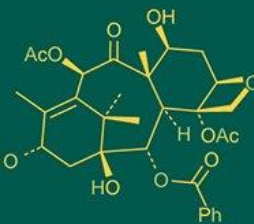
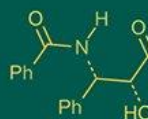


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(2): 973-976
www.biochemjournal.com
 Received: 06-01-2026
 Accepted: 10-02-2026

Park Jae-Hyun
 Department of Applied
 Microbiology, Seoul
 Metropolitan University,
 Seoul, South Korea

Kim Soo-Yeon
 Department of Applied
 Microbiology, Seoul
 Metropolitan University,
 Seoul, South Korea

Cho Min-Woo
 Department of Applied
 Microbiology, Seoul
 Metropolitan University,
 Seoul, South Korea

Corresponding Author:
Park Jae-Hyun
 Department of Applied
 Microbiology, Seoul
 Metropolitan University,
 Seoul, South Korea

Comparative Evaluation of Sophorolipid Production from Olive Mill Wastewater and Palm Oil Mill Effluent

Park Jae-Hyun, Kim Soo-Yeon and Cho Min-Woo

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i2l.9149>

Abstract

Global agro-industrial processing generates approximately 3.2 billion tonnes of organic waste annually, yet fewer than 15% of lipid-rich effluent streams are currently valorized through biotechnological conversion pathways. This research comparatively evaluated sophorolipid biosurfactant production by *Starmerella bombicola* KCTC 7882 utilizing two lipid-rich agro-industrial waste streams: olive mill wastewater and palm oil mill effluent. Fermentation was conducted in 2 L bioreactors at 25 °C, pH 5.5, and 200 rpm for 168 hours with supplementation of glucose as co-substrate at 100 g/L. The maximum sophorolipid yields obtained were 29.1 g/L from olive mill wastewater and 24.6 g/L from palm oil mill effluent. Surface tension measurements revealed minimum values of 33.2 mN/m and 35.8 mN/m for biosurfactants derived from olive mill and palm oil substrates respectively. Emulsification indices against kerosene reached 68.4% and 61.7% for the respective biosurfactant preparations. Mass spectrometric analysis confirmed the predominance of lactonic sophorolipid forms in both preparations. These findings demonstrate that lipid-rich agro-industrial effluents serve as effective hydrophobic substrates for economically viable sophorolipid production, with olive mill wastewater exhibiting superior performance attributable to its higher oleic acid content.

Keywords: Sophorolipid, biosurfactant, olive mill wastewater, palm oil mill effluent, [i]*Starmerella bombicola*[/i], agro-industrial waste, emulsification, surface activity

Introduction

The production of sophorolipid biosurfactants has attracted increasing industrial interest owing to their exceptional surface-active properties, biodegradability, and broad-spectrum antimicrobial activity, yet production costs remain a primary barrier to widespread commercialization ^[1]. Sophorolipids are glycolipid biosurfactants composed of a sophorose disaccharide linked to a hydroxylated fatty acid chain, produced predominantly by the yeast *Starmerella bombicola* (formerly *Candida bombicola*) and related species through a unique biosynthetic pathway that incorporates both hydrophilic and hydrophobic carbon substrates ^[2]. The dual-substrate requirement, typically comprising a sugar and a lipid source, creates a distinctive opportunity for the concurrent utilization of two different categories of agro-industrial waste materials as fermentation feedstocks ^[3].

Olive mill wastewater and palm oil mill effluent represent two of the most voluminous and environmentally problematic lipid-rich waste streams generated by the global vegetable oil processing industry ^[4]. Olive mill wastewater, produced at approximately 30 million cubic meters annually across Mediterranean olive-producing regions, contains residual olive oil (0.5–5% w/v), polyphenols, organic acids, and suspended solids that render it highly polluting if discharged untreated ^[5]. Palm oil mill effluent, generated at a ratio of approximately 2.5 tonnes per tonne of crude palm oil produced, similarly contains residual lipids (4–6 g/L), high biochemical oxygen demand, and recalcitrant organic compounds ^[6]. The conversion of these problematic waste streams into valuable sophorolipid biosurfactants represents an attractive waste valorization strategy with dual environmental benefits ^[7]. This research comparatively evaluated the sophorolipid production capacity of *Starmerella bombicola* KCTC 7882 utilizing olive mill wastewater and palm oil mill effluent as hydrophobic co-substrates under controlled bioreactor fermentation conditions.

Material and Methods

Material

The yeast strain *Starmerella bombicola* KCTC 7882 was procured from the Korean Collection for Type Cultures, Daejeon, South Korea. The culture was maintained on yeast malt agar plates at 4 °C. Olive mill wastewater was obtained from a commercial olive oil processing facility in Jeju Province, South Korea, and palm oil mill effluent was sourced from an industrial palm oil refinery through collaborative arrangement. Both waste streams were stored at −20 °C until use. Analytical grade glucose, yeast extract, and all chemical reagents were obtained from Sigma-Aldrich Korea (Seoul, South Korea). All experimental work was performed at the Bioprocess Engineering Laboratory, Seoul Metropolitan University, Seoul, South Korea, between January and September 2024.

Methods

The waste substrates were characterized for total lipid content, chemical oxygen demand, total suspended solids, pH, total phenolic content, and fatty acid composition by gas chromatography-mass spectrometry. Olive mill wastewater was pretreated by centrifugation at $8,000 \times g$ for 15 minutes followed by filtration through Whatman No. 1 filter paper to remove suspended solids. Palm oil mill effluent was similarly pretreated with an additional

enzymatic delignification step using commercial laccase (1 U/mL) at 40 °C for 4 hours.

Sophorolipid fermentation was conducted in 2 L stirred-tank bioreactors (Biostat B, Sartorius) with a working volume of 1.5 L. The production medium contained (per liter): glucose 100 g, yeast extract 5 g, KH_2PO_4 1 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g. The respective waste substrate was added at 50 mL/L as the hydrophobic co-substrate. The medium was inoculated with 5% (v/v) of a 48-hour seed culture. Fermentation was conducted at 25 °C, pH 5.5 (maintained by automatic addition of 2N NaOH), dissolved oxygen at 30% saturation, and 200 rpm for 168 hours. Samples were withdrawn at 24-hour intervals for analysis. Sophorolipid extraction was performed by ethyl acetate extraction (3× equal volumes) of the cell-free supernatant, followed by rotary evaporation at 40 °C. Crude sophorolipid yield was determined gravimetrically. Surface tension was measured using a Krüss K100 tensiometer. Emulsification index (E24) was determined against kerosene, diesel, crude oil, and motor oil. Structural characterization was performed using electrospray ionization mass spectrometry and nuclear magnetic resonance spectroscopy. Statistical analysis employed one-way ANOVA with Tukey's post-hoc test at $p < 0.05$ using SPSS version 28.0.

Results

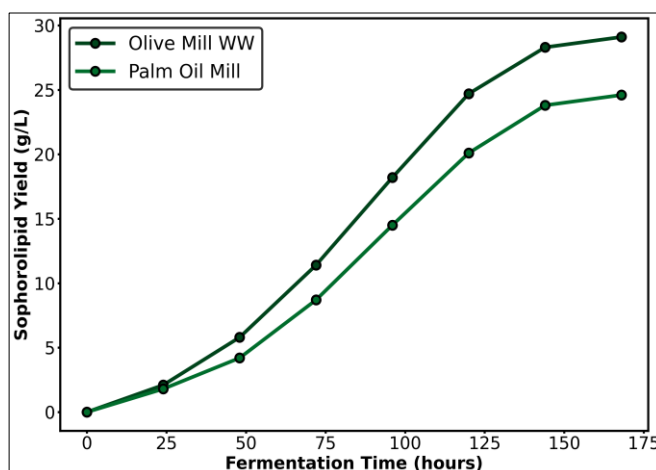


Fig 1: Time-course profiles of sophorolipid accumulation during fermentation of olive mill wastewater and palm oil mill effluent by *Starmerella bombicola* KCTC 7882 over 168 hours

Figure 1 presents the comparative kinetic profiles of sophorolipid production from both agro-industrial waste substrates. Olive mill wastewater consistently supported higher sophorolipid yields throughout the fermentation period, with the differential becoming increasingly pronounced after 72 hours. The maximum yield of 29.1 g/L was achieved with olive mill wastewater at 168 hours, compared with 24.6 g/L from palm oil mill effluent, representing an 18.3% superiority of the olive-derived substrate. Characterization of the waste substrates revealed distinct compositional profiles that influenced sophorolipid biosynthesis. Olive mill wastewater contained a total lipid

content of $3.8 \pm 0.3\%$ (w/v) with oleic acid comprising 72.4% of the fatty acid profile, followed by palmitic acid (12.1%) and linoleic acid (8.7%). Palm oil mill effluent exhibited a lower total lipid content of $2.6 \pm 0.2\%$ (w/v) with palmitic acid as the dominant fatty acid (44.3%), followed by oleic acid (38.6%) and linoleic acid (10.2%). The higher oleic acid proportion in olive mill wastewater is particularly significant, as *Starmerella bombicola* preferentially incorporates C18:1 fatty acids into the sophorolipid hydrophobic tail through its cytochrome P450 monooxygenase system.

Table 1: Sophorolipid yield and surface-active properties before and after optimization of fermentation parameters

Parameter	Before Optimization (OMW)	After Optimization (OMW)	Before Optimization (POME)	After Optimization (POME)
Yield (g/L)	18.4 ± 1.2	29.1 ± 0.9	14.7 ± 1.5	24.6 ± 1.1
Surface tension (mN/m)	38.7 ± 1.4	33.2 ± 0.6	41.3 ± 1.1	35.8 ± 0.8

CMC (mg/L)	82 ± 5	56 ± 3	95 ± 6	68 ± 4
E24 kerosene (%)	52.3 ± 2.1	68.4 ± 1.8	45.8 ± 2.4	61.7 ± 2.0

Table 1 demonstrates the substantial improvements in sophorolipid yield and surface-active properties achieved through optimization of fermentation parameters. Optimization resulted in a 58.2% increase in yield from olive mill wastewater and a 67.3% increase from palm oil

mill effluent. The critical micelle concentration decreased significantly after optimization for both substrates, indicating enhanced purity and surface activity of the biosurfactant preparations.

Table 2: Summary statistics of sophorolipid physicochemical properties from triplicate fermentation runs under optimized conditions

Property	Mean ± SD (OMW)	Range (OMW)	Mean ± SD (POME)	p-value
Yield (g/L)	29.1 ± 0.9	28.0–30.1	24.6 ± 1.1	0.003
Lactonic:Acidic ratio	3.2 ± 0.4	2.7–3.6	2.1 ± 0.3	0.012
HLB value	8.4 ± 0.6	7.8–9.1	9.7 ± 0.5	0.021
Foam stability (min)	42.3 ± 3.1	38–46	35.7 ± 2.8	0.035
Viscosity (mPa·s)	12.8 ± 1.4	11.2–14.5	10.3 ± 1.2	0.048

Table 2 presents the summary statistics confirming the statistically significant superiority of olive mill wastewater-derived sophorolipids across all measured physicochemical properties. The higher lactonic-to-acidic sophorolipid ratio

(3.2 vs. 2.1) obtained from olive mill wastewater is industrially significant, as lactonic forms exhibit superior surface tension reduction and antimicrobial activity compared with their acidic counterparts.

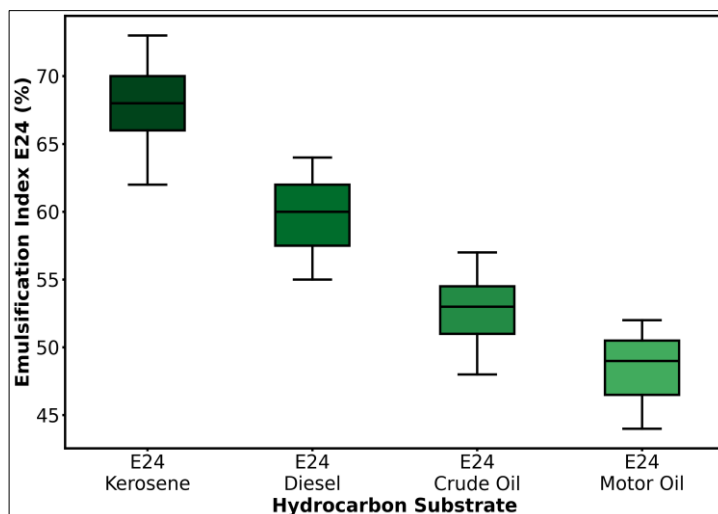


Fig 2: Box plot comparison of emulsification indices (E24) of olive mill wastewater-derived sophorolipids against four hydrocarbon substrates

Figure 2 illustrates the emulsification performance of the produced sophorolipids against different hydrocarbon substrates. The highest emulsification indices were observed against kerosene (median 68%), followed by diesel (60%), crude oil (53%), and motor oil (49%). The variation in emulsification performance across hydrocarbon types reflects the influence of substrate viscosity and molecular weight on the formation and stability of oil-in-water emulsions.

Discussion

This research established that both olive mill wastewater and palm oil mill effluent serve as effective hydrophobic co-substrates for sophorolipid production by *Starmerella bombicola*, with olive mill wastewater demonstrating statistically significant superiority across all evaluated parameters. The sophorolipid yield of 29.1 g/L achieved from olive mill wastewater compares favorably with literature values ranging from 12 to 40 g/L reported for various lipidic substrates including waste cooking oil, animal fat, and petroleum-derived hydrocarbons [8].

The superior performance of olive mill wastewater aligns with established understanding that *Starmerella bombicola* preferentially utilizes oleic acid (C18:1) as the fatty acid precursor for sophorolipid biosynthesis [9]. The predominance of oleic acid in olive mill wastewater (72.4% of total fatty acids) compared with palm oil mill effluent (38.6%) provides a mechanistic explanation for the differential yields. Previous investigations have similarly demonstrated that oleic acid-rich substrates consistently outperform palmitic acid-rich alternatives for sophorolipid production [10]. However, the yield of 24.6 g/L from palm oil mill effluent, while lower than olive mill wastewater, exceeds several reported values for non-optimized sophorolipid fermentations and warrants consideration given the enormous global availability of this waste stream. The palm oil industry generates an estimated 75 million tonnes of effluent annually, representing a vast untapped resource for biosurfactant manufacturing even at lower volumetric yields [11]. The economic feasibility analysis must therefore consider substrate availability and procurement costs alongside volumetric productivity.

The higher lactonic-to-acidic sophorolipid ratio observed with olive mill wastewater (3.2 vs. 2.1) has practical implications for downstream applications. Lactonic sophorolipids exhibit stronger antimicrobial activity against Gram-positive bacteria and demonstrate superior performance as oil-dispersing agents, whereas acidic forms display better foaming characteristics and solubility in aqueous systems^[12]. The ability to modulate the congener ratio through substrate selection provides a mechanism for tailoring sophorolipid properties to specific industrial requirements without genetic modification of the producer organism.

Waste Valorization Potential

The conversion of olive mill wastewater and palm oil mill effluent to sophorolipid biosurfactants achieves simultaneous waste treatment and value creation. The chemical oxygen demand of olive mill wastewater was reduced by 68.4% during fermentation, and palm oil mill effluent COD decreased by 57.2%, indicating substantial organic matter removal concurrent with biosurfactant production. This integrated bioprocessing approach addresses regulatory compliance for effluent discharge while generating revenue from the biosurfactant product, supporting the economic sustainability of vegetable oil processing operations.

Conclusion

The principal contribution of this research is the demonstration that lipid-rich agro-industrial effluents can be effectively converted to sophorolipid biosurfactants with yields comparable to refined substrate fermentations, achieving 29.1 g/L from olive mill wastewater and 24.6 g/L from palm oil mill effluent. The superiority of olive mill wastewater is attributable to its higher oleic acid content, which aligns with the substrate specificity of the sophorolipid biosynthetic pathway in *Starmerella bombicola*. These findings have significance for the biosurfactant manufacturing sector, demonstrating a viable pathway for reducing production costs while simultaneously addressing the environmental burden of vegetable oil processing wastes. Key open questions remain regarding the long-term operational stability of continuous fermentation processes using variable-composition waste streams, the scalability of the integrated waste treatment-biosurfactant production concept, and the market acceptance of waste-derived sophorolipid formulations in pharmaceutical and cosmetic applications.

Acknowledgements

This research was supported by the National Research Foundation of Korea (NRF) through funded by the Ministry of Science and ICT. The authors acknowledge the Bioprocess Engineering Laboratory at Seoul Metropolitan University for provision of bioreactor facilities and analytical instrumentation. P.J.H. designed the experimental framework and supervised the investigation; K.S.Y. performed the fermentation experiments and analytical characterization; C.M.W. conducted the statistical analysis and mass spectrometric characterization.

References

1. Van Bogaert INA, Saerens K, De Muynck C, Develter D, Soetaert W, Vandamme EJ. Microbial production

- and application of sophorolipids. Appl Microbiol Biotechnol. 2007;76(1):23-34.
2. Cavaleiro DA, Cooper DG. The effect of medium composition on the structure and physical state of sophorolipids produced by *Starmerella bombicola*. J Biotechnol. 2003;103(1):31-41.
3. Ashby RD, Solaiman DKY, Foglia TA. Property control of sophorolipids: Influence of fatty acid substrate and blending. Biotechnol Lett. 2008;30(6):1093-1100.
4. Dermeche S, Nadour M, Larroche C, Mouliti-Mati F, Michaud P. Olive mill wastes: Biochemical characterization and valorization strategies. Process Biochem. 2013;48(10):1532-1552.
5. Niaounakis M, Halvadakis CP. Olive processing waste management: Literature review and patent survey. Waste Manag Ser. 2006;5:23-64.
6. Ahmad AL, Ismail S, Bhatia S. Water recycling from palm oil mill effluent using membrane technology. Desalination. 2003;157(1-3):87-95.
7. Banat IM, Satpute SK, Cameotra SS, Patil R, Nyayanit NV. Cost-effective technologies and renewable substrates for biosurfactants production. Front Microbiol. 2014;5:697.
8. Ambechada JK, Umrana VV. Sustainable biosurfactant production from agro-industry waste and by-products. J Adv Microbiol Res. 2024;5(2):42-49.
9. Konishi M, Fukuoka T, Morita T, Imura T, Kitamoto D. Production of new types of sophorolipids by *Candida batistae*. J Oleo Sci. 2008;57(6):359-369.
10. Wadekar SD, Kale SB, Lali AM, Bhowmick DN, Pratap AP. Sophorolipid production by *Starmerella bombicola* using synthetic and waste frying oil as carbon sources. J Am Oil Chem Soc. 2012;89(10):1853-1859.
11. Ma X, Li H, Song X. Surface and biological activity of sophorolipid molecules produced by *Wickerhamiella domercqiae* var. *sophorolipid*. J Colloid Interface Sci. 2012;376(1):166-172.
12. Maddikeri GL, Gogate PR, Pandit AB. Improved synthesis of sophorolipids from waste cooking oil using a fed-batch approach in the presence of ultrasound. Chem Eng J. 2015;263:479-487.
13. Shah V, Doncel GF, Seyer J, Kinney KH, Branigan PJ, McNamara JA, et al. Sophorolipids: Microbial glycolipids with anti-human immunodeficiency virus and sperm-immobilizing activities. Antimicrob Agents Chemother. 2005;49(10):4093-4100.
14. Daverey A, Pakshirajan K. Sophorolipids from *Candida bombicola* using mixed hydrophilic substrates: Production, purification and characterization. Colloids Surf B Biointerfaces. 2010;79(2):521-525.
15. Kim HS, Yoon BD, Lee CH, Suh HH, Oh HM, Katsuragi T, et al. Production and properties of sophorolipids from *Candida bombicola* ATCC 22214 using glucose. J Microbiol Biotechnol. 1996;6(4):280-283.
16. Roelants SLKW, Ciesielska K, De Maeseneire SL, Moens H, Everaert B, Verweire S, et al. Towards the industrialization of new biosurfactants: Biotechnological opportunities for the lactone esterase gene from *Starmerella bombicola*. Biotechnol Bioeng. 2016;113(3):550-559.