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PRODUCTION OF BIOSURFACTANT FROM BIODIESEL SIDE-STREAM GLYCERINE BY Pseudomonas aeruginosa RS6

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Chair : Assoc. Prof. Mohd Rafein Zakaria, PhD
Faculty : Biotechnology and Biomolecular Sciences

Biosurfactant is an amphipathic compound produced by microorganisms as a secondary metabolite. Since biosurfactants are biologically produced compounds that have been shown to be less harmful to the environment, biodegradable, and safe as opposed to conventional synthetic surfactants because they can be produced from renewable substrates. Biosurfactants showed an efficient and successful application in numerous industries such as in agriculture (biopesticide), medical (antimicrobial agent) and washing industry (cleaning agent). Waste glycerine is a by-product of the biodiesel industry, and the current glycerol market is unable to accommodate the excess amounts produced; as a result, new markets for refined glycerol must be developed. This study aims to obtain the optimized fermentation condition for the production of rhamnolipids biosurfactant by *Pseudomonas aeruginosa* RS6 using waste glycerine as a substrate. In this study, the effect of temperature, initial pH medium, waste glycerine concentrations, nitrogen sources and nitrogen concentrations on the production of rhamnolipid biosurfactant was studied. The rhamnolipid produced was determined by high-performance liquid chromatography (HPLC) method, emulsification index, and surface tension measurement. Rhamnolipids produced under the optimized fermentation conditions were further characterized using liquid chromatography-mass spectrometry (LC-MS) and Fourier transform infrared spectrometry (FT-IR) and evaluated on the antimicrobial activity and toxicity of rhamnolipids produced by strain RS6 using selected pathogens to see the potential of using rhamnolipids in agricultural industry. The optimized conditions for the production of rhamnolipids was obtained when *P. aeruginosa* RS6 was grown in a basal salt medium at initial pH 6.5 supplemented with 1.0% waste glycerine (v/v) and 0.2 M sodium nitrate at 35°C. Moreover, the fermentation was carried out in a 2 L stirred tank bioreactor to see the difference in rhamnolipids production compared to in shake flask. About 2.72 g/L and 3.0 g/L of rhamnolipids were obtained from shake flask and stirred tank bioreactor, respectively. In the crude extract of biosurfactant obtained, FT-IR confirmed the presence of rhamnolipids and

LC-MS confirmed the crude consists of both mono- and di-rhamnolipids. Rhamnolipids produced can inhibit the growth of both Gram-positive (*Bacillus pumilus*) and Gram-negative (*Pantoea stewartii*, *Pantoea ananatis* and *Erwinia mallotivora*) plant pathogens (ranging from 37% to 77% of inhibition) and showed low toxicity on fish embryonic toxicity test (91.67% of zebrafish survivals). Results obtained showed that waste glycerine from biodiesel industry could be used as a renewable carbon source for the production of rhamnolipids by *P. aeruginosa* RS6. The rhamnolipids produce can be applied in the agricultural industry as an antimicrobial agent to combat the pathogenic microorganisms causing diseases to plants.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN BIOSURFAKTAN DARIPADA GLISERIN ALIRAN SISI BIODIESEL OLEH *Pseudomonas aeruginosa* RS6

Oleh

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Pengerusi : Prof. Madya. Mohd Rafein Zakaria, PhD
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Biosurfaktan adalah sebatian amfipatik yang dihasilkan oleh mikroorganisma sebagai metabolit sekunder. Oleh kerana sebatian biosurfaktan dihasilkan secara biologi yang telah terbukti kurang berbahaya kepada alam sekitar, terbiodegradasi, dan selamat berbanding dengan surfaktan sintentik konvensional kerana ia boleh dihasilkan dari substrat yang boleh diperbaharui. Biosurfaktan menunjukkan aplikasi yang cekap dan berjaya dalam pelbagai industri seperti dalam industri pertanian (biopestisid), perubatan (agen antimikrob) dan basuh (agen pembersih). Sisa gliserin adalah bahan buangan dari industri biodiesel, dan pasaran gliserol semasa tidak dapat menampung jumlah lebihan yang dihasilkan; maka, pasaran baru untuk gliserol tulen mesti dibangunkan. Kajian ini bertujuan untuk memperoleh keadaan fermentasi yang optimum bagi penghasilan biosurfaktan rhamnolipid oleh *Pseudomonas aeruginosa* RS6 menggunakan sisa gliserin sebagai substrat. Dalam kajian ini, kesan suhu, medium pH awal, kepekatan sisa glyserin, sumber nitrogen dan kepekatan nitrogen ke atas penghasilan biosurfaktan rhamnolipid telah dikaji. Rhamnolipid yang dihasilkan ditentukan dengan kaedah kromatografi cecair prestasi tinggi (HPLC), indeks emulsifikasi dan pengukuran ketegangan permukaan. Rhamnolipid yang dihasilkan dalam keadaan fermentasi yang optimum kemudian dicirikan dengan menggunakan kromatografi cecair spektrometri jisim (LC-MS) dan spektrometri infra-merah transformasi Fourier (FT-IR), dan dinilai untuk aktiviti antimikrobial dan ketoksikan rhamnolipid yang dihasilkan oleh strain RS6 menggunakan organisma terpilih untuk melihat potensi penggunaan rhamnolipid dalam industri pertanian. Keadaan optimum untuk penghasilan rhamnolipid diperoleh ketika *P. aeruginosa* RS6 dihidupkan di dalam medium garam asas pada pH awal media 6.5 dengan penambahan 1.0% sisa gliserin (v/v) dan 0.2 M natrium nitrat pada 35°C. Selain itu, fermentasi dilakukan dalam bioreaktor tangki teraduk 2 L untuk melihat perbezaan penghasilan rhamnolipid berbanding dengan dalam kelalang goncang. Kira-kira 2.72 g/L dan 3.0 g/L rhamnolipid diperolehi daripada

kelalang goncang dan bioreaktor tangki teraduk, masing-masing. Dalam ekstrak kasar biosurfaktan yang diperolehi, FT-IR mengesahkan adanya rhamnolipid dan LC-MS pula mengesahkan ekstrak kasar tersebut terdiri daripada mono- dan di-rhamnolipid. Rhamnolipid yang dihasilkan dapat merencatkan pertumbuhan kedua-dua patogen tumbuhan Gram-positif (*Bacillus pumilus*) dan Gram-negatif (*Pantoea stewartii*, *Pantoea ananatis* and *Erwinia mallotivora*) (antara 37% hingga 77% perencatan) dan menunjukkan ketoksikan yang rendah pada ujian ketoksikan embrio ikan (91.67% zebrafish hidup). Keputusan yang diperolehi menunjukkan bahawa sisa gliserin dari industri biodiesel boleh digunakan sebagai sumber karbon yang boleh diperbaharui untuk penghasilan rhamnolipid oleh *P. aeruginosa* RS6. Rhamnolipid yang dihasilkan boleh digunakan dalam industri pertanian sebagai agen antimikrob untuk memerangi mikroorganisma patogenik yang menyebabkan penyakit kepada tumbuhan.

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I certify that a Thesis Examination Committee has met on **19 November 2020** to conduct the final examination of Shobanah Menon Baskaran on her thesis entitled **" Production of Biosurfactant from Biodiesel Side-Stream Glycerine by *Pseudomonas aeruginosa* RS6"** in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15th March 1998. The Committee recommends that the student be awarded the Master of Science.

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I hereby confirm that:

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Declaration by the Members of Supervisory Committee

This is to certify that:

- The research conducted and the writing of the thesis was under our supervision
- Supervision of responsibilities as slated in Rule 41 in Rule 2003 (Revision 2012-2013) were adhered to.

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LIST OF ABBREVIATIONS

%	Percentage
±	Plus-minus
°C	Degree Celsius
β	Beta
µg/mL	Microgram per millilitre
µL	Microliter
µm	Micrometre
ACP	Acyl carrier protein
B. pumilus	*Bacillus pumilus*
BSM	Basal salt medium
C/N	Carbon-to-nitrogen
$CaCl_2$	Calcium chloride
cm^{-1}	Per cubic centimetre
CMC	Critical micelle concentration
$CoCl_2 \cdot 6\ H_2O$	Cobalt (II) chloride hexahydrate
$CuSO_4 \cdot 5\ H_2O$	Copper sulfate pentahydrate
DMSO	Dimethyl sulfoxide
dTDPL-rhamnose	Deoxy thymidine diphosphate L-rhamnose
E. coli	*Escherichia coli*
E. mallotivora	*Erwinia mallotivora*
E_{24}	Emulsification index after 24 h
E_{48}	Emulsification index after 48 h
E_{72}	Emulsification index after 72 h
ESI	Electrospray ionization
FAME	Fatty acid methyl ester
$FeCl_3 \cdot 6\ H_2O$	Ferric chloride hexahydrate
FT-IR	Fourier transform infrared
g/L	Gram per litre
GC-FID	Gas chromatography with flame ionization detector
h	Hour
HAA	Hydroxyalkanoyloxy alkanoate
HCl	Hydrochloric acid
hpe	Hour post-exposure
hpf	Hour post-fertilization
HPLC	High-performance liquid chromatography
HPLC-UV	High-Performance Liquid Chromatography-Ultraviolet
HSD	Honestly Significant Difference
K_2HPO_4	Dipotassium hydrogen phosphate
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
L	Litre
LC	Lethal concentration
LC-MS	Liquid chromatography-mass spectrometry
M	Molarity
m/z	Mass to charge
m^3	Cubic metre

MEL	Mannosylerythritol-lipids
MEOR	Microbial enhanced oil recovery
mg	Milligram
mg/mL	Milligram per millilitre
$MgSO_4$	Magnesium sulphate
min	Minute
mL	Millilitre
mL/min	Millilitre per minute
mm	Millilitre
mN/m	Millinewton per meter
$MnSO_4 \cdot H_2O$	Manganese sulfate monohydrate
NA	Nutrient agar
$Na_3C_6H_5O_7 \cdot 2H_2O$	Sodium citrate dihydrate
NaCl	Sodium chloride
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
NB	Nutrient broth
NTWG	Non-treated waste glycerine
OD	Optical density
P. aeruginosa	*Pseudomonas aeruginosa*
P. ananatis	*Pantoea ananatis*
P. stewartii	*Pantoea stewartii*
RhlA	3-hydroxyacyl-ACP:3- hydroxyacyl-ACP O-3hydroxyl-acyl-transferase
RhlB	Rhamnosyltransferase I
RhlC	Rhamnosyltransferase II
rpm	Rotation per minute
SDS	Sodium dodecyl sulphate
sp.	Species
SPE	Solid-phase extraction
TLC	Thin layer chromatography
TNTC	Too numerous to count
TWG	Treated waste glycerine
UK	United Kingdom
UPM	Universiti Putra Malaysia
USA	United States of America
v/v	Volume per volume
vvm	Volume of air per volume of liquid per minute
wt	Weight
$ZnSO_4 \cdot 7H_2O$	Zinc sulfate heptahydrate

CHAPTER 1

INTRODUCTION

The word surfactant was derived from the phrase 'surface active agents' and defined the behaviour of amphiphilic molecules at the interfaces between different phases, gas, liquid, and solid (Marchant & Banat, 2012). The surface-active compounds self-aggregate in both polar and non-polar solvent systems and are therefore used in countless industrial and consumer products as emulsifiers, dispersants, wetting agents, and foaming agents (Bhadani *et al*., 2020). Surfactants are essential components for most consumer products, such as detergents, fabric softeners, shampoos, cosmetics, body lotions, and personal care products. They are also commonly used in papermaking industry, dyeing and textile manufacturing, food and beverage processing, pigments, paints and coatings industries, and so forth (Kumar & Tyagi, 2014). In the pharmaceutical industries, surfactants are an essential component for the solubilization of hydrophobic drugs in aqueous media, as components of emulsions, surfactant self-assembly vehicles for oral and transdermal drug delivery, and as agents for enhancing drug absorption and penetration (Pandey *et al*., 2014). In the agricultural industries, the agrochemical formulations include the usage of surfactant, which is not only important for its preparation and long-term physically stability maintenance, but also important for improving the biological efficiency of agrochemicals, enhancing the foliar absorption of herbicides, growth boosters and defoliants (Castro *et al*., 2014).

The efficacy of the surfactant is defined by its ability to lower the surface tension, which is a measure of the surface free energy per unit area needed to bring the molecule from the bulk phase to the surface (Soberón-Chávez & Maier, 2011). The physicochemical characteristics that characterize a surfactant are its abilities to enhance the apparent water solubility of hydrophobic compounds, to create water-hydrocarbon emulsions, and to reduce surface tension (Desai & Banat, 1997). At present, commercial surfactants are synthesized from petrochemicals, animal fats, plants, and microorganisms (Akbari *et al*., 2018). Surfactants were initially produced using renewable resources like fats and oil, while today, the majorities are of petrochemical origin (Rufino *et al*., 2014). Since synthetic surfactant reduces worldwide non-renewable petrochemical resources, has low biodegradability and also can contribute to the environmental pollution, a green alternative which is microbial surfactant has been introduced as this surfactant is biologically produced. Besides, microbial surfactant also exhibits special characteristic which is constant effectiveness over a wide range of pH and temperature (Oliveira *et al*., 2009).

Biosurfactants are produced by plants and animals as well as microorganisms. A wide range of research been conducted on surfactants produced by bacteria, yeast, and fungi (Soberón-Chávez & Maier, 2011). Biosurfactants are derived from

renewable resources and are low or non-toxic, biodegradable, exhibit excellent surface activity, possess high specificity, demonstrate efficacy under extreme conditions and can also be reused as opposed to conventional surfactants (Koglin *et al*., 2010; Lima *et al*., 2011; Xu *et al*., 2011). Among biosurfactants, rhamnolipids, a class of glycolipid mainly known from bacteria of the *Pseudomonas* genus are mostly studied (Henkel *et al*., 2012). Rhamnolipids have been shown to reduce the water surface tension from 72 to 28 mN/m, and the water-oil system interfacial tension from 43 to <1 mN/m (Kaskatepe & Yildiz, 2016). Nevertheless, the values are in the range of an effective and often used surfactant, sodium dodecyl sulfate (SDS). Antimicrobial properties are another additional feature of the rhamnolipids that makes them more attentive. There are several reports stated that the rhamnolipids produced have been demonstrated to be effective against Gram-positive bacteria, Gram-negative bacteria, fungi (Benincasa *et al*., 2004; Haba *et al*., 2003) and insect pest (Kim *et al*., 2011). According to Chen *et al*. (2017), there is a dual mechanism of action for rhamnolipids where they have antimicrobial properties and also activate defensive responses against pathogens in plants. This dual property would lead to productivity improvements and the creation of new biopesticides.

Crude glycerine, also known as crude glycerol, is the by-product produced during biodiesel production. Crude glycerine is generally refined to pure glycerol, valuable raw material for industries, for example, food and beverages, pharmaceutical, cosmetics, tobacco, and textiles (Luo *et al*., 2016). The amount of waste glycerine being produced is at a volumetric ratio of 1:10 to the amount of biodiesel production, which is for every 1 m^3 of biodiesel, 0.1 m^3 of crude glycerine is produced (Monteiro *et al*., 2018). Crude glycerine purification is a tedious approach, and so the use of crude glycerine as it is as a source for any industrial product is a value-added approach (Garlapati *et al*., 2016). Not only does the abundance of crude glycerine generated by the biodiesel industry affect the cost of the production of biodiesel, but it also critically creates an environmental problem (Anuar & Abdullah, 2016). As biodiesel production rises worldwide, glycerine generation will increase accordingly, creating a new issue which is the disposal of waste glycerine (Luo *et al*., 2016; Quispe *et al*., 2013). It can be not only expensive but also wasteful to dispose crude glycerine (He *et al*., 2017). In this manner, it is necessary to find new uses for crude glycerine to improve the economic sustainability of the biodiesel industry and reduce the environmental effects of crude glycerine waste disposal. In recent years, intense research into the use and processing of crude glycerine is on the way with increased yields (Ethier *et al*., 2011; Moon *et al*., 2010; Sabourin-Provost & Hallenbeck, 2009). Many known microorganisms can use glycerol naturally as a sole source of carbon and energy. The low-cost glycerol can be used as a water-soluble substrate for the production of biosurfactants (Makkar *et al*., 2011).

Biosurfactants have already been used in the formulations of a few commercial products, mostly from far east in Asia, but there are still a few issues to overcome before they can be used more widely (Marchant & Banat, 2012). One of the problem is the low yield of biosurfactant being produced. The types and yields of biosurfactants produced are influenced by culture medium, carbon source, and the

growth conditions (pH, temperature, limiting nutrients, and trace elements) (Batista *et al*., 2006). This study aims to determine the optimal environmental factors: temperature, initial pH medium, concentration of waste glycerine, nitrogen source and its concentration for maximum production of rhamnolipids biosurfactant by *Pseudomonas aeruginosa* RS6 with the fermentation of waste glycerine obtained from biodiesel production. The main objectives for this study are;

1. To obtain an optimum fermentation condition for the production of rhamnolipids in shake flask and stirred tank bioreactor by utilizing waste glycerine as a carbon source.

2. To determine the properties of rhamnolipids produced from *Pseudomonas aeruginosa* RS6.

REFERENCES

Abalos, A., Pinazo, A., Infante, M. R., Casals, M., García, F., & Manresa, A. (2001). Physicochemical and antimicrobial properties of new rhamnolipids produced by *Pseudomonas aeruginosa* AT10 from soybean oil refinery wastes. *Langmuir*, *17*(5), 1367–1371.

Abbasi, H., Hamedi, M. M., Lotfabad, T. B., Zahiri, H. S., Sharafi, H., Masoomi, F., Moosavi-Movahedi, A. A., Ortiz, A., Amanlou, M., & Noghabi, K. A. (2012). Biosurfactant-producing bacterium, *Pseudomonas aeruginosa* MA01 isolated from spoiled apples: Physicochemical and structural characteristics of isolated biosurfactant. *Journal of Bioscience and Bioengineering*, *113*(2), 211–219.

Abdel-Mawgoud, A. M., Lépine, F., & Déziel, E. (2010). Rhamnolipids: Diversity of structures, microbial origins and roles. In *Applied Microbiology and Biotechnology* (Vol. 86, Issue 5, pp. 1323–1336).

Abidin, N., Ismail, S. I., Vadamalai, G., Yusof, M. T., Hakiman, M., Karam, D. S., Ismail-Suhaimy, N. W., Ibrahim, R., & Zulperi, D. (2020). Genetic diversity of *Pantoea stewartii* subspecies *stewartii* causing jackfruit-bronzing disease in Malaysia. *PLoS ONE*, *15*(6). h

Abouseoud, M., Maachi, R., & Amrane, A. (2007). Biosurfactant Production from olive oil by *Pseudomonas fluorescens*. *Trends in Applied Microbiology*, 340–347.

Abouseoud, M., Yataghene, A., Amrane, A., & Maachi, R. (2010). Effect of pH and salinity on the emulsifying capacity and naphthalene solubility of a biosurfactant produced by *Pseudomonas fluorescens*. *Journal of Hazardous Materials*, *180*(1–3), 131–136.

Aghajani, M., Rahimpour, A., Amani, H., & Taherzadeh, M. J. (2018). Rhamnolipid as new bio-agent for cleaning of ultrafiltration membrane fouled by whey. *Engineering in Life Sciences*, *18*(5), 272–280.

Akbari, S., Abdurahman, N. H., Yunus, R. M., Fayaz, F., & Alara, O. R. (2018). Biosurfactants—a new frontier for social and environmental safety: a mini review. *Biotechnology Research and Innovation*, *2*(1), 81–90.

Ali, S., Mil, H. G. J. van, & Richardson, M. K. (2011). Large-scale assessment of the zebrafish embryo as a possible predictive model in toxicity testing. *PLoS One*, *6*(6: e21076).

Alwadani, N., & Fatehi, P. (2018). Synthetic and lignin-based surfactants: Challenges and opportunities. *Carbon Resources Conversion*, *1*(2), 126–138.

Amani, H. (2018). Application of a dynamic method for the volumetric mass transfer coefficient determination in the scale-up of rhamnolipid biosurfactant production. *Journal of Surfactants and Detergents*, *August*, 827–833.

Amani, H., Mehrnia, M. R., Sarrafzadeh, M. H., Haghighi, M., & Soudi, M. R. (2010). Scale up and application of biosurfactant from bacillus subtilis in enhanced oil recovery. *Applied Biochemistry and Biotechnology*, *162*(2), 510–523.

Andersen, K. K., & Otzen, D. E. (2014). Folding of outer membrane protein A in the anionic biosurfactant rhamnolipid. *FEBS Letters*, *588*(10), 1955–1960.

Anuar, M. R., & Abdullah, A. Z. (2016). Challenges in biodiesel industry with regards to feedstock, environmental, social and sustainability issues: A critical review. In *Renewable and Sustainable Energy Reviews* (Vol. 58, pp. 208–223). Elsevier Ltd.

Aparna, A., Srinikethan, G., & Smitha, H. (2012). Production and characterization of biosurfactant produced by a novel *Pseudomonas* sp. 2B. *Colloids and Surfaces B: Biointerfaces*, *95*, 23–29.

Aransiola, E. F., Ojumu, T. V., Oyekola, O. O., Madzimbamuto, T. F., & Ikhu-Omoregbe, D. I. O. (2014). A review of current technology for biodiesel production: State of the art. *Biomass and Bioenergy*, *61*, 276–297.

Arino, S., Marchal, R., & Vandecasteele, J. P. (1996). Identification and production of a rhamnolipidic biosurfactant by a *Pseudomonas* species. *Applied Microbiology and Biotechnology*, *45*(1–2), 162–168.

Asadinezhad, A., Novák, I., Lehocký, M., Sedlařík, V., Vesel, A., Junkar, I., Sáha, P., & Chodák, I. (2010). An in vitro bacterial adhesion assessment of surface-modified medical-grade PVC. *Colloids and Surfaces B: Biointerfaces*, *77*(2), 246–256.

Ashby, R. D., Nuñez, A., Solaiman, D. K. Y., & Foglia, T. A. (2005). Sophorolipid biosynthesis from a biodiesel co-product stream. *JAOCS, Journal of the American Oil Chemists' Society*, *82*(9), 625–630.

Ballot, F. (2009). Bacterial Production of Antimicrobial Biosurfactants (Unpublished master's thesis) (Issue March). University of Stellenbosch, South Africa.

Banat, I. M., Satpute, S. K., Cameotra, S. S., Patil, R., & Nyayanit, N. V. (2014). Cost effective technologies and renewable substrates for biosurfactants' production. *Frontiers in Microbiology*, *5*(DEC), 1–18.

Barber, W. P., & Stuckey, D. C. (2000). Nitrogen removal in a modified anaerobic baffled reactor (ABR): 1, denitrification. *Water Research*, *34*(9), 2413–2422.

Batista, S. B., Mounteer, A. H., Amorim, F. R., & Totola, M. R. (2006). Isolation and characterization of biosurfactant / bioemulsifier-producing bacteria from petroleum contaminated sites. *Bioresource Technology*, *97*(6), 868–875.

Bazire, A., Dheilly, A., Diab, F., Morin, D., Jebbar, M., Haras, D., & Dufour, A. (2005). Osmotic stress and phosphate limitation alter production of cell-to-cell signal molecules and rhamnolipid biosurfactant by *Pseudomonas aeruginosa*. *FEMS Microbiology Letters*, *253*(1), 125–131.

Befkadu, A. A., & Chen, Q. (2018). Surfactant-enhanced soil washing for removal of petroleum hydrocarbons from contaminated soils: a review. *Pedosphere*, *28*(3), 383–410.

Benincasa, M., Abalos, A., Oliveira, I., & Manresa, A. (2004). Chemical structure, surface properties and biological activities of the biosurfactant produced by *Pseudomonas aeruginosa* LBI from soapstock. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *85*(1), 1–8.

Benincasa, M., Contiero, J., Manresa, M. ., & Moraes, I. . (2002). Rhamnolipid production by *Pseudomonas aeruginosa* LBI growing on soapstock as the sole carbon source. *Journal of Food Engineering*, *54*(4), 283–288.

Bergstorm, S., Toerell, H., & Davide, H. (1946). Pyolipic acid, a metabolic product of

Pseudomonas pyocyanea, active against *Mycobacterium tuberculosis*. *Arch Biochem*, *23*(A), 1–12.

Bewley, B. R., Berkaliev, A., Henriksen, H., Ball, D. B., & Ott, L. S. (2015). Waste glycerol from biodiesel synthesis as a component in deep eutectic solvents. *Fuel Processing Technology*, *138*, 419–423.

Bhadani, A., Kafle, A., Ogura, T., Akamatsu, M., Sakai, K., Sakai, H., & Abe, M. (2020). Current perspective of sustainable surfactants based on renewable building blocks. *Current Opinion in Colloid & Interface Science*, *45*, 124–135.

Bharali, P., Saikia, J. P., Ray, A., & Konwar, B. K. (2013). Rhamnolipid (RL) from *Pseudomonas aeruginosa* OBP1: A novel chemotaxis and antibacterial agent. *Colloids and Surfaces B: Biointerfaces*, *103*, 502–509.

Bharali, P., Singh, S. P., Dutta, N., Gogoi, S., Bora, L. C., Debnath, P., & Konwar, B. K. (2014). Biodiesel derived waste glycerol as an economic substrate for biosurfactant production using indigenous *Pseudomonas aeruginosa*. *RSC Advances*, *4*(73), 38698–38706.

Biswal, N. R., & Paria, S. (2014). Interfacial and wetting behavior of natural-synthetic mixed surfactant systems. *RSC Advances*, *4*(18), 9182–9188.

Bragg, J. R., Prince, R. C., Harner, E. J., & Atlas, R. M. (1994). Effectiveness of bioremediation for the Exxon Valdez oil spill. *Nature*, *368*(6470), 413–418.

Bratovcic, A., Nazdrajic, S., Odobasic, A., & Sestan, I. (2018). The influence of type of surfactant on physicochemical properties of liquid soap. *Int. J. Mat. Chem*, *8*, 31–37.

Braunbeck, T., Böttcher, M., Hollert, H., Kosmehl, T., Lammer, E., Leist, E., Rudolf, M., & Seitz, N. (2005). Towards an alternative for the acute fish lc50 test in chemical assessment: the fish embryo toxicity test goes multi-species-an update. *Alternatives to Animal Experimentation*, *22*(2), 87–102.

Braunbeck, T., Kais, B., Lammer, E., Otte, J., Schneider, K., Stengel, D., & Strecker, R. (2014). The fish embryo test (FET): origin, applications, and future. *Environmental Science and Pollution Research*, *22*(21), 16247–16261.

Campos, J. M., Montenegro Stamford, T. L., Sarubbo, L. A., de Luna, J. M., Rufino, R. D., & Banat, I. M. (2013). Microbial biosurfactants as additives for food industries. *Biotechnology Progress*, *29*(5), 1097–1108.

Castro, M., Ojeda, C., & Cirelli, A. (2014). Advances in surfactants for agrochemicals. *Environmental Chemistry Letters*, *12*, 85–95.

Chatzifragkou, A., & Papanikolaou, S. (2012). Effect of impurities in biodiesel-derived waste glycerol on the performance and feasibility of biotechnological processes. In *Applied Microbiology and Biotechnology* (Vol. 95, Issue 1, pp. 13–27).

Chaudhari, S. P., & Dugar, R. P. (2017). Application of surfactants in solid dispersion technology for improving solubility of poorly water soluble drugs. *Journal of Drug Delivery Science and Technology*, *41*, 68–77.

Chayabutra, C., Wu, J., & Ju, L. K. (2001). Rhamnolipid production by *Pseudomonas aeruginosa* under denitrification: Effects of limiting nutrients and carbon substrates. *Biotechnology and Bioengineering*, *72*(1), 25–33.

Chen, J., Wu, Q., Hua, Y., Chen, J., Zhang, H., & Wang, H. (2017). Potential applications of biosurfactant rhamnolipids in agriculture and biomedicine. In *Applied Microbiology and Biotechnology* (Vol. 101, Issues 23–24, pp. 8309–8319). Springer Verlag.

Chen, S.-Y., Lu, W.-B., Wei, Y.-H., Chen, W.-M., & Chang, J.-S. (2007). Improved production of biosurfactant with newly isolated *Pseudomonas aeruginosa* S2. *Biotechnology Progress*, *23*(3), 661–666.

Chiu, C. W., Dasari, M. A., Sutterlin, W. R., & Suppes, G. J. (2006). Removal of residual catalyst from simulated biodiesel's crude glycerol for glycerol hydrogenolysis to propylene glycol. *Industrial and Engineering Chemistry Research*, *45*(2), 791–795.

Chong, H., & Li, Q. (2017). Microbial production of rhamnolipids: Opportunities, challenges and strategies. *Microbial Cell Factories*, *16*(1), 1–12.

Chrzanowski, Ł., Dziadas, M., Ławniczak, Ł., Cyplik, P., Białas, W., Szulc, A., Lisiecki, P., & Jeleń, H. (2012). Biodegradation of rhamnolipids in liquid cultures: Effect of biosurfactant dissipation on diesel fuel/B20 blend biodegradation efficiency and bacterial community composition. *Bioresource Technology*, *111*, 328–335.

Costa, S. G. V. A. O., Nitschke, M., Haddad, R., Eberlin, M. N., & Contiero, J. (2006). Production of *Pseudomonas aeruginosa* LBI rhamnolipids following growth on Brazilian native oils. *Process Biochemistry*, *41*(2), 483–488.

Coutinho, T. A., & Venter, S. N. (2009). Pantoea ananatis: An unconventional plant pathogen. *Molecular Plant Pathology*, *10*(3), 325–335.

Dasari, M. A., Kiatsimkul, P. P., Sutterlin, W. R., & Suppes, G. J. (2005). Low-pressure hydrogenolysis of glycerol to propylene glycol. *Applied Catalysis A: General*, *281*(1–2), 225–231.

De, S., Malik, S., Ghosh, A., Saha, R., & Saha, B. (2015). A review on natural surfactants. *RSC Advances*, *5*(81), 65757–65767.

de Koster, C. G., Vos, B., Versluis, C., Heerma, W., & Haverkamp, J. (1994). High-performance thin-layer chromatography/fast atom bombardment (tandem) mass spectrometry of *Pseudomonas* rhamnolipids. *Biological Mass Spectrometry*, *23*(4), 179–185.

De Rienzo, M. A. D., Kamalanathan, I. D., & Martin, P. J. (2016). Comparative study of the production of rhamnolipid biosurfactants by *B. thailandensis* E264 and *P. aeruginosa* ATCC 9027 using foam fractionation. *Process Biochemistry*, *51*(7), 820–827.

de Sousa, J. R., da Costa Correia, J. A., De Almeida, J. G. L., Rodrigues, S., Pessoa, O. D. L., Melo, V. M. M., Gonalves, L. R. B., & Gonçalves, L. R. B. (2011). Evaluation of a co-product of biodiesel production as carbon source in the production of biosurfactant by *P. aeruginosa* MSIC02. *Process Biochemistry*, *46*(9), 1831–1839.

Deleu, M., Paquot, M., & Blecker, C. (2015). Surface tension measurements on micellar systems. *Encyclopedia of Surface and Colloid Science, Third Edition*, 5119–5126.

Demirbas, A. (2008). Biodiesel: A realistic fuel alternative for diesel engines. In

Biodiesel: A Realistic Fuel Alternative for Diesel Engines.

Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews : MMBR*, *61*(1), 47–64.

Déziel, E., Lépine, F., Dennie, D., Boismenu, D., Mamer, O. A., & Villemur, R. (2000). Liquid chromatography/mass spectrometry analysis of mixtures of rhamnolipids produced by *Pseudomonas aeruginosa* strain 57RP grown on mannitol or naphthalene. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1440*(2–3), 244–252.

Dharmadi, Y., Murarka, A., & Gonzalez, R. (2006). Anaerobic fermentation of glycerol by *Escherichia coli*: A new platform for metabolic engineering. *Biotechnology and Bioengineering*, *94*(5), 821–829.

Díaz De Rienzo, M. A., Stevenson, P., Marchant, R., & Banat, I. M. (2016). Antibacterial properties of biosurfactants against selected Gram-positive and -negative bacteria. *FEMS Microbiology Letters*, *363*(2).

Diaz De Rienzo, M. A., Stevenson, P. S., Marchant, R., & Banat, I. M. (2016). Effect of biosurfactants on *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms in a BioFlux channel. *Applied Microbiology and Biotechnology*, *100*(13), 5773–5779.

dos Santos, A. S., Pereira Jr, N., & Freire, D. M. G. (2016). Strategies for improved rhamnolipid production by *Pseudomonas aeruginosa* PA1. *PeerJ*, *4*, e2078.

Ebbensgaard, A., Mordhorst, H., Aarestrup, F. M., & Hansen, E. B. (2018). The role of outer membrane proteins and lipopolysaccharides for the sensitivity of *Escherichia coli* to antimicrobial peptides. *Frontiers in Microbiology*, *9*(SEP), 2153.

Edwards, K. R., Lepo, J. E., & Lewis, M. A. (2003). Toxicity comparison of biosurfactants and synthetic surfactants used in oil spill remediation to two estuarine species. *Marine Pollution Bulletin*, *46*(10), 1309–1316.

Ehinmitola, E. O., Aransiola, E. F., & Adeagbo, O. P. (2018). Comparative study of various carbon sources on rhamnolipid production. *South African Journal of Chemical Engineering*, *26*, 42–48.

Eraqi, W. A., Yassin, A. S., Ali, A. E., & Amin, M. A. (2016). Utilization of crude glycerol as a substrate for the production of rhamnolipid by *Pseudomonas aeruginosa*. *Biotechnology Research International*, *2016*, 1–9.

Ethier, S., Woisard, K., Vaughan, D., & Wen, Z. (2011). Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid. *Bioresource Technology*, *102*(1), 88–93.

Farid, M. A. A., Hassan, M. A., Roslan, A. M., Ariffin, H., Norrrahim, M. N. F., Othman, M. R., & Yoshihito, S. (2021). Improving the decolorization of glycerol by adsorption using activated carbon derived from oil palm biomass. *Environmental Science and Pollution Research*, 1–12.

Farid, M. A. A., Hassan, M. A., Taufiq-Yap, Y. H., Shirai, Y., Hasan, M. Y., & Zakaria, M. R. (2017). Waterless purification using oil palm biomass-derived bioadsorbent improved the quality of biodiesel from waste cooking oil. *Journal of Cleaner*

Production, *165*, 262–272.

Farobie, O., & Matsumura, Y. (2017). State of the art of biodiesel production under supercritical conditions. *Progress in Energy and Combustion Science*, *63*, 173–203.

Ferreira, J. de F., Vieira, E. A., & Nitschke, M. (2019). The antibacterial activity of rhamnolipid biosurfactant is pH dependent. *Food Research International*, *116*, 737–744.

Franzetti, A., Di Gennaro, P., Bevilacqua, A., Papacchini, M., & Bestetti, G. (2006). Environmental features of two commercial surfactants widely used in soil remediation. *Chemosphere*, *62*(9), 1474–1480.

Fu, J., Sharma, P., Spicer, V., Krokhin, O. V, Zhang, X., Fristensky, B., Wilkins, J. A., Cicek, N., Sparling, R., & Levin, D. B. (2015). Effects of impurities in biodiesel-derived glycerol on growth and expression of heavy metal ion homeostasis genes and gene products in *Pseudomonas putida* LS46. *Applied Microbiology and Biotechnology*, *99*(13), 5583–5592.

Funston, S. J., Tsaousi, K., Rudden, M., Smyth, T. J., Stevenson, P. S., Marchant, R., & Banat, I. M. (2016). Characterising rhamnolipid production in *Burkholderia thailandensis* E264, a non-pathogenic producer. *Applied Microbiology and Biotechnology*, *100*(18), 7945–7956.

Gao, Z., Ma, Y., Wang, Q., Zhang, M., Wang, J., & Liu, Y. (2016). Effect of crude glycerol impurities on lipid preparation by *Rhodosporidium toruloides* yeast 32489. *Bioresource Technology*, *218*, 373–379.

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

Garlapati, V. K., Shankar, U., & Budhiraja, A. (2016). Bioconversion technologies of crude glycerol to value added industrial products. In *Biotechnology Reports* (Vol. 9, pp. 9–14). Elsevier B.V.

Gazem, A., Laheb, M., Kumar, S., & Rajesh, A. (2018). Surface and interfacial tension for various liquids. *International Refereed Journal of Engineering and Science*, *7*(1), 64–66.

Gong, Z., Peng, Y., & Wang, Q. (2015). Rhamnolipid production , characterization and fermentation scale-up by *Pseudomonas aeruginosa* with plant oils. *Biotechnology Letters*, *37*(10), 2033–2038.

González-Pajuelo, M., Meynial-Salles, I., Mendes, F., Soucaille, P., & Vasconcelos, I. (2006). Microbial conversion of glycerol to 1,3-propanediol: Physiological comparison of a natural producer, *Clostridium butyricum* VPI 3266, and an engineered strain, *Clostridium acetobutylicum* DG1(pSPD5). *Applied and Environmental Microbiology*, *72*(1), 96–101.

Gudiña, E. J., Rodrigues, A. I., de Freitas, V., Azevedo, Z., Teixeira, J. A., & Rodrigues, L. R. (2016). Valorization of agro-industrial wastes towards the production of rhamnolipids. *Bioresource Technology*, *212*, 144–150.

Gunther, N. W., Nuñez, A., Fett, W., & Solaiman, D. K. Y. (2005). Production of

rhamnolipids by *Pseudomonas chlororaphis*, a nonpathogenic bacterium. *Applied and Environmental Microbiology*, *71*(5), 2288–2293.

Haba, E., Pinazo, A., Jauregui, O., Espuny, M. J., Infante, M. R., & Manresa, A. (2003). Physicochemical characterization and antimicrobial properties of rhamnolipids produced by *Pseudomonas aeruginosa* 47T2 NCBIM 40044. *Biotechnology and Bioengineering*, *81*(3), 316–322.

Hamzah, A., Sabturani, N., & Radiman, S. (2013). Screening and optimization of biosurfactant production by the hydrocarbon-degrading bacteria. *Sains Malaysiana*, *42*(5), 615–623.

He, Q. (Sophia), McNutt, J., & Yang, J. (2017). Utilization of the residual glycerol from biodiesel production for renewable energy generation. In *Renewable and Sustainable Energy Reviews* (Vol. 71, pp. 63–76).

Henkel, M., Müller, M. M., Kügler, J. H., Lovaglio, R. B., Contiero, J., Syldatk, C., & Hausmann, R. (2012). Rhamnolipids as biosurfactants from renewable resources: Concepts for next-generation rhamnolipid production. *Process Biochemistry*, *47*(8), 1207–1219.

Henn, K., & Braunbeck, T. (2011). Dechorionation as a tool to improve the fish embryo toxicity test (FET) with the zebrafish (Danio rerio). *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *153*(1), 91–98.

Hermsen, S. A. B., van den Brandhof, E.-J., van der Ven, L. T. M., & Piersma, A. H. (2011). Relative embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test and comparison with their in vivo potencies. *Toxicology in Vitro*, *25*(3), 745–753.

Hogan, D. E., Tian, F., Malm, S. W., Olivares, C., Palos Pacheco, R., Simonich, M. T., Hunjan, A. S., Tanguay, R. L., Klimecki, W. T., Polt, R., Pemberton, J. E., Curry, J. E., & Maier, R. M. (2019). Biodegradability and toxicity of monorhamnolipid biosurfactant diastereomers. *Journal of Hazardous Materials*, *364*, 600–607.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., Collins, J. E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J. C., Koch, R., Rauch, G. J., White, S., Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*(7446), 498–503.

Hu, S., Luo, X., Wan, C., & Li, Y. (2012). Characterization of crude glycerol from biodiesel plants. *Journal of Agricultural and Food Chemistry*, *60*(23), 5915–5921.

Irie, Y., O'Toole, G. A., & Yuk, M. H. (2005). *Pseudomonas aeruginosa* rhamnolipids disperse *Bordetella bronchiseptica* biofilms. *FEMS Microbiology Letters*, *250*(2), 237–243.

Irorere, V. U., Tripathi, L., Marchant, R., McClean, S., & Banat, I. M. (2017). Microbial rhamnolipid production: a critical re-evaluation of published data and suggested future publication criteria. *Applied Microbiology and Biotechnology*, *101*(10), 3941–3951.

Ismail, W., Shammary, S. Al, El-Sayed, W. S., Obuekwe, C., El Nayal, A. M., Abdul Raheem, A. S., & Al-Humam, A. (2015). Stimulation of rhamnolipid biosurfactants production in *Pseudomonas aeruginosa* AK6U by organosulfur

compounds provided as sulfur sources. *Biotechnology Reports*, *7*, 55–63.

Ivanković, T., & Hrenović, J. (2010). Surfactants in the environment. In *Arhiv za Higijenu Rada i Toksikologiju* (Vol. 61, Issue 1, pp. 95–110).

Jadhav, V. V., Yadav, A., Shouche, Y. S., Aphale, S., Moghe, A., Pillai, S., Arora, A., & Bhadekar, R. K. (2013). Studies on biosurfactant from *Oceanobacillus* sp. BRI 10 isolated from antarctic sea water. *Desalination*, *318*, 64–71.

Jahan, R., Bodratti, A. M., Tsianou, M., & Alexandridis, P. (2019). Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications. *Advances in Colloid and Interface Science*, 102061.

Jimoh, A. A., & Lin, J. (2019). Biosurfactant: A new frontier for greener technology and environmental sustainability. *Ecotoxicology and Environmental Safety*, *184*, 109607.

Johann, S., Seiler, T.-B., Tiso, T., Bluhm, K., Blank, L. M., & Hollert, H. (2016). Mechanism-specific and whole-organism ecotoxicity of mono-rhamnolipids. *Science of The Total Environment*, *548–549*, 155–163.

Joice, P. A., & Parthasarathi, R. (2014). Optimization of biosurfactant production from *Pseudomonas aeruginosa* PBSC1. *International Journal of Current Microbiology and Applied Sciences*, *3*(9), 140–151.

Joshi, S. J., Geetha, S. J., Yadav, S., & Desai, A. J. (2013). Optimization of bench-scale production of biosurfactant by *Bacillus licheniformis* R2. *APCBEE Procedia*, *5*, 232–236.

Karthick, A., Roy, B., & Chattopadhyay, P. (2019). A review on the application of chemical surfactant and surfactant foam for remediation of petroleum oil contaminated soil. *Journal of Environmental Management*, *243*, 187–205.

Kaskatepe, B., & Yildiz, S. (2016). Rhamnolipid biosurfactants produced by *Pseudomonas* Species. *Brazilian Archives of Biology and Technology*, *59*, 1–16.

Katoh, S., Horiuchi, J., & Yoshida, F. (2015). Instrumentation and control of bioprocesses. In S. Katoh, J. Horiuchi, & F. Yoshida (Eds.), *Biochemical Engineering* (2nd ed., pp. 217–234). Wiley-VCH Verlag GmbH & Co. KGaA.

Khan, A. H. A., Tanveer, S., Alia, S., Anees, M., Sultan, A., Iqbal, M., & Yousaf, S. (2017). Role of nutrients in bacterial biosurfactant production and effect of biosurfactant production on petroleum hydrocarbon biodegradation. *Ecological Engineering*, *104*, 158–164.

Khanna, S., Goyal, A., & Moholkar, V. S. (2012). Microbial conversion of glycerol: Present status and future prospects. In *Critical Reviews in Biotechnology* (Vol. 32, Issue 3, pp. 235–262).

Kim, B. S., Lee, J. Y., & Hwang, B. K. (2000). In vivo control and in vitro antifungal activity of rhamnolipid B, a glycolipid antibiotic, against *Phytophthora capsici* and *Colletotrichum orbiculare*. *Pest Management Science*, *56*(12), 1029–1035.

Kim, H. S., Jeon, J. W., Kim, B. H., Ahn, C. Y., Oh, H. M., & Yoon, B. D. (2006). Extracellular production of a glycolipid biosurfactant, mannosylerythritol lipid, by *Candida* sp. SY16 using fed-batch fermentation. *Applied Microbiology and Biotechnology*, *70*(4), 391–396.

Kim, S. K., Kim, Y. C., Lee, S., Kim, J. C., Yun, M. Y., & Kim, I. S. (2011). Insecticidal activity of rhamnolipid isolated from *Pseudomonas* sp. EP-3 against green peach aphid (*Myzus persicae*). *Journal of Agricultural and Food Chemistry*, *59*(3), 934–938.

Kiran, G. S., Ninawe, A. S., Lipton, A. N., Pandian, V., & Selvin, J. (2016). Rhamnolipid biosurfactants: Evolutionary implications, applications and future prospects from untapped marine resource. In *Critical Reviews in Biotechnology* (Vol. 36, Issue 3, pp. 399–415).

Koch, A. K., Kappeli, O., Fiechter, A., & Reiser, J. (1991). Hydrocarbon assimilation and biosurfactant production in *Pseudomonas aeruginosa* mutants. *Journal of Bacteriology*, *173*(13), 4212–4219.

Koglin, A., Doetsch, V., & Bernhard, F. (2010). Molecular engineering aspects for the production of new and modified biosurfactants. *Advances in Experimental Medicine and Biology*, *672*, 158–169.

Kondo, T., Kamikawa, M., & Oka, K. (2015). Glycerin purification method. In *US Patent App. 14/* http://www.google.com/patents/US20140114095

Kong, P. S., Aroua, M. K., & Daud, W. M. A. W. (2016). Conversion of crude and pure glycerol into derivatives: A feasibility evaluation. *Renewable and Sustainable Energy Reviews*, *63*, 533–555.

Kongjao, S., Damronglerd, S., & Hunsom, M. (2010). Purification of crude glycerol derived from waste used-oil methyl ester plant. *Korean Journal of Chemical Engineering*, *27*(3), 944–949.

Kronemberger, F. D. A., Santa Anna, L. M. M., Fernandes, A. C. L. B., Menezes, R. R. De, Borges, C. P., & Freire, D. M. G. (2008). Oxygen-controlled biosurfactant production in a bench scale bioreactor. *Applied Biochemistry and Biotechnology*, *147*(1–3), 33–45.

Kumar, L. R., Yellapu, S. K., Tyagi, R. D., & Zhang, X. (2019). A review on variation in crude glycerol composition, bio-valorization of crude and purified glycerol as carbon source for lipid production. *Bioresource Technology*, *293*, 122155.

Kumar, N., & Tyagi, R. (2014). Industrial applications of dimeric surfactants: a review. *Journal of Dispersion Science and Technology*, *35*(2), 205–214.

Laarman, A. J., Bardoel, B. W., Ruyken, M., Fernie, J., Milder, F. J., van Strijp, J. A. G., & Rooijakkers, S. H. M. (2012). *Pseudomonas aeruginosa* alkaline protease blocks complement activation via the classical and lectin pathways. *The Journal of Immunology*, *188*(1), 386–393.

LaBauve, A. E., & Wargo, M. J. (2012). Growth and laboratory maintenance of *Pseudomonas aeruginosa*. *Current Protocols in Microbiology*, *SUPPL.25*.

Lan, G., Fan, Q., Liu, Y., Liu, Y., Liu, Y., Yin, X., & Luo, M. (2015). Effects of the addition of waste cooking oil on heavy crude oil biodegradation and microbial enhanced oil recovery using *Pseudomonas* sp. SWP- 4. *Biochemical Engineering Journal*, *103*, 219–226.

Ławniczak, Ł., Marecik, R., & Chrzanowski, Ł. (2013). Contributions of biosurfactants to natural or induced bioremediation. In *Applied Microbiology and Biotechnology*

(Vol. 97, Issue 6, pp. 2327–2339).

Lee, K. M., Hwang, S. H., Ha, S. D., Jang, J. H., Lim, D. J., & Kong, J. Y. (2004). Rhamnolipid production in batch and fed-batch fermentation using *Pseudomonas aeruginosa* BYK-2 KCTC 18012P. *Biotechnology and Bioprocess Engineering*, *9*(4), 267–273.

Leitermann, F., Syldatk, C., & Hausmann, R. (2008). Fast quantitative determination of microbial rhamnolipids from cultivation broths by ATR-FTIR Spectroscopy. *Journal of Biological Engineering*, *2*.

Lima, T. M. S., Procópio, L. C., Brandão, F. D., Carvalho, A. M. X., Tótola, M. R., & Borges, A. C. (2011). Biodegradability of bacterial surfactants. *Biodegradation*, *22*(3), 585–592.

Liu, G., Zhong, H., Yang, X., Liu, Y., Shao, B., & Liu, Z. (2018). Advances in applications of rhamnolipids biosurfactant in environmental remediation: A review. *Biotechnology and Bioengineering*, *115*(4), 796–814.

Lotfabad, T. B., Abassi, H., Ahmadkhaniha, R., Roostaazad, R., Masoomi, F., Zahiri, H. S., Ahmadian, G., Vali, H., & Noghabi, K. A. (2010). Structural characterization of a rhamnolipid-type biosurfactant produced by *Pseudomonas aeruginosa* MR01: Enhancement of di-rhamnolipid proportion using gamma irradiation. *Colloids and Surfaces B: Biointerfaces*, *81*(2), 397–405.

Lotfabad, T. B., Ebadipour, N., & Roostaazad, R. (2016). Evaluation of a recycling bioreactor for biosurfactant production by *Pseudomonas aeruginosa* MR01 using soybean oil waste. *Journal of Chemical Technology and Biotechnology*, *91*(5), 1368–1377.

Lotfabad, T. B., Shahcheraghi, F., & Shooraj, F. (2013). Assessment of antibacterial capability of rhamnolipids produced by two indigenous *Pseudomonas aeruginosa* strains. *Jundishapur Journal of Microbiology*, *6*(1), 29–35.

Lotfabad, T. B., Shourian, M., Roostaazad, R., Najafabadi, A. R., Adelzadeh, M. R., & Noghabi, K. A. (2009). An efficient biosurfactant-producing bacterium *Pseudomonas aeruginosa* MR01, isolated from oil excavation areas in south of Iran. *Colloids and Surfaces B: Biointerfaces*, *69*(2), 183–193.

Lukic, M., Pantelic, I., & Savic, S. (2016). An overview of novel surfactants for formulation of cosmetics with certain emphasis on acidic active substances. In *Tenside, Surfactants, Detergents* (Vol. 53, Issue 1, pp. 7–19).

Luna, J. M., Rufino, R. D., Sarubbo, L. A., Lígia, R. R., José, A. T., Galba, M. de C.-T., Rodrigues, L. R. M., Teixeira, J. A. C., & de Campos-Takaki, G. M. (2011). Evaluation antimicrobial and antiadhesive properties of the biosurfactant lunasan produced by *Candida sphaerica* UCP 0995. *Current Microbiology*, *62*(5), 1527–1534.

Luo, X., Ge, X., Cui, S., & Li, Y. (2016). Value-added processing of crude glycerol into chemicals and polymers. *Bioresource Technology*, *215*, 144–154.

Magalhães, L., & Nitschke, M. (2013). Antimicrobial activity of rhamnolipids against *Listeria monocytogenes* and their synergistic interaction with nisin. *Food Control*, *29*(1), 138–142.

Makkar, R. S., Cameotra, S. S., & Banat, I. M. (2011). Advances in utilization of renewable substrates for biosurfactant production. In *AMB Express* (Vol. 1, Issue 1, pp. 1–19). Springer Verlag.

Mańko, D., & Zdziennicka, A. (2015). Sugar-based surfactants as alternative to synthetic ones. *Annales UMCS, Chemia*, *70*(1), 161–168.

Marchant, R., & Banat, I. M. (2012). Microbial biosurfactants: challenges and opportunities for future exploitation. *Trends in Biotechnology*, *30*(11), 558–565.

Marchant, R., & Banat, I. M. (2014). Protocols for measuring biosurfactant production in microbial cultures. In T. J. McGenity, K. N. Timmis, & B. Nogales (Eds.), *Hydrocarbon and Lipid Microbiology Protocols* (pp. 119–128). Springer Berlin Heidelberg.

Mata-Sandoval, J. C., Karns, J., & Torrents, A. (2001). Effect of nutritional and environmental conditions on the production and composition of rhamnolipids by *P. aeruginosa* UG2. *Microbiological Research*, *155*(4), 249–256.

Md, F. (2012). Biosurfactant: Production and application. *Journal of Petroleum & Environmental Biotechnology*, *3*(4).

Meesters, P. A. E. P., Huijberts, G. N. M., & Eggink, G. (1996). High-cell-density cultivation of the lipid accumulating yeast *Cryptococcus curvatus* using glycerol as a carbon source. *Applied Microbiology and Biotechnology*, *45*(5), 575–579.

Mehling, A., Kleber, M., & Hensen, H. (2007). Comparative studies on the ocular and dermal irritation potential of surfactants. *Food and Chemical Toxicology*, *45*(5), 747–758.

Mirsalehian, A., Kalantar-Neyestanaki, D., Taherikalani, M., Jabalameli, F., & Emaneini, M. (2017). Determination of carbapenem resistance mechanism in clinical isolates of *Pseudomonas aeruginosa* isolated from burn patients, in Tehran, Iran. *Journal of Epidemiology and Global Health*, *7*(3), 155–159.

Mnif, I., & Ghribi, D. (2016). Glycolipid biosurfactants: main properties and potential applications in agriculture and food industry. *Journal of the Science of Food and Agriculture*, *96*(13), 4310–4320.

Mohan, P. K., Nakhla, G., & Yanful, E. K. (2006). Biokinetics of biodegradation of surfactants under aerobic, anoxic and anaerobic conditions. *Water Research*, *40*(3), 533–540.

Mohd Taha, M. D., Mohd Jaini, M. F., Saidi, N. B., Abdul Rahim, R., Md Shah, U. K., & Mohd Hashim, A. (2019). Biological control of *Erwinia mallotivora*, the causal agent of papaya dieback disease by indigenous seed-borne endophytic lactic acid bacteria consortium. *PLOS ONE*, *14*(12), e0224431.

Monteiro, M. R., Kugelmeier, C. L., Pinheiro, R. S., Batalha, M. O., & da Silva César, A. (2018). Glycerol from biodiesel production: Technological paths for sustainability. In *Renewable and Sustainable Energy Reviews* (Vol. 88, pp. 109–122).

Moon, C., Ahn, J. H., Kim, S. W., Sang, B. I., & Um, Y. (2010). Effect of biodiesel-derived raw glycerol on 1,3-propanediol production by different microorganisms. *Applied Biochemistry and Biotechnology*, *161*(1–8), 502–510.

Moussa, T. A. A., Mohamed, M. S., & Samak, N. (2014). Production and characterization of di-rhamnolipid produced by *Pseudomonas aeruginosa* TMN. *Brazilian Journal of Chemical Engineering*, *31*(4), 867–880.

Muhammad, M. T., & Khan, M. N. (2018). Eco-friendly, biodegradable natural surfactant (Acacia Concinna): An alternative to the synthetic surfactants. *Journal of Cleaner Production*, *188*, 678–685.

Mukherjee, S., Das, P., & Sen, R. (2006). Towards commercial production of microbial surfactants. *Trends in Biotechnology*, *24*(11), 509–515.

Müller, M. M., Hörmann, B., Syldatk, C., & Hausmann, R. (2010). *Pseudomonas aeruginosa* PAO1 as a model for rhamnolipid production in bioreactor systems. *Applied Microbiology and Biotechnology*, *87*(1), 167–174.

Mulligan, C. N. (2005). Environmental applications for biosurfactants. *Environmental Pollution*, *133*(2), 183–198.

Muntaha, S. T., & Khan, M. N. (2015). Natural surfactant extracted from *Sapindus mukurossi* as an eco-friendly alternate to synthetic surfactant - A dye surfactant interaction study. *Journal of Cleaner Production*, *93*, 145–150.

Narayan, M. S., Manoj, G. P., Vatchravelu, K., Bhagyalakshmi, N., & Mahadevaswamy, M. (2005). Utilization of glycerol as carbon source on the growth, pigment and lipid production in Spirulina platensis. *International Journal of Food Sciences and Nutrition*, *56*(7), 521–528.

Negin, C., Ali, S., & Xie, Q. (2017). Most common surfactants employed in chemical enhanced oil recovery. In *Petroleum* (Vol. 3, Issue 2, pp. 197–211). KeAi Communications Co.

Nitschke, M., & Costa, S. G. V. A. O. (2007). Biosurfactants in food industry. In *Trends in Food Science and Technology* (Vol. 18, Issue 5, pp. 252–259).

Noll, P., Treinen, C., Müller, S., Senkalla, S., Lilge, L., Hausmann, R., & Henkel, M. (2019). Evaluating temperature-induced regulation of a ROSE-like RNA-thermometer for heterologous rhamnolipid production in *Pseudomonas putida* KT2440. *AMB Express*, *9*(1).

Nordin, N., Zakaria, M., Halmi, M., Ariff, A., Zawawi, R., & Wasoh, H. (2013). Isolation and screening of high efficiency biosurfactant-producing *Pseudomonas* sp. *Journal of Biochemistry, Microbiology and Biotechnology*, *1*(1), 25–31.

Oliveira, F. J. S., Vazquez, L., de Campos, N. P., & de França, F. P. (2009). Production of rhamnolipids by a *Pseudomonas alcaligenes* strain. *Process Biochemistry*, *44*(4), 383–389.

Onwosi, C. O., & Odibo, F. J. C. (2012). Effects of carbon and nitrogen sources on rhamnolipid biosurfactant production by *Pseudomonas nitroreducens* isolated from soil. *World Journal of Microbiology and Biotechnology*, *28*(3), 937–942.

Ortiz, A., Teruel, J. A., Espuny, M. J., Marqués, A., Manresa, Á., & Aranda, F. J. (2006). Effects of dirhamnolipid on the structural properties of phosphatidylcholine membranes. *International Journal of Pharmaceutics*, *325*(1–2), 99–107.

Pacwa-Płociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: Recent advances. In *International*

Journal of Molecular Sciences (Vol. 12, Issue 1, pp. 633–654).

Pandey, A. (2014). Role of surfactants as penetration enhancer in transdermal drug delivery system. *Journal of Molecular Pharmaceutics & Organic Process Research*, *2*(2).

Papanikolaou, S., & Aggelis, G. (2002). Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. *Bioresource Technology*, *82*(1), 43–49.

Papanikolaou, S., Fakas, S., Fick, M., Chevalot, I., Galiotou-Panayotou, M., Komaitis, M., Marc, I., & Aggelis, G. (2008). Biotechnological valorisation of raw glycerol discharged after bio-diesel (fatty acid methyl esters) manufacturing process: Production of 1,3-propanediol, citric acid and single cell oil. *Biomass and Bioenergy*, *32*(1), 60–71.

Pardo-Martin, C., Chang, T. Y., Koo, B. K., Gilleland, C. L., Wasserman, S. C., & Yanik, M. F. (2010). High-throughput in vivo vertebrate screening. *Nature Methods*, *7*(8), 634–636.

Paulino, B. N., Pessôa, M. G., Mano, M. C. R., Molina, G., Neri-Numa, I. A., & Pastore, G. M. (2016). Current status in biotechnological production and applications of glycolipid biosurfactants. *Applied Microbiology and Biotechnology*, *100*(24), 10265–10293.

Pei, X., Zhan, X., & Zhou, L. (2009). Effect of biosurfactant on the sorption of phenanthrene onto original and H2O2-treated soils. *Journal of Environmental Sciences*, *21*(10), 1378–1385.

Pereira, A. G., Pacheco, G. J., Tavares, L. F., Neves, B. C., Kronemberger, F. de A., Reis, R. S., & Freire, D. M. G. (2013). Optimization of biosurfactant production using waste from biodiesel industry in a new membrane assisted bioreactor. *Process Biochemistry*, *48*(9), 1271–1278.

Pi, G., Mao, L., Bao, M., Li, Y., Gong, H., & Zhang, J. (2015). Preparation of oil-in-seawater emulsions based on environmentally benign nanoparticles and biosurfactant for oil spill remediation. *ACS Sustainable Chemistry & Engineering*, *3*(11), 2686–2693.

Pieper, D. H., & Reineke, W. (2000). Engineering bacteria for bioremediation. *Current Opinion in Biotechnology*, *11*(3), 262–270.

Pornsunthorntawee, O., Wongpanit, P., Chavadej, S., Abe, M., & Rujiravanit, R. (2008). Structural and physicochemical characterization of crude biosurfactant produced by *Pseudomonas aeruginosa* SP4 isolated from petroleum-contaminated soil. *Bioresource Technology*, *99*(6), 1589–1595.

Pradima, J., Kulkarni, M. R., & Archna. (2017). Review on enzymatic synthesis of value added products of glycerol, a by-product derived from biodiesel production. *Resource-Efficient Technologies*, *3*(4), 394–405.

Praveesh, B. V., Soniyamby, A. R., Mariappan, C., Kavithakumari, P., Palaniswamy, M., & Lalitha, S. (2011). Biosurfactant production by *Pseudomonas* Sp from soil using whey as carbon source. *4*(4).

Putri, M., & Hertadi, R. (2015). Effect of glycerol as carbon source for biosurfactant

production by halophilic bacteria *Pseudomonas stutzeri* BK-AB12. *Procedia Chemistry*, *16*, 321–327.

Quispe, C. A. G., Coronado, C. J. R., & Carvalho Jr., J. A. (2013). Glycerol: Production, consumption, prices, characterization and new trends in combustion. *Renewable and Sustainable Energy Reviews*, *27*, 475–493.

Rahim, R., Ochsner, U. A., Olvera, C., Graninger, M., Messner, P., Lam, J. S., & Soberón-Chávez, G. (2001). Cloning and functional characterization of the *Pseudomonas aeruginosa* rhlC gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. *Molecular Microbiology*, *40*(3), 708–718.

Rahman, K. S. M., Rahman, T. J., McClean, S., Marchant, R., & Banat, I. M. (2002). Rhamnolipid biosurfactant production by strains of *Pseudomonas aeruginosa* using low-cost raw materials. *Biotechnology Progress*, *18*(6), 1277–1281.

Randhawa, K. K. S., & Rahman, P. K. S. M. (2014). Rhamnolipid biosurfactants-past, present, and future scenario of global market. *Frontiers in Microbiology*, *5*, 1–7.

Reddy, K. S., Khan, M. Y., Archana, K., Reddy, M. G., & Hameeda, B. (2016). Utilization of mango kernel oil for the rhamnolipid production by *Pseudomonas aeruginosa* DR1 towards its application as biocontrol agent. *Bioresource Technology*, *221*, 291–299.

Rikalović, M. G., Vrvić, M. M., & Karadžić, I. M. (2015). Rhamnolipid biosurfactant from *Pseudomonas aeruginosa* - From discovery to application in contemporary technology. *Journal of the Serbian Chemical Society*, *80*(3), 279–304.

Ron, E. Z., & Rosenberg, E. (2001). Natural roles of biosurfactants. *Environmental Microbiology*, *3*(4), 229–236.

Roy, S., Chandni, S., Das, I., Karthik, L., Kumar, G., & Bhaskara Rao, K. V. (2015). Aquatic model for engine oil degradation by rhamnolipid producing Nocardiopsis VITSISB. *3 Biotech*, *5*(2), 153–164.

Rufino, R. D., de Luna, J. M., de Campos Takaki, G. M., & Sarubbo, L. A. (2014). Characterization and properties of the biosurfactant produced by *Candida lipolytica* UCP 0988. *Electronic Journal of Biotechnology*, *17*(1), 34–38.

Sabourin-Provost, G., & Hallenbeck, P. C. (2009). High yield conversion of a crude glycerol fraction from biodiesel production to hydrogen by photofermentation. *Bioresource Technology*, *100*(14), 3513–3517.

Sachdev, D. P., & Cameotra, S. S. (2013). Biosurfactants in agriculture. In *Applied Microbiology and Biotechnology* (Vol. 97, Issue 3, pp. 1005–1016).

Sadeghnejad, A., Aroujalian, A., Raisi, A., & Fazel, S. (2014). Antibacterial nano silver coating on the surface of polyethylene films using corona discharge. *Surface and Coatings Technology*, *245*, 1–8.

Saikia, R. R., Deka, S., Deka, M., & Sarma, H. (2012). Optimization of environmental factors for improved production of rhamnolipid biosurfactant by *Pseudomonas aeruginosa* RS29 on glycerol. *Journal of Basic Microbiology*, *52*(4), 446–457.

Samul, D., Leja, K., & Grajek, W. (2014). Impurities of crude glycerol and their effect on metabolite production. *Annals of Microbiology*, *64*(3), 891–898.

Santa Ana, L. M., Soriano, A. U., Gomes, A. C., Menezes, E. P., Gutarra, M. L. E., Freire, D. M. G., & Pereira, N. (2007). Use of biosurfactant in the removal of oil from contaminated sandy oil. *Journal of Chemical Technology and Biotechnology*, *82*(7), 687–691.

Santos, D. K. F., Meira, H. M., Rufino, R. D., Luna, J. M., & Sarubbo, L. A. (2017). Biosurfactant production from *Candida lipolytica* in bioreactor and evaluation of its toxicity for application as a bioremediation agent. *Process Biochemistry*, *54*, 20–27.

Santos, D. K. F., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). Biosurfactants: Multifunctional biomolecules of the 21st century. *International Journal of Molecular Sciences*, *17*(3), 1–31.

Schenk, T., Schuphan, I., & Schmidt, B. (1995). High-performance liquid chromatographic determination of the rhamnolipids produced by *Pseudomonas aeruginosa*. *Journal of Chromatography A*, *693*(1), 7–13.

Scholz, S., Fischer, S., Gündel, U., Küster, E., Luckenbach, T., & Voelker, D. (2008). The zebrafish embryo model in environmental risk assessment - Applications beyond acute toxicity testing. In *Environmental Science and Pollution Research* (Vol. 15, Issue 5, pp. 394–404). Springer.

Schooling, S. R., Charaf, U. K., Allison, D. G., & Gilbert, P. (2004). A role for rhamnolipid in biofilm dispersion. *Biofilms*, *1*(2), 91–99.

Sdrula, N. (2010). A study using classical or membrane separation in the biodiesel process. *Desalination*, *250*(3), 1070–1072.

Sezer, N., Atieh, M. A., & Koç, M. (2019). A comprehensive review on synthesis, stability, thermophysical properties, and characterization of nanofluids. *Powder Technology*, *344*, 404–431.

Sha, R., Jiang, L., Meng, Q., Zhang, G., & Song, Z. (2012). Producing cell-free culture broth of rhamnolipids as a cost-effective fungicide against plant pathogens. *Journal of Basic Microbiology*, *52*(4), 458–466.

Shah, S. K., Bhattarai, A., & Chatterjee, S. K. (2013). Applications of surfactants in modern science and technology. *Modern Trends in Science and Technology*, 147–158.

Sharma, D. (2016). Classification and properties of biosurfactants. *Surfactant Science Series*, 21–42.

Sharma, R., Singh, J., & Verma, N. (2018). Optimization of rhamnolipid production from *Pseudomonas aeruginosa* PBS towards application for microbial enhanced oil recovery. *3 Biotech*, *8*(1).

Silva, S. N. R. L., Farias, C. B. B., Rufino, R. D., Luna, J. M., & Sarubbo, L. A. (2010). Glycerol as substrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP0992. *Colloids and Surfaces B: Biointerfaces*, *79*(1), 174–183.

Sim, L., Ward, O. P., & Li, Z. Y. (1997). Production and characterisation of a biosurfactant isolated from *Pseudomonas aeruginosa* UW-1. *Journal of Industrial Microbiology and Biotechnology*, *19*(4), 232–238.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2006).

Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples: Laboratory Analytical Procedure (LAP*); Issue Date: 12/08/2006*. *January*. www.nrel.gov

Smyth, T. J. P., Guazzaroni, M. E., & Corte, N. L. (2010). Isolation and analysis of low molecular weight microbial glycolipids. *Handbook of Hydrocarbon and Lipid Microbiology*, *1*(Iii), 1–6.

Soberón-Chávez, G., Aguirre-Ramírez, M., & Sánchez, R. (2005). The *Pseudomonas aeruginosa* RhlA enzyme is involved in rhamnolipid and polyhydroxyalkanoate production. *Journal of Industrial Microbiology and Biotechnology*, *32*(11), 675.

Soberón-Chávez, G., & Maier, R. M. (2011). Biosurfactants: A General Overview (G. Soberón-Chávez (ed.); pp. 1–11). Springer Berlin Heidelberg.

Sperandio, F., Huang, Y.-Y., & Hamblin, M. (2013). Antimicrobial photodynamic therapy to kill gram-negative bacteria. *Recent Patents on Anti-Infective Drug Discovery*, *8*(2), 108–120.

Supardan, M. D., Adisalamun, Lubis, Y. M., Annisa, Y., Satriana, & Mustapha, W. A. W. (2017). Effect of co-solvent addition on glycerolysis of waste cooking oil. *Pertanika Journal of Science and Technology*, *25*(4).

Tiso, T., Zauter, R., Tulke, H., Leuchtle, B., Li, W. J., Behrens, B., Wittgens, A., Rosenau, F., Hayen, H., & Blank, L. M. (2017). Designer rhamnolipids by reduction of congener diversity: Production and characterization. *Microbial Cell Factories*, *16*(1), 1–14.

Trummler, K., Effenberger, F., & Syldatk, C. (2003). An integrated microbial/enzymatic process for production of rhamnolipids and L-(+)-rhamnose from rapeseed oil with *Pseudomonas* sp. DSM 2874. *European Journal of Lipid Science and Technology*, *105*(10), 563–571.

Truong, L., Reif, D. M., St Mary, L., Geier, M. C., Truong, H. D., & Tanguay, R. L. (2013). Multidimensional in vivo hazard assessment using zebrafish. *Toxicological Sciences*, *137*(1), 212–233.

Varjani, S. J., & Upasani, V. N. (2016). Carbon spectrum utilization by an indigenous strain of *Pseudomonas aeruginosa* NCIM 5514: Production, characterization and surface active properties of biosurfactant. *Bioresource Technology*, *221*, 510–516.

Varjani, S. J., & Upasani, V. N. (2017). Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. *Bioresource Technology*, *232*, 389–397.

Varvaresou, A., & Iakovou, K. (2015). Biosurfactants in cosmetics and biopharmaceuticals. *Letters in Applied Microbiology*, *61*(3), 214–223.

Vatsa, P., Sanchez, L., Clement, C., Baillieul, F., & Dorey, S. (2010). Rhamnolipid biosurfactants as new players in animal and plant defense against microbes. *International Journal of Molecular Sciences*, *11*(12), 5095–5108.

Vaughan, M., & Van Egmond, R. (2010). The use of the zebrafish (Danio rerio) embryo for the acute toxicity testing of surfactants, as a possible alternative to the acute fish test. *ATLA Alternatives to Laboratory Animals*, *38*(3), 231–238.

Vigneshwaran, C., Vasantharaj, M. J. K., & Sivasubramanian, V. (2016). A review on

biosurfactants and its environmental applications. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *10*(8), 152–160.

Vivek, N., Sindhu, R., Madhavan, A., Anju, A. J., Castro, E., Faraco, V., Pandey, A., & Binod, P. (2017). Recent advances in the production of value added chemicals and lipids utilizing biodiesel industry generated crude glycerol as a substrate – Metabolic aspects, challenges and possibilities: An overview. *Bioresource Technology*, *239*, 507–517.

Wang, Y., Zhang, Y., Li, X., Sun, M., Wei, Z., Wang, Y., Gao, A., Chen, D., Zhao, X., & Feng, X. (2015). Exploring the effects of different types of surfactants on zebrafish embryos and larvae. *Scientific Reports*, *5*.

Wei, Y.-H., Chou, C.-L., & Chang, J.-S. (2005). Rhamnolipid production by indigenous *Pseudomonas aeruginosa* J4 originating from petrochemical wastewater. *Biochemical Engineering Journal*, *27*(2), 146–154.

Whang, L. M., Liu, P. W. G., Ma, C. C., & Cheng, S. S. (2008). Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. *Journal of Hazardous Materials*, *151*(1), 155–163.

Williams, P., & Cámara, M. (2009). Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules. In *Current Opinion in Microbiology* (Vol. 12, Issue 2, pp. 182–191).

Wu, J.-Y., Yeh, K.-L., Lu, W.-B., Lin, C.-L., & Chang, J.-S. (2008). Rhamnolipid production with indigenous *Pseudomonas aeruginosa* EM1 isolated from oil-contaminated site. *Bioresource Technology*, *99*(5), 1157–1164.

Xia, W. J., Luo, Z. Bin, Dong, H. P., Yu, L., Cui, Q. F., & Bi, Y. Q. (2012). Synthesis, characterization, and oil recovery application of biosurfactant produced by indigenous *Pseudomonas aeruginosa* WJ-1 using waste vegetable oils. *Applied Biochemistry and Biotechnology*, *166*(5), 1148–1166.

Xiao, Y., Xiao, G., & Varma, A. (2013). A universal procedure for crude glycerol purification from different feedstocks in biodiesel production: Experimental and simulation study. *Industrial and Engineering Chemistry Research*, *52*(39), 14291–14296.

Xu, Q., Nakajima, M., Liu, Z., & Shiina, T. (2011). Biosurfactants for microbubble preparation and application. *International Journal of Molecular Sciences*, *12*(1), 462–475.

Yang, F., Hanna, M. A., & Sun, R. (2012). Value-added uses for crude glycerol - A byproduct of biodiesel production. *Biotechnology for Biofuels*, *5*(1), 13.

Ying, G.-G. (2006). Fate, behavior and effects of surfactants and their degradation products in the environment. *Environment International*, *32*(3), 417–431.

Yuan, Y., & Gao, M. (2015). Genomic analysis of a ginger pathogen *Bacillus pumilus* providing the understanding to the pathogenesis and the novel control strategy. *Scientific Reports*, *5*.

Zhang, L., Jiao, L., Zhong, J., Guan, W., & Lu, C. (2017). Lighting up the interactions between bacteria and surfactants with aggregation-induced emission characteristics. *Materials Chemistry Frontiers*, *1*(9), 1829–1835.

Zhang, X., Xu, D., Zhu, C., Lundaa, T., & Scherr, K. E. (2012). Isolation and identification of biosurfactant producing and crude oil degrading *Pseudomonas aeruginosa* strains. *Chemical Engineering Journal*, *209*, 138–146.

Zheng, P., Wereath, K., Sun, J., van den Heuvel, J., & Zeng, A. P. (2006). Overexpression of genes of the dha regulon and its effects on cell growth, glycerol fermentation to 1,3-propanediol and plasmid stability in *Klebsiella pneumoniae*. *Process Biochemistry*, *41*(10), 2160–2169.

Zheng, T., Xu, Y. S., Yong, X. Y., Li, B., Yin, D., Cheng, Q. W., Yuan, H. R., & Yong, Y. C. (2015). Endogenously enhanced biosurfactant production promotes electricity generation from microbial fuel cells. *Bioresource Technology*, *197*, 416–421.

Zhou, J., Xue, R., Liu, S., Xu, N., Xin, F., Zhang, W., Jiang, M., & Dong, W. (2019). High di-rhamnolipid production using *Pseudomonas aeruginosa* KT1115, separation of mono/di-rhamnolipids, and evaluation of their properties. *Frontiers in Bioengineering and Biotechnology*, *7*, 245.

Zhu, C., Chiu, S., Nakas, J. P., & Nomura, C. T. (2013). Bioplastics from waste glycerol derived from biodiesel industry. *Journal of Applied Polymer Science*, *130*(1), 1–13.

APPENDICES

Appendix A

Extraction of biosurfactant

(a) Before vigorous shaking of acidified cell-free supernatant and ethyl acetate.
(b) After two times of extraction process.

(a)

(b)

Appendix B

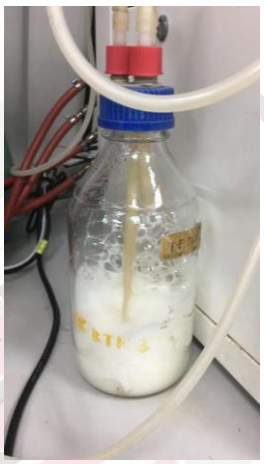
Crude extract

This is the crude extract obtained after concentrating using a rotary evaporator.

Appendix C

2 L bioreactor

Fermentation of biosurfactant in a 2 L bioreactor with recycling accessory.



Appendix D

Antimicrobial activity using the microdilution method

This is the 96-well flat-bottom microdilution plates used to determine the antimicrobial activity of rhamnolipids against plant pathogens.

Appendix E

Results of HSD function of SAS (version 9)

This is the example of analysis output from the SAS for the parameter temperature.

```
The SAS System          16:25 Tuesday, July 27, 2020   1

 Obs    trt    rep      Rl       Ed        Ec

  1     25      1     1.117    30.000    37.931
  2     25      2     1.831    26.667    26.667
  3     25      3     1.504    46.667    21.530
  4     30      1     1.423    58.065    13.333
  5     30      2     1.417    60.000    10.345
  6     30      3     1.420    58.065    15.333
  7     35      1     1.735    60.000    60.000
  8     35      2     1.889    60.000    43.000
  9     35      3     1.664    60.000    51.852
 10     37      1     0.944    56.667    16.667
 11     37      2     0.985    61.290    29.667
 12     37      3     1.115    56.667    14.286

                    The ANOVA Procedure

                  Class Level Information

                Class        Levels    Values

               trt                 4    25 30 35 37

                rep                3    1 2 3

                 Number of observations    12

                    The ANOVA Procedure

                 Dependent Variable: Rl

                                 Sum of
Source                    DF         Squares     Mean Square    F Value    Pr
                                                                           > F

  Model                    3      0.85743333      0.28581111       7.67
                                                                    0.0097

        Error              8      0.29791733      0.03723967

          Corrected Total             11      1.15535067

        R-Square     Coeff Var      Root MSE       Rl Mean

        0.742141      13.58666      0.192976      1.420333
```

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	0.85743333	0.28581111	7.67	0.0097

The ANOVA Procedure
Dependent Variable: Ed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1358.778632	452.926211	14.71	0.0013
Error	8	246.376829	30.797104		
Corrected Total	11	1605.155461			

R-Square	Coeff Var	Root MSE	Ed Mean
0.846509	10.50235	5.549514	52.84067

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	1358.778632	452.926211	14.71	0.0013

The ANOVA Procedure
Dependent Variable: Ec

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2529.951160	843.317053	15.51	0.0011
Error	8	435.019895	54.377487		
Corrected Total	11	2964.971054			

R-Square	Coeff Var	Root MSE	Ec Mean
0.853280	25.97958	7.374109	28.38425

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	2529.951160	843.317053	15.51	0.0011

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Rl

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.03724
Critical Value of Studentized Range	4.52880
Minimum Significant Difference	0.5046

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt
A	1.7627	3	35
B A	1.4840	3	25
B A	1.4200	3	30
B	1.0147	3	37

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Ed

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	30.7971
Critical Value of Studentized Range	4.52880
Minimum Significant Difference	14.51

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt
A	60.000	3	35
A	58.710	3	30
A	58.208	3	37
B	34.445	3	25

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for Ec

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	54.37749
Critical Value of Studentized Range	4.52880
Minimum Significant Difference	19.281

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt
A	51.617	3	35
B	28.709	3	25
B			
B	20.207	3	37
B			
B	13.004	3	30

BIODATA OF STUDENT

Shobanah Menon Baskaran was born on 10th of January 1995 at Hospital Kajang, Kajang, Selangor, Malaysia. Her educational journey started at Sekolah Kebangsaan Cina Yu Hua, Kajang from 2002 to 2007. She then furthered her secondary education at Sekolah Menengah Kebangsaan Jalan Empat, Bandar Baru Bangi, Selangor from 2008 to 2012. Upon receiving her Sijil Pelajaran Malaysia (SPM) result in 2013, she was offered to do Foundation in Agricultural Science in Universiti Putra Malaysia. The author graduated with a Bachelor of Science with Honours in Biotechnology from Universiti Putra Malaysia in 2018. Right after completing her bachelor's degree, she started her master's degree in September 2018 in Environmental Biotechnology under the supervision of Associate Professor Dr. Mohd Rafein Zakaria.

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