PRODUCTION OF BIOSURFACTANT FROM USED COOKING OIL BY LOCAL BACTERIAL ISOLATES FOR HEAVY METALS REMOVAL

NURUL HANISAH MD BADRUL HISHAM

FBSB 2019 4

PRODUCTION OF BIOSURFACTANT FROM USED COOKING OIL BY LOCAL BACTERIAL ISOLATES FOR HEAVY METALS REMOVAL

By

NURUL HANISAH MD BADRUL HISHAM

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

January 2019

COPYRIGHT

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

PRODUCTION OF BIOSURFACTANT FROM USED COOKING OIL BY LOCAL BACTERIAL ISOLATES FOR HEAVY METALS REMOVAL

By

NURUL HANISAH MD BADRUL HISHAM

January 2019

Chairman : Professor Suraini Abd-Aziz, PhD
Faculty : Biotechnology and Biomolecular Sciences

Heavy metals from industrial effluents and sewage contribute to serious water pollution in most developing countries. The constant penetration and contamination of heavy metals into natural water sources may substantially raise the chances of human exposure to these metals through ingestion, inhalation or skin contact which could lead to liver damage, cancer and other severe conditions in the long term. Biosurfactant as an efficient biological surface active agent may provide an alternative solution for the removal of heavy metals from the industrial wastes. Biosurfactants exhibit the properties of reducing surface and interfacial tension, stabilizing emulsions, promoting foaming, high selectivity and specific activity at extreme temperatures, pH, and salinity, and ability to be synthesized from renewable resources. The eco-friendly and biodegradable nature of biosurfactants make their usage more favourable over chemical surfactants. Therefore, this study aimed to produce biosurfactant from renewable feedstock which is used cooking oil (UCO) by local isolates for heavy metals removal. Four bacterial isolates capable of utilizing UCO as a carbon source were isolated using Bushnell and Haas agar and screened for biosurfactant production with the help of oil spreading assay and surface tension activity. Out of the four Gram-positive isolates, the strain HIP3 showed the highest oil displacement area with the lowest surface tension of 38 mN/m after 7 days of culturing in mineral salt medium and 2% (v/v)

UCO at temperature of 30°C and agitation of 200 rpm. Strain HIP3 was identified as *Bacillus* sp. based on 16S rRNA gene sequencing and was selected as a potential biosurfactant producing microorganism. Extraction method using chloroform: methanol (2:1) as the solvents gave the highest biosurfactant yield which is 9.5 g/L. High Performance Liquid Chromatography (HPLC) analysis confirmed that the biosurfactant produced by *Bacillus* sp. HIP3 consists of a lipopeptide similar to standard surfactin. The biosurfactant capable of removing 13.57%, 12.71%, 2.91%, 1.68%, and 0.7% of copper, lead, zinc, chromium, and cadmium, respectively from artificially contaminated water, highlighting its potential for bioremediation.

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

PENGHASILAN BIOSURFAKTAN DARIPADA MINYAK MASAK TERPAKAI OLEH BAKTERIA PENCILAN TEMPATAN UNTUK PENYINGKIRAN LOGAM BERAT

Oleh

NURUL HANISAH MD BADRUL HISHAM

Januari 2019

Pengerusi : Profesor Suraini Abd-Aziz, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Logam berat dari efluen dan kumbahan perindustrian menyumbang kepada pencemaran air yang serius di kebanyakan negara membangun. Pencemaran berterusan logam berat ke dalam sumber air semulajadi boleh meningkatkan pendedahan manusia terhadap logam ini melalui penghadaman, penyedutan atau sentuhan kulit yang boleh mengakibatkan keadaan teruk dalam jangka panjang. Biosurfaktan sebagai kaedah biologi untuk penyingkiran logam berat dari sisa industri memberikan penyelesaian alternatif. Biosurfaktan adalah sebatian permukaan aktif yang mempamerkan pengurangan ketegangan permukaan dan antara muka, menstabilkan emulsi, menggalakkan pembentukan buih, selektiviti dan aktiviti khusus yang tinggi pada suhu, pH, dan kemasinan melampau, dan keupayaan untuk disintesis daripada sumber diperbaharui. Sifat biosurfaktan yang mesra alam dan terbiodegradasikan menjadikan lebih baik daripada surfaktan kimia. Kajian ini bertujuan menghasilkan biosurfaktan dari bahan mentah diperbaharui; minyak masak terpakai (MMT) untuk penyingkiran logam berat. Empat pencilan bakteria yang menggunakan MMT sebagai sumber karbon telah diasingkan menggunakan agar *Bushnell* dan *Haas* dan disaring untuk penghasilan biosurfaktan dengan ujian penyebaran minyak dan aktiviti ketegangan permukaan. Strain HIP3 menunjukkan kawasan anjakan minyak

tertinggi dengan ketegangan permukaan terendah iaitu 38 mN/m selepas 7 hari membiak dalam medium garam mineral pada suhu 30°C, agitasi 200 rpm dan 2% (v/v) MMT. Strain HIP3 adalah sebagai spesis *Bacillus* berdasarkan turutan rRNA 16s. Kaedah pengekstrakan menggunakan kloroform: metanol (2:1) memberikan hasil biosurfaktan tertinggi iaitu 9.5 g/L. Analisis kromatografi cecair prestasi tinggi (HPLC) mengesahkan biosurfaktan yang dihasilkan oleh spesis *Bacillus* HIP3 terdiri dari lipopeptida yang serupa dengan surfaktin standard. Biosurfaktan menyingkirkan 13.57%, 12.71%, 2.91%, 1.68%, dan 0.7% masing-masing tembaga, plumbum, zink, kromium dan kadmium dari air tercemar buatan, menonjolkan potensi bioremediasi.

ACKNOWLEDGEMENTS

Praise to Allah SWT, whom with His willing I was able to complete this research thesis entitled "Characterization of Biosurfactant from Used Cooking Oil by Local Bacterial Isolates for Heavy Metals Removal". I take this opportunity to express my profound gratitude to my project supervisor, Professor Dr. Suraini Abd. Aziz for her exemplary guidance, monitoring and throughout the course of this thesis. A special thanks to my co-supervisors, Dr. Mohamad Faizal Ibrahim and Dr. Norhayati Ramli for their cordial support and valuable information which helped me in completing this task through various stages. I also would like to convey my gratitude to Universiti Putra Malaysia and Ministry of Higher Education Malaysia for the funding throughout my study.

Not forgotten the members of Environmental Biotechnology Group (EB Group), Universiti Putra Malaysia for their never-ending support, advices and suggestions in completing this research especially Hazwani, Arisyah, Farhana, Fatin, Atheera, Suhailah, Marahaini, Ruqayyah, Nabilah, Enis, Siti and Jamilah. Lastly, I would like to express my deepest appreciation to all my family members for their moral supports especially my parents, Mr. Md Badrul Hisham and Mrs. Nor Azmina; my siblings, Khairul, Hawa, Hanani, Amin, Haziqah and Hafizah; and also my fiancée, Syahid. Without their endless love, support, and encouragement, I probably would not have this strength to complete the research.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Suraini Abd. Aziz, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohamad Faizal Ibrahim, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Norhayati Ramli, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced; this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No : Nurul Hanisah Md Badrul Hisham, GS40893

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Suraini Abd. Aziz

Signature: _____
Name of Member of Supervisory Committee: Dr. Mohamad Faizal Ibrahim

Signature: _____
Name of Member of Supervisory Committee: Dr. Norhayati Ramli

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µL	Microliter
µL/min	Microliter per Minute
µm	Micrometer
AHLs	Acylhomoserine Lactones
ATCC	American Type Culture Collection
ATP-P_i	Adenosine Triphosphate Inorganic Phosphate
AU	Absorbance Unit
BH	Bushnell and Haas
bp	Base Pair
BS	Biosurfactant
C/N	Carbon to Nitrogen Ratio
Ca	Calcium
Cd	Cadmium
cm	Centimeter
cm^2	Centimeter x Centimeter
CMC	Critical Micelle Concentration
Cr	Chromium
Da	Dalton
dH_2O	Distilled Water

DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribonucleotide Triphosphate
EOR	Enhanced Oil Recovery
FAME	Fatty Acid Methyl Ester
FFAs	Free Fatty Acids
g	Gram
g/L	Gram per Litre
GC-MS	Gas Chromatography–Mass Spectrometry
h	Hour
HPLC	High Performance Liquid Chromatography
HPLC-MS	High Performance Liquid Chromatography-Mass Spectrometry
HPLC-UV	High Performance Liquid Chromatography-Ultraviolet
id	Internal Diameter
IFT	Interfacial Tension
kb	Kilo Bases
kg	Kilogram
MEGA	Molecular Evolutionary Genetics Analysis
MEOR	Microbial Enhanced Oil Recovery
mg	Milligram
mg/L	Milligram per Liter

min	Minute
mL	Milliliter
mL/min	Milliliter per Minute
mN/m	Millinewton per Meter
MSM	Mineral Salt Medium
MTCC	Microbial Type Culture Collection
MW	Molecular Weight
NA	Nutrient Agar
NCBI	National Center for Biotechnology Information
nm	Nanometer
NRPSs	Nonribosomal Peptide Synthetases
OD	Optical Density
v/v	Volume per Volume
Pb	Lead
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDA	Photo-Diode Array
POME	Palm Oil Mill Effluent
ppm	Part per Million
QSS	Quorum Sensing System
rpm	Rotation per Minute

rRNA	Ribosomal Ribonucleic Acid
SDBS	Sodium Dodecyl Benzene Sulfonate
sec	Second
sp.	Species
ST	Surface Tension
TFA	Trifluoroacetic Acid
UCO	Used Cooking Oil
UPM	Universiti Putra Malaysia
USA	United States of America
UV	Ultraviolet
w/v	Weight per Volume
w/w	Weight per Weight
YPD	Yeast Potato Dextrose

CHAPTER 1

INTRODUCTION

Vegetable oils are used globally especially for food preparation and the most common cooking oil in Malaysia is made from oil palm because of its availability and low price relative to other sources, such as olive, corn or coconut plants (Yaakob et al., 2013). These vegetable oils are used in cooking due to its contribution to good taste, attractive colors, and better presentation. As this trend becomes increasingly popular, accumulation of waste generated from cooking oil also increases. There is a growing concern regarding the environmental impact of the rise in the production of used cooking oil in homes and restaurants. Kheang et al. (2006) conveyed that around 50,000 tons of used cooking oil generated from vegetable oils and/or animal fats are disposed of to the environment without proper treatment annually in Malaysia only. This act in the long run contributes to water and soil contamination, causes aquatic life distraction, sewer system blockages and overflow, increases water treatment and waste management cost, and consequently generates undesirable impacts to the entire environmental system (Yaakob et al., 2013).

The interest in microbial surfactants or biosurfactants has been steadily increasing in recent years mainly due to the possibility of production from wastes other than diversity and environmentally friendly nature (Banat, 1995). In addition, the production of biosurfactants from renewable substrates could reduce one of the limiting factors: the high production costs, which is related with incompetent methods for product recovery and purification (dos Santos et al., 2010). Oily substrate such as used cooking oil which may cause severe environmental problems has been proven to be a good and cheap renewable carbon source for the production of biosurfactants (Jamal et al., 2011; Oliveira & Garcia-Cruz, 2013; Haba et al., 2000). The used cooking oil from domestic waste also contains appropriate balance of nutrients to support optimum bacterial growth and synthesis of biosurfactants (Makkar & Cameotra, 1999; Oliveira & Garcia-Cruz, 2013).

Biosurfactants have various chemical compositions generally consisting of fatty acids, glycolipids, lipopeptides, lipopolysaccharides and lipoproteins depending on the producing microorganism, raw material and process conditions. It is an amphiphilic molecule with both hydrophilic and

hydrophobic (generally hydrocarbon) moieties that partition preferentially at the interface between fluid phases with different degrees of polarity and hydrogen bonding such as oil/water or air/water interfaces. Numerous characteristics of biosurfactants such as foaming, dispersion, wetting, emulsification/de-emulsification, and coating make them suitable to be applied in the physicochemical and biological remediation technologies of both organic and metal contaminants (Pacwa-Płociniczak et al., 2011).

Heavy metals, which are generally more persistent than organic pollutants due to their non-biodegradability and high toxicity even at trace concentrations, are becoming one of the most serious environmental problems today. It may lead to bioaccumulation in living organisms, causing health problems in animals, plants and human beings if it was left untreated in the environment (Argun et al., 2007). The property of microbial surfactant to chelate toxic heavy metals and form an insoluble precipitate was seen as the perfect solution to the treatment of heavy metals in wastewater due to its greater environmental compatibility, lower toxicity and higher biodegradability than synthetic surfactants (Mulligan, 2009).

One of the most popular biosurfactant used in bioremediation of sites contaminated with toxic heavy metals is surfactin since it was said to have the powerful surface activity. Surfactin is the cyclic lipopeptide biosurfactants produced by *Bacillus* sp. and it can reduce the surface tension of water from 72 to 27mN/m at concentrations as low as 0.005% (Arima et al., 1968). The potential advantages of using the lipopeptide surfactin include the presence of two charges due to glutamic and aspartic amino acids as part of its peptide structure, thus the binding of heavy metals would be expected (Thimon et al., 1992).

Therefore, the objectives of this study were:

1. To screen, isolate, and characterize biosurfactant producing bacteria
2. To produce, characterize and evaluate the efficiency of biosurfactant from used cooking oil as a substrate by locally isolated bacteria for heavy metals removal

REFERENCES

Affandi, I. E., Suratman, N. H., Abdullah, S., Ahmad, W. A., & Zakaria, Z. A. (2014). Degradation of oil and grease from high-strength industrial effluents using locally isolated aerobic biosurfactant-producing bacteria. *International Biodeterioration Biodegradation*, pp. 33–40. Elsevier Ltd.

Al-Bahry, S. N., Al-Wahaibi, Y. M., Elshafie, A. E., Al-Bemani, A. S., Joshi, S. J., Al-Makhmari, H. S., & Al-Sulaimani, H. S. (2013). Biosurfactant production by *Bacillus subtilis* B20 using date molasses and its possible application in enhanced oil recovery. *International Biodeterioration and Biodegradation*, *81*, 141–146.

Amiriyan, A., Assadi, M. M., Saggadian, V. A., & Noohi, A. (2004). Bioemulsan Production by Iranian Oil Reservoirs. *Iranian Journal of Environmental Health Science & Engineering*, *1*(2), 28–35.

Vinithkumar, N. V., … Kirubagaran, R. (2014). Heterologous expression, purification, and phylogenetic analysis of oil-degrading biosurfactant biosynthesis genes from the marine sponge-associated *Bacillus licheniformis* NIOT-06. *Bioprocess and Biosystems Engineering*, *38*(6), 1009–1018.

Atlas, R. M. (2010). Atlas Oil Agar. In *Handbook of Microbiological Media, Fourth Edition* (Fourth Edi). United States: CRC Press.

Baker, G. C., Smith, J. J., & Cowan, D. A. (2003). Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods*, *55*(3), 541–555.

Baker, S. C., & Chen, C. Y. (2010). Enrichment and purification of lipopeptide biosurfactants. In R. Sen (Ed.), *Advances in Experimental Medicine and Biology* (First, Vol. 672, pp. 281–288). New York, USA: Landes Bioscience and Springer Science+Business Media.

Banat, I. M. (1995). Biosurfactants Production and Possible Uses in Microbial Enhanced Oil Recovery and Oil Pollution Remediation: A Review. *Bioresource Technology*, *51*, 1–12.

Banat, I. M., Makkar, R. S., & Cameotra, S. S. (2000). Potential commercial applications of microbial surfactants. *Applied Microbiology and Biotechnology*, *53*(5), 495–508.

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

Biria, D., Maghsoudi, E., Roostaazad, R., Dadafarin, H., Lotfi, S. S., & Amoozegar, M. a. (2010). Purification and characterization of a novel biosurfactant produced by *Bacillus licheniformis* MS3. *World Journal of Microbiology and Biotechnology*, *26*(5), 871–878.

Cameotra, S. S., & Makkar, R. S. (1998). Synthesis of biosurfactants in extreme conditions. *Applied Microbiology and Biotechnology*, *50*(5), 520–9.

Chandankere, R., Yao, J., Choi, M. M. F., Masakorala, K., & Chan, Y. (2013). An efficient biosurfactant-producing and crude-oil emulsifying bacterium *Bacillus methylotrophicus* USTBa isolated from petroleum reservoir. *Biochemical Engineering Journal*, *74*, 46–53.

Chen, H. R., Chen, C. C., Reddy, a. S., Chen, C. Y., Li, W. R., Tseng, M. J., … Atla, S. B. (2011). Removal of mercury by foam fractionation using surfactin, a biosurfactant. *International Journal of Molecular Sciences*, *12*(11), 8245–8258.

Das, A. J., Lal, S., Kumar, R., & Verma, C. (2017, June 3). Bacterial biosurfactants can be an ecofriendly and advanced technology for remediation of heavy metals and co-contaminated soil. *International Journal of Environmental Science and Technology*.

De Franca, I. W. L., Lima, A. P., Lemos, J. A. M., Lemos, C. G. F., Melo, V. M. M., De Sant'ana, H. B., & Goncalves, L. R. B. (2015). Production of a biosurfactant by *Bacillus subtilis* ICA56 aiming bioremediation of impacted soils. *Catalysis Today*, *255*, 10–15.

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.

Dong, K., & Lee, S. (2011). *Bacillus kyonggiensis* sp. nov., isolated from soil of a lettuce field. *Journal of Microbiology*, *49*(5), 776–781.

dos Santos, S. C., Fernandez, L. G., Rossi-Alva, J. C., & de Abreu Roque, M. R. (2010). Evaluation of substrates from renewable-resources in biosurfactants production by *Pseudomonas strains. African Journal of Biotechnology*, *9*(35), 5704–5711.

Elazzazy, A. M., Abdelmoneim, T. S., & Almaghrabi, O. A. (2015). Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia. *Saudi Journal of Biological Sciences*, *22*(4), 466–475.

Georgiou, G., Lin, S.-C., & Sharma, M. M. (1992). Surface-active Compounds from Microorganisms. *Bio/Technology*, *10*, 60–65.

Gertz, C. (2000). Chemical and physical parameters as quality Deep-Frying Process – Changes at Elevated Temperature. *European Journal of Lipid Science and Technology*, *102*, 566–572.

Haba, E., Espuny, M. J., Busquets, M., & Manresa, a. (2000). Screening and production of rhamnolipids by P*seudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. *Journal of Applied Microbiology*, *88*(3), 379–387.

Helmy, Q., Kardena, E., Funamizu, N., & Wisjnuprapto. (2011). Strategies toward commercial scale of biosurfactant production as potential substitute for it's chemically counterparts. *International Journal of Biotechnology*, *12*(1/2), 66.

Innis, M. A., Gelfand, D. H., Sninsky, J. J., & White, T. J. (1990). Molecular Biology). In *Amplifications or Applications*. Academic Press.

Jamal, P., Alam, M. Z., Zainuddin, E., & Wan Nawawi, W. M. F. (2011). Production of Biosurfactant in 2L Bioreactor Using Sludge Palm Oil as a Substrate. *IIUM Engineering*, *12*(4), 109–114.

Kavitha, V., Mandal, A. B., & Gnanamani, A. (2014). Microbial biosurfactant mediated removal and / or solubilization of crude oil contamination from soil and aqueous phase : An approach with *Bacillus licheniformis* MTCC 5514. *International Biodeterioration & Biodegradation*, *94*, 24–30.

Li, J., Deng, M., Wang, Y., & Chen, W. (2016). Production and characteristics of biosurfactant produced by *Bacillus pseudomycoides* BS6 utilizing soybean oil waste. *International Biodeterioration and Biodegradation*, *112*, 72–79.

Li, J., Yang, G., Wu, M., Zhao, Y., & Zhou, S. (2014). *Bacillus huizhouensis* sp. nov., isolated from a paddy field soil. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *106*(2), 357–363.

Madhaiyan, M., Poonguzhali, S., Lee, J. S., Lee, K. C., & Hari, K. (2011). *Bacillus rhizosphaerae* sp. nov., an novel diazotrophic bacterium isolated from sugarcane rhizosphere soil. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *100*(3), 437–444.

Maikudi Usman, M., Dadrasnia, A., Tzin Lim, K., Fahim Mahmud, A., & Ismail, S. (2016). Application of biosurfactants in environmental biotechnology; remediation of oil and heavy metal. *AIMS Bioengineering*, *3*(3), 289–304.

Makkar, R. S., & Cameotra, S. S. (1999). Biosurfactant Production by Microorganisms on Unconventional Carbon Sources. *Journal of Surfactants and Detergents*, *2*(2), 237–241.

Makkar, R. S., & Cameotra, S. S. (2002). An update on the use of unconventional substrates for biosurfactant production and their new applications. *Applied Microbiology and Biotechnology*, *58*(4), 428–34.

Makkar, R. S., Cameotra, S. S., & Banat, I. M. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB Express*, *1*(1), 5.

Mnif, I., & Ghribi, D. (2015). Review lipopeptides biosurfactants: Mean classes and new insights for industrial, biomedical, and environmental applications. *Biopolymers*, *104*(3), 129–147.

Mohammed, A. S., Kapri, A., & Goel, R. (2011). *Use of Biosurfactants in the Removal of Heavy Metal Ions from Soils*. *Biomanagement of Metal-Contaminated Soils* (Vol. 20).

Morikawa, M., Hirata, Y., & Imanaka, T. (2000). A Study on the structure-function relationship of lipopeptides biosurfactants. *Biochimica et Biophysica Acta 1488*, 211–218.

Mukherjee, A. K., & Das, K. (2010). Microbial surfactants and their potential applications: An overview. In *Advances in Experimental Medicine and Biology* (Vol. 672, pp. 54–64).

Mulligan, C. N. (2005). Environmental applications for biosurfactants. *Environmental Pollution*, *133*(2), 183–98.

Nerurkar, A. S. (2010). Structural and Molecular Characteristics of Lichenysin and Its Relationship with Surface Activity. *Biosurfactants*, 304–315.

Neves, L. C. M., Oliveira, K. S., Kobayashi, M. J., Penna, T. C. V., & Converti, A. (2007). Biosurfactant produdion by cultivation of *Bacillus atrophaeus* ATCC 9372 in semidefined Glucose/casein-based media. *Applied Biochemistry and Biotechnology*, *137*, 539–554.

Oliveira, J. G. De, & Garcia-Cruz, C. H. (2013). Properties of a biosurfactant produced by *Bacillus pumilus* using vinasse and waste frying oil as alternative carbon sources. *Brazilian Archives of Biology and Technology*, *56*(1), 155–160.

Pacwa-Płociniczak, M., Płaza, G. a, Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: recent advances. *International Journal of Molecular Sciences*, *12*(1), 633–54.

Pathak, K. V., Bose, A., & Keharia, H. (2014). Characterization of novel lipopeptides produced by *Bacillus tequilensis* P15 using liquid chromatography coupled electron spray ionization tandem mass spectrometry (LC-ESI-MS/MS). *International Journal of Peptide Research and Therapeutics*, *20*(2), 133–143.

Peng, L., Huihui, L., Rong, L., Shunpeng, L., & Xing, H. (2009). Biodegradation of fomesafan by *Lysinibacillus* sp.ZB-1 isolated from soil. *Chemosphere*, *77*, 1614–1619.

Pereira, J. F. B., Gudiña, E. J., Costa, R., Vitorino, R., Teixeira, J. A., Coutinho, J. A. P., & Rodrigues, L. R. (2013). Optimization and characterization of biosurfactant production by *Bacillus subtilis* isolates towards microbial enhanced oil recovery applications. *Fuel*, *111*, 259–268.

Plaza Grazyna, A., Pacwa-Plociniczak, M., Piotrowska-Seget, Z., Brigmon, R., & Krol, E. (2015). Characterization of *Bacillus* Strains Producing Biosurfactants. *Environmental Sustainability*, 173–183.

Pruthi, V., & Cameotra, S. S. (1997). Production and properties of a biosurfactant synthesized by *Arthrobacter protophormiae* - an Antarctic strain. *World Journal of Microbiology & Biotechnology*, *13*, 137–139.

Rangarajan, V., & Clarke, K. G. (2016). Towards bacterial lipopeptide products for specific applications — a review of appropriate downstream processing schemes. *Process Biochemistry*, *51*(12), 2176–2185.

Rocha e Silva, N. M. P., Rufino, R. D., Luna, J. M., Santos, V. a., & Sarubbo, L. a. (2014). Screening of *Pseudomonas* species for biosurfactant production using low-cost substrates. *Biocatalysis and Agricultural Biotechnology*, *3*(2), 132–139.

Ron, E. Z., & Rosenberg, E. (2002). Biosurfactants and oil bioremediation. *Current Opinion in Biotechnology*, *13*(3), 249–252.

Sansinenea, E., & Ortiz, A. (2011). Secondary metabolites of soil *Bacillus* sp. *Biotechnology Letters*, *33*(8), 1523–1538.

Sarubbo, L. A., Brasileiro, P. P. F., Silveira, G. N. M., & Juliana, M. (2018). Application of a Low Cost Biosurfactant in the Removal of Heavy Metals in Soil. *Chemical Engineering Transactions*, *64*, 433–438.

Satpute, S. K., Banpurkar, A. G., Dhakephalkar, P. K., Banat, I. M., & Chopade, B. A. (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. *Critical Reviews in Biotechnology*, *30*(2), 127–144.

Satpute, S. K., Bhuyan, S. S., Pardesi, K. R., Mujumdar, S. S., Dhakephalkar, P. K., Shete, A. M., & Chopade, B. A. (2010). *Molecular Genetics of Biosurfactant Synthesis in Microorganisms*. (R. Sen, Ed.) (First). Texas, USA: Landes Bioscience.

Shaligram, N. S., & Singhal, R. S. (2010). Surfactin - a review on biosynthesis, fermentation, purification and applications. *Food Technology and Biotechnology*, *48*(2), 119–134.

Silva, R. de C. F. S., Almeida, D. G., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2014). Applications of biosurfactants in the petroleum industry and the remediation of oil spills. *International Journal of Molecular Sciences*, *15*(7), 12523–12542. h

Singh, A. K., & Cameotra, S. S. (2013). Efficiency of lipopeptide biosurfactants in removal of petroleum hydrocarbons and heavy metals from contaminated soil. *Environmental Science and Pollution Research*, *20*(10), 7367–7376.

Smyth, T. J. P., Perfumo, A., McClean, S., Marchant, R., & Banat, I. M. (2010). Isolation and Analysis of Lipopeptides and High Molecular Weight Biosurfactants. In K. N. Timmis (Ed.), *Handbook of Hydrocarbon and Lipid Microbiology* (Vol. 78, pp. 3687–3704). Northern Ireland, UK: Springer-Verlag Berlin Heidelberg.

Sousa, M., Dantas, I. T., Feitosa, F. X., Alencar, A. E. V, Soares, S. A., & Melo, V. M. M. (2014). Performance of a Biosurfactant Produced by *Bacillus subtilis* LAMI005 on the Formation of Oil / Biosurfactant / Water Emulsion: Study of the Phase Behaviour of Emulsified Systems. *Brazilian Journal of Chemical Engineering*, *31*(03), 613–623.

Souza, E. C., Oliveira, P., Azevedo, D. S. De, Manuel, J., Converti, A., Pinheiro, R., & Oliveira, D. S. (2017). Influence of temperature and pH on the production of biosurfactant , bacteriocin and lactic acid by *Lactococcus lactis* CECT-4434. *CyTA - Journal of Food*, *15*(4), 525–530.

Swapna, T. H., Papathoti, N. K., Khan, M. Y., Reddy, G., & Hameeda, B. (2016). Bioreduction of Cr (VI) by biosurfactant producing Marine bacterium *Bacillus subtilis* SHB 13. *Journal of Scientific and Industrial Research*, *75*(7), 432–438.

Tarntip, R., & Sirichom, T. (2011). Isolation of Proteolytic , Lipolytic , and Bioemulsifying Bacteria for Improvement of The Aerobic Treatment of Poultry Processing Wastewater. *African Journal of Micriobiology Research*, *5*(30), 5493–5497.

Thavasi, R., Jayalakshmi, S., Balasubramanian, T., & Banat, I. M. (2008). Production and characterization of a glycolipid biosurfactant from *Bacillus megaterium* using economically cheaper sources. *World Journal of Microbiology and Biotechnology*, *24*(7), 917–925.

Varadavenkatesan, T., & Murty, V. R. (2013). Production of a Lipopeptide Biosurfactant by a Novel *Bacillus* sp. and Its Applicability to Enhanced Oil Recovery. *ISRN Microbiology*, *2013*, 621519.

Walia, N. K., & Cameotra, S. S. (2015). Lipopeptides: Biosynthesis and Applications. *Journal of Microbial and Biochemical Technology*, *7*(2), 103–107.

Wu, S. J., Hu, Z. H., Zhang, L. L., Yu, X., & Chen, J. M. (2009). A Novel Dichloromethane-degrading *Lysinibacillus Sphaericus* Strain Wh22 and its Degradative Plasmid. *Applied Microbiology Biotechnology*, *82*, 731–740.

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.

Yateem, A., Balba, M. ., Al-Shayji, Y., & Al-Awadhi, N. (2002). Isolation and Characterization of Biosurfactant-Producing Bacteria from Oil-Contaminated Soil. *Soil and Sediment Contamination*, *11*(1), 41–55.

Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M., & McInerney, M. J. (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. *Journal of Microbiological Methods*, *56*(3), 339–47.

Zhou, H., Chen, J., Yang, Z., Qin, B., Li, Y., & Kong, X. (2015). Biosurfactant production and characterization of *Bacillus* sp. ZG0427 isolated from oil-contaminated soil. *Annals of Microbiology*.