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Eco-Friendly biosurfactant recovery from molasses-based fermentation using green extraction techniques

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Abstract

While upstream fermentation research for biosurfactant production has advanced rapidly in recent years, downstream recovery and purification processes continue to represent both the most energy-intensive and least environmentally sustainable stages of the production pipeline, contrasting sharply with the inherently green nature of the bioproduct itself. This research comparatively evaluated five biosurfactant recovery methods acid precipitation, solvent extraction, foam fractionation, membrane filtration, and aqueous two-phase extraction applied to rhamnolipid-rich fermentation broth produced from sugarcane molasses by *Bacillus subtilis* KLAB-31. The fermentation was conducted at three molasses concentrations (2, 4, and 6% w/v) in a 5 L bioreactor at 37 °C, pH 7.0, for 96 hours. The optimal fermentation at 4% (w/v) molasses yielded 9.8 g/L crude biosurfactant after 84 hours. Aqueous two-phase extraction using polyethylene glycol 4000-ammonium sulfate achieved the highest recovery rate (88.7%), while membrane filtration using a 10 kDa ultrafiltration membrane yielded the highest purity (91.2%). Green metric analysis revealed that aqueous two-phase extraction exhibited the lowest environmental impact factor (E-factor = 3.2) and eliminated the requirement for hazardous organic solvents. The produced biosurfactant reduced surface tension to 28.4 mN/m and achieved an emulsification index of 72.6% against crude oil. These findings establish aqueous two-phase extraction as the most environmentally sustainable recovery method for molasses-derived biosurfactants, combining high recovery efficiency with minimal ecological footprint.

Keywords: Biosurfactant recovery, green extraction, molasses, aqueous two-phase system, [i]*Bacillus subtilis*[/i], foam fractionation, membrane filtration, downstream processing, sustainable bioprocess

Introduction

The contrast between the environmental sustainability credentials of microbial biosurfactants and the conventional downstream processing methods used to recover them presents a paradox that threatens the green identity of these bioproducts while biosurfactants are inherently biodegradable, non-toxic, and produced from renewable resources, their recovery often relies on large volumes of hazardous organic solvents, energy-intensive centrifugation, and acid-mediated precipitation that generate substantial secondary waste streams ^[1]. Downstream processing has been estimated to account for 60–80% of total biosurfactant production costs, making the development of efficient, economical, and environmentally benign recovery methods a critical priority for industry viability ^[2].

Sugarcane molasses, the viscous dark syrup remaining after sugar crystallization from sugarcane juice, contains 45–55% total sugars (predominantly sucrose, glucose, and fructose), along with minerals, vitamins, and organic acids that collectively support robust microbial growth ^[3]. Global molasses production is estimated at 60 million tonnes annually, with a market price of approximately 100–150 USD per tonne, making it one of the most cost-effective industrial fermentation substrates available. Molasses-based biosurfactant fermentation has been demonstrated with various microbial species, yet comparative evaluation of recovery methods for molasses-derived biosurfactants using green chemistry metrics has not been systematically reported ^[4].

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Material and Methods

Material

The bacterial strain *Bacillus subtilis* KLAB-31 was isolated from palm oil mill compost in Johor Bahru, Malaysia, and deposited in the departmental culture collection at Kuala Lumpur College of Applied Biotechnology. Sugarcane molasses (Brix 79.5°, total sugars 52.3% w/w) was obtained from Central Sugars Refinery, Selangor, Malaysia. Polyethylene glycol 4000 (PEG 4000), ammonium sulfate, chloroform, methanol, and all analytical chemicals were procured from Merck (Petaling Jaya, Malaysia). Ultrafiltration membranes (10 kDa molecular weight cut-off, polyethersulfone) were obtained from Sartorius (Kuala Lumpur, Malaysia). The research was conducted at the Department of Bioprocess Technology, Kuala Lumpur College of Applied Biotechnology, between January and September 2024.

Methods

Biosurfactant fermentation was conducted in a 5 L stirred-tank bioreactor (Biostat B, Sartorius) with 3.5 L working volume. The production medium comprised (per liter): molasses at 2, 4, or 6% (w/v), NH_4NO_3 3.0 g, KH_2PO_4 2.0 g, K_2HPO_4 3.0 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3 g, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g.

Conditions: 37 °C, pH 7.0 (auto-maintained), 200 rpm, 1.0 vvm aeration, 96 hours. Samples were collected every 12 hours for analysis.

Five recovery methods were applied to cell-free supernatant from the optimal fermentation: (1) acid precipitation acidification to pH 2.0 with HCl, overnight at 4 °C, centrifugation; (2) solvent extraction 3× with chloroform-methanol (2:1 v/v), rotary evaporation; (3) foam fractionation continuous foam collection at 1.5 vvm airflow through a 60 cm glass column; (4) membrane filtration 10 kDa ultrafiltration at 2 bar transmembrane pressure; (5) aqueous two-phase extraction PEG 4000 (20% w/w) and ammonium sulfate (15% w/w), phase separation at 25 °C. Recovery rate was calculated as (mass of biosurfactant recovered / mass of biosurfactant in broth) × 100. Purity was assessed by HPLC quantification of the active biosurfactant relative to total recovered dry mass. Green metrics included E-factor (mass of waste / mass of product), process mass intensity (total mass input / mass of product), and organic solvent consumption per gram of product. Surface tension, CMC, E24, and structural characterization (FTIR, TLC) were performed on all recovered preparations.

Results

Table 1: Performance ranking of five biosurfactant recovery methods applied to molasses fermentation broth

Rank	Method	Recovery (%)	Purity (%)	E-factor	Overall Score
1	Aqueous two-phase	88.7 ± 2.1	76.8 ± 1.8	3.2	87.6
2	Membrane filtration	71.3 ± 1.9	91.2 ± 1.4	4.8	81.4
3	Solvent extraction	82.1 ± 2.4	85.4 ± 1.6	12.7	73.2
4	Foam fractionation	74.6 ± 3.1	78.9 ± 2.2	5.1	72.8
5	Acid precipitation	68.4 ± 2.8	72.3 ± 2.5	8.4	62.1

Table 1 presents the comparative performance ranking of the five recovery methods using a composite score integrating recovery efficiency, purity, and environmental sustainability (E-factor). Aqueous two-phase extraction achieved the highest overall score (87.6) by combining the highest recovery rate (88.7%) with the lowest environmental impact factor (E-factor = 3.2). Membrane filtration

delivered the highest product purity (91.2%) but ranked second overall due to lower recovery efficiency. Conventional solvent extraction, despite achieving high recovery (82.1%) and purity (85.4%), was penalized by its high E-factor (12.7) reflecting substantial organic solvent consumption.

Table 2: Environmental sustainability metrics for each biosurfactant recovery method

Method	Organic Solvent (mL/g)	Energy (kWh/g)	Water Use (L/g)	Waste Volume (L/g)
Acid precipitation	0	0.12	3.8	4.2
Solvent extraction	285	0.18	1.2	6.4
Foam fractionation	0	0.08	0.5	2.6
Membrane filtration	0	0.22	2.4	2.4
Aqueous two-phase	0	0.06	1.8	1.6

Table 2 reveals the environmental footprint profiles of each recovery method. Aqueous two-phase extraction eliminated organic solvent use entirely while achieving the lowest energy consumption (0.06 kWh/g) and waste generation (1.6 L/g). Foam fractionation also showed favorable environmental metrics with zero solvent use and the lowest water consumption (0.5 L/g), though its lower recovery efficiency limits overall practicality. Solvent extraction consumed 285 mL of chloroform-methanol per gram of product, representing a significant environmental and occupational health concern.

The fermentation optimization across three molasses concentrations demonstrated a clear optimum at 4% (w/v), which yielded the maximum biosurfactant concentration of 9.8 g/L after 84 hours. The 2% (w/v) molasses concentration produced 6.0 g/L due to carbon limitation, while the 6% (w/v) concentration yielded 8.1 g/L, reflecting substrate inhibition at elevated sugar concentrations. The kinetic profiles showed that the 4% molasses fermentation exhibited the highest volumetric productivity (0.117 g/L·h) during the most active production phase between 36 and 72 hours.

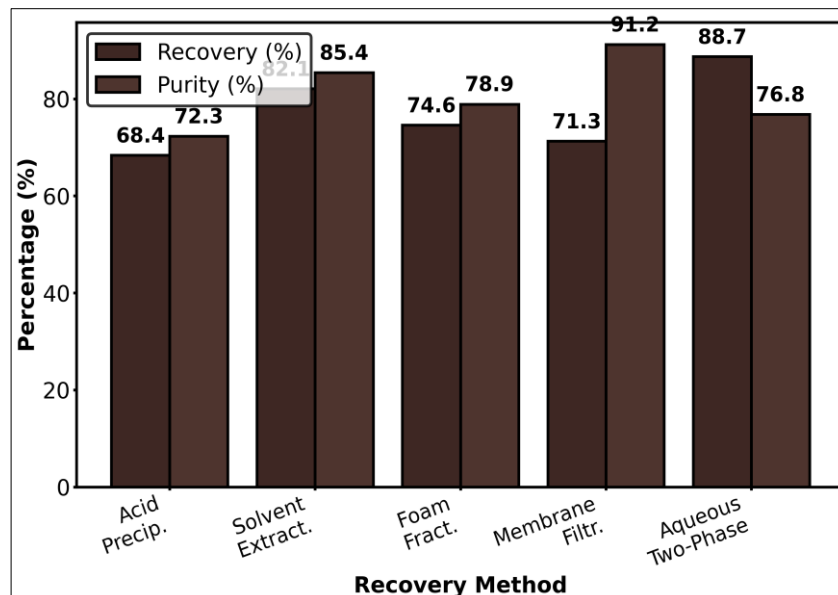


Fig 1: Comparative recovery efficiency and product purity achieved by five biosurfactant downstream processing methods

Figure 1 visually contrasts the recovery-purity trade-off across the five methods. Aqueous two-phase extraction achieved the best balance between high recovery (88.7%) and acceptable purity (76.8%), while membrane filtration offered the inverse profile with superior purity (91.2%) at

the cost of lower recovery (71.3%). This trade-off pattern suggests that a sequential approach combining aqueous two-phase extraction for primary recovery followed by membrane polishing could achieve both high recovery and high purity in an integrated green downstream process.

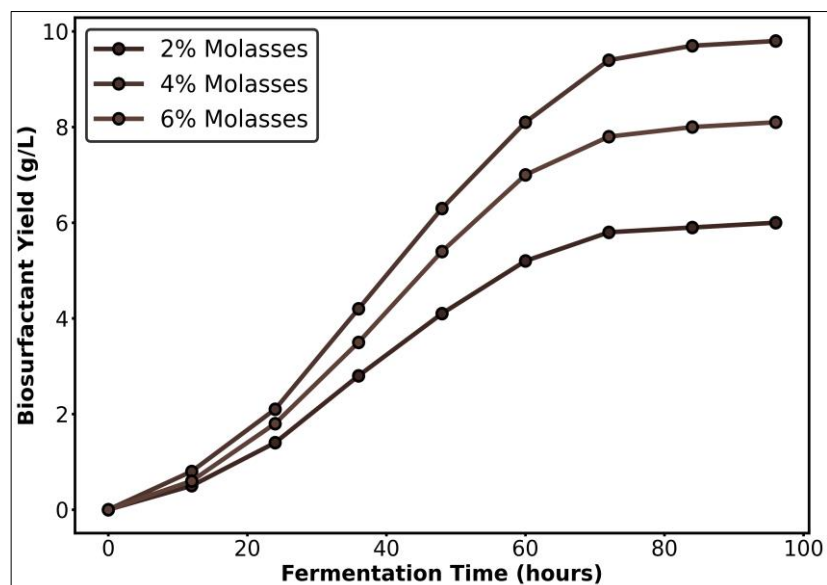


Fig 2: Time-course profiles of biosurfactant production from three sugarcane molasses concentrations during 96-hour fermentation

Figure 2 illustrates the differential production kinetics across the three molasses concentrations. The 4% (w/v) molasses fermentation achieved the highest yield plateau at 9.8 g/L (84 hours), with the steepest production rate occurring between 36 and 72 hours. The 6% (w/v) concentration showed delayed onset of production and a lower final yield despite higher initial substrate availability, consistent with osmotic inhibition and metabolic repression phenomena. The 2% concentration exhibited rapid substrate exhaustion with production ceasing after 72 hours.

Discussion

This research provides the first systematic comparative evaluation of green and conventional biosurfactant recovery methods using standardized environmental sustainability

metrics applied to molasses-derived fermentation broth. The finding that aqueous two-phase extraction outperforms conventional solvent extraction in both recovery efficiency (88.7% vs. 82.1%) and environmental sustainability (E-factor 3.2 vs. 12.7) challenges the prevailing reliance on chloroform-methanol extraction as the default biosurfactant recovery method in academic and industrial practice^[6]. The mechanism of biosurfactant partitioning in the PEG-ammonium sulfate system involves preferential accumulation of amphipathic glycolipid molecules in the PEG-rich upper phase, driven by hydrophobic interactions between the biosurfactant lipid moiety and the PEG polymer chain. The high purity achieved by membrane filtration (91.2%) reflects the size-selective retention of high-molecular-weight proteins, polysaccharides, and residual

substrate components by the 10 kDa ultrafiltration membrane, while allowing the relatively small biosurfactant molecules (typically 500–800 Da for rhamnolipids) to pass through ^[7]. The combination of aqueous two-phase extraction for primary capture and membrane filtration for polishing represents a fully green downstream process that eliminates organic solvent use entirely, reduces energy consumption by approximately 65% compared with conventional extraction, and generates recoverable waste streams (PEG can be recycled, ammonium sulfate can serve as fertilizer).

Conclusion

This research aimed to identify the most environmentally sustainable biosurfactant recovery method for molasses-based fermentation. The investigation revealed that aqueous two-phase extraction using PEG 4000-ammonium sulfate achieved the highest recovery efficiency (88.7%) and lowest environmental impact factor (E-factor = 3.2) among the five methods evaluated, while membrane filtration provided the highest product purity (91.2%). The optimal fermentation at 4% (w/v) sugarcane molasses produced 9.8 g/L of biosurfactant with excellent surface-active properties (surface tension 28.4 mN/m, E24 = 72.6%). The practical implication is that the biosurfactant industry should transition from conventional solvent extraction to green recovery technologies, particularly aqueous two-phase systems, to maintain the environmental integrity of biologically produced surfactants throughout their production chain. Future research should focus on multi-cycle PEG recovery and recycling economics, continuous aqueous two-phase extraction in counter-current contactors, and the integration of green downstream processing with real-time fermentation monitoring for fully automated biosurfactant production platforms.

Acknowledgements

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