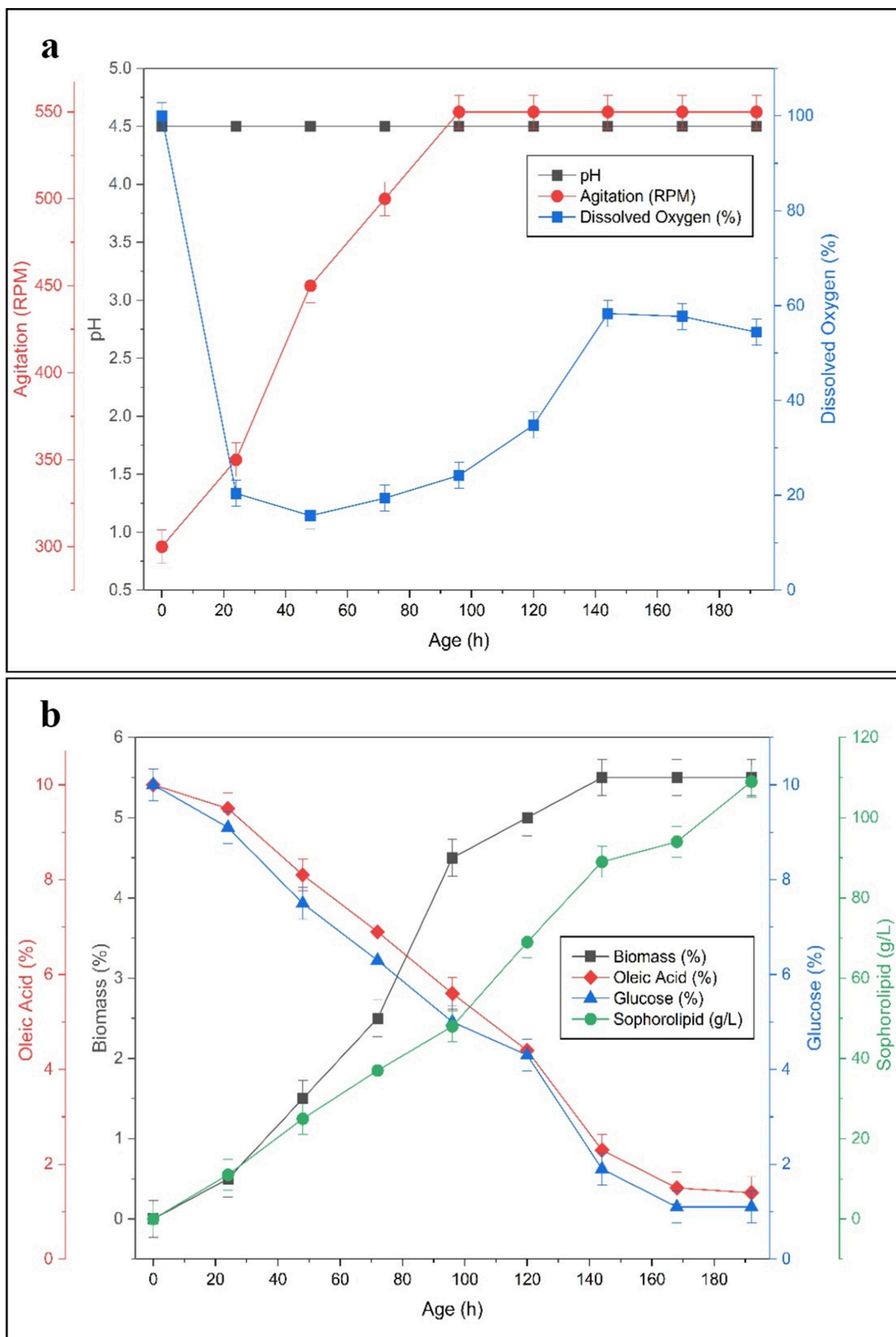


Figure 2

Bench top Lab scale 5 L fermenter (a) Batch processing parameters, (b) the substrate utilization Oleic acid and glucose consumption vs sophorolipid and biomass production. pH maintained between 4.3 to 4.7, and aeration maintained 1 VVM throughout the batches. Total 3 batches were taken on bench top scale fermenter

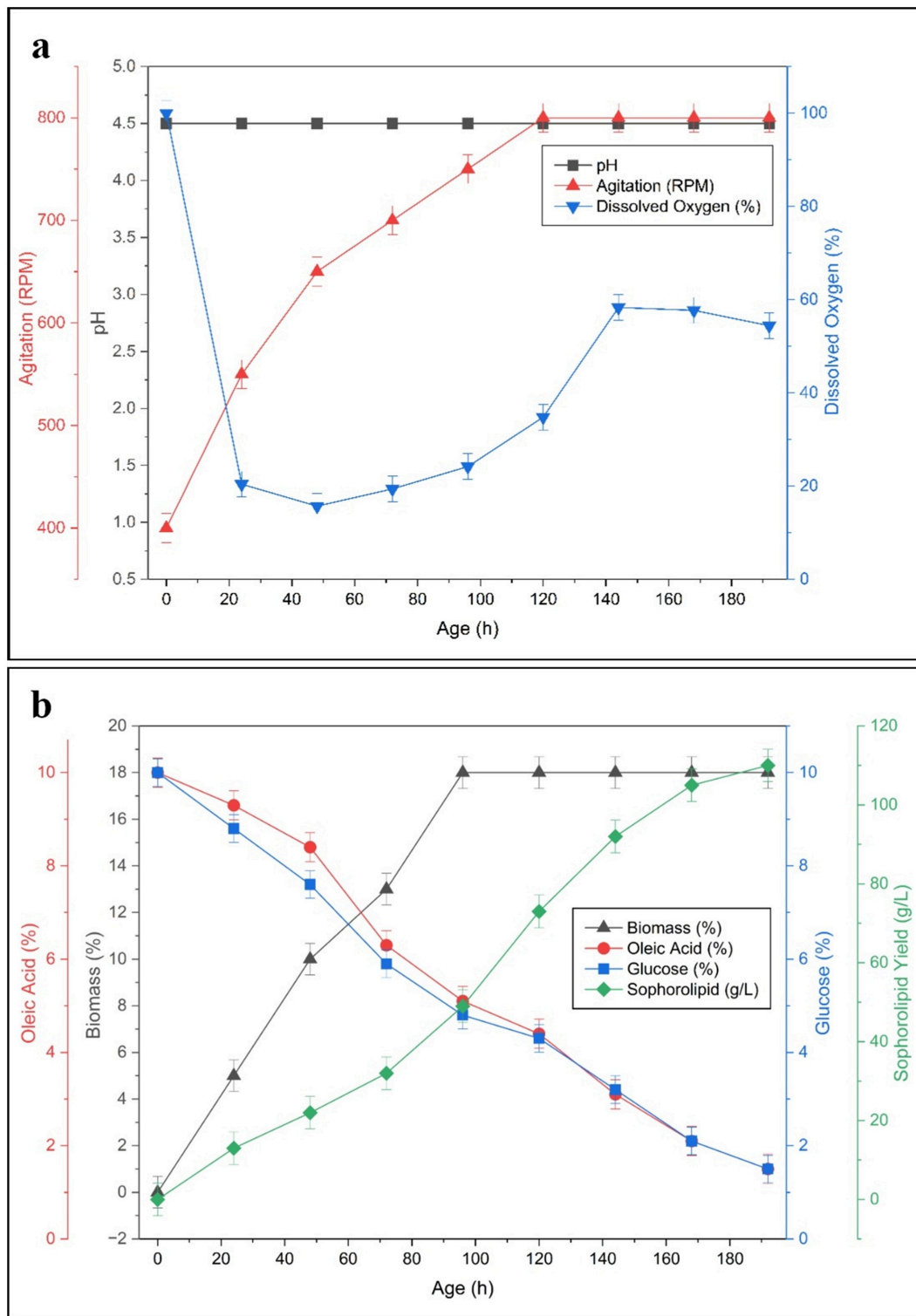


Figure 3

100 L Pilot scale fermenter (a) Batch processing parameters, (b) the substrate utilization Oleic acid and glucose consumption vs sophorolipid and biomass production. pH maintained between 4.3 to 4.7, and aeration maintained 1 VVM throughout the batches. Total 10 batches were taken on 100 L pilot scale fermenter.

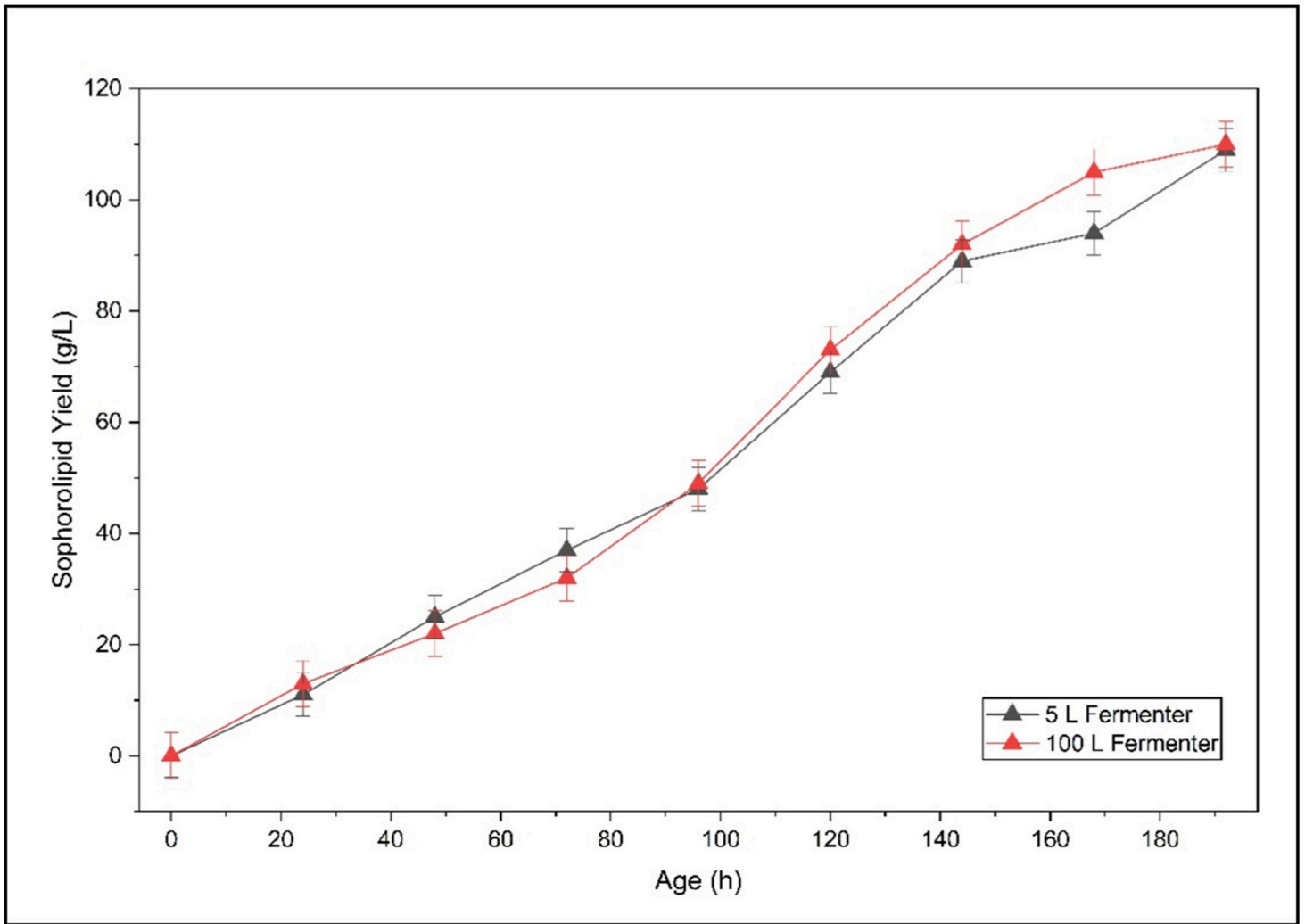


Figure 4

Sophorolipid yield comparison between Bench top scale 5 L and pilot scale 100 L fermenter scale.

Supplementary Files

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- [SupplementaryData.docx](#)