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OPTIMIZATION OF RHAMNOLIPID PRODUCTION USING OIL EXTRACTED FROM OIL PALM BIOMASS AS CARBON SOURCES BY *PSEUDOMONAS AERUGINOSA* STRAIN K

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "Optimization of rhamnolipid production using oil extracted from oil palm biomass as carbon sources by *Pseudomonas aeruginosa* strain K" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Zahraa' Nur Sabrina Binti Mohd Mubarak (161960) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Rhamnolipids are one of the important biosurfactants that are useful in many industries like pharmaceutical, bioremediation and agriculture. *Pseudomonas aeruginosa* is one of the microorganisms that can produce rhamnolipid. The aim of this study is to test the ability of our *P. aeruginosa* strain K to utilize oil palm biomasses as the substrate to produce rhamnolipids. Based on the qualitative test on cetyltrimethyl ammonium bromide agar, *P. aeruginosa* strain K was identified as a rhamnolipids producer. The emulsification index (E_{24}) of the rhamnolipids produced was 56.80%. Oil from three types of oil palm biomasses; palm oil mill effluent (POME), decanter cake (DC) and mesocarp were extracted and used as the substrates for rhamnolipids production. Oil from POME gave the highest E_{24} value (43.02%). The rhamnolipids can be detected after 48 hours of incubation time with an E24 value of 41.42% when POME oil was used as a carbon source. After 48 hours of incubation time, 1% concentration of oil from POME produced 45.97% of E_{24} value. This research showed that oil palm biomasses could be used to produce rhamnolipids from *Pseudomonas aeruginosa* strain K. This offers an opportunity to produce rhamnolipids at low cost and safer manner which meets industrial and ecological needs.

ABSTRAK

Rhamnolipids adalah salah satu biosurfaktan penting yang digunakan dalam pelbagai industri seperti farmaseutikal, bioremediasi dan pertanian. *Pseudomonas aeruginosa* adalah salah satu daripada mikroorganisma yang boleh menghasilkan rhamnolipid. Tujuan kajian ini adalah untuk menguji keupayaan *P. aeruginosa* strain K kami untuk menggunakan biomas kelapa sawit sebagai substrat untuk menghasilkan rhamnolipids. Berdasarkan ujian kualitatif pada agar cetyltrimethyl ammonium bromide, *P. aeruginosa* strain K telah dikenal pasti sebagai penghasil rhamnolipids. Indeks pengemulsian (E_{24}) daripada rhamnolipids adalah 56.80%. Minyak daripada tiga jenis biomas kelapa sawit; efluen kilang minyak sawit (POME), kek botol (DC) dan mesokarpa ini dipilih dan digunakan sebagai substrat untuk penghasilan rhamnolipids. Minyak daripada POME memberikan nilai E_{24} tertinggi iaitu 43.01%. Penghasilannya rhamnolipids boleh dikesan selepas 48 jam masa pengeraman dengan nilai E_{24} daripada 41.41% apabila minyak POME telah digunakan sebagai sumber karbon. Selepas 48 jam masa inkubasi, 1% kepekatan minyak dari POME menghasilkan 45.97% nilai E_{24}. Kajian ini menunjukkan bahawa biomas minyak kelapa sawit boleh digunakan untuk menghasilkan rhamnolipids menggunakan *Pseudomonas aeruginosa* strain K. Ini menawarkan peluang untuk menghasilkan rhamnolipids dengan kos rendah dan cara yang lebih selamat serta memenuhi keperluan industri dan ekologi.

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CHAPTER 1

INTRODUCTION

Surfactants, or surface active agents, can be classified into two main groups: synthetic surfactants and biosurfactants. Synthetic surfactants are produced by organic chemical reactions, while biosurfactants are produced by biological processes, being excreted extracellular by microorganisms such as bacteria, fungi and yeast. When compared to synthetic surfactants, biosurfactants have several advantages, including high biodegradability, low toxicity, low irritancy and compatibility with human skin (Banat et al., 2000).

Rhamnolipids produced from different *Pseudomonas* sp. strains can effectively emulsify and stabilize emulsions with various types of hydrocarbons and oils (Wei et al., 2005). Rhamnolipid are glycosides that are composed, of a glycon part and an aglycon part linked to each other via O-glycosidic linkage (Abdel-mawgoud et al., 2011). Rhamnolipids contain a hydrophilic head formed by one or two rhamnose molecules and a hydrophobic tail that contains one or two fatty acid chains (Sánchez et al., 2007).

Nowadays, an increase in concerns about environmental protection has led to the consideration of biosurfactants as alternatives to synthetic surfactants and the development of cost-effective bioprocesses to the biosurfactant production is of great interest (Nischke and Pastore, 2006). The use of the alternative substrates such as agro based industrial wastes like palm oil mill effluent (POME), decanter cake (DC) and empty fruit bunch are some of the attractive strategies for economical biosurfactants production (Makkar, Cameotra, & Banat, 2011). The development of low-cost processes and raw material can account for 10-30% of the final product cost. Further optimization of culture medium and growth conditions can significantly increase the yield of biosurfactant (Mutalik et al., 2008).

The objectives of this study were (1) to test the rhamnolipids production in *Pseudomonas aeruginosa* strain K; (2) to determine the rhamnolipids production in *P. aeruginosa* strain K using media containing different types of oil extracted palm oil mill effluent (POME), decanter cake (DC) and Mesocarp that act as a sole carbon source; and (3) to optimize the rhamnolipids production in *P. aeruginosa* strain K.

REFERENCES

A.N. Ma (1999). The Planters, Kuala Lumpur Innovations in Management of Palm Effluent, Palm Oil Research Institute of Malaysia (PORIM)

Abalos A, Pinazo A, Infante MR et al. (2001). Physicochemical and antimicrobial properties of new rhamnolipids produced by *Pseudomonas aeruginosa* AT10 from soybean oil refinery wastes. *Langmuir*. 17:1367-1371.

Abdel-Mawgoud, A. M., Hausmann, R., Le, F., De, E., & Markus, M. M. (2011). *Biosurfactants*. (G. Soberón-Chávez, Ed.) (Vol. 20, pp. 13–56). Berlin, Heidelberg: Springer Berlin Heidelberg.

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

Abubakr AR, Alimon AR, Yaakub H, Abdullah N, Ivan M (2013). Growth, nitrogen metabolism and carcass composition of goats fed palm oil by-products. Small Ruminant Research. 112:91–96.

Agamuthu, P. (1995). Palm oil mill effluent and utilization. Waste treatment plant. Narosa Publishing House, New Delhi. pp: 338-360.

Al-araji, L. I. Y., Rahman, RNZRA., Basri, M., & Salleh, A. B. (2007). Optimisation of rhamnolipids produced by *Pseudomonas aeruginosa* 181 using Response Surface Modeling. *57*(4):571–575.

Anderson, G. E. Solvent extraction. Retrieved 14 APRIL 2015 from http://lipidlibrary.aocs.org/processing/solventextract/index.htm

Benincasa M, Abalos M, Oliveira I et al. (2004). Chemical structure, surface properties and biological activities of the biosurfactant produced by *Pseudomonas aeruginosa* LBI from soapstock. 85:1-8.

Benincasa M, Contiero J, Manresa MA, Moraes IO (2002). Rhamnolipid production by *Pseudomonas aeru*ginosa LBI growing on soap stock as the sole carbon source. *Journal of Food Engineering.*

Benincasa M, Contiero J, Manresa MA, Moraes IO (2002). Rhamnolipid production by *Pseudomonas aeruginosa* LBI growing on soap stock as the sole carbon source. *Journal of Food Engineering*.

Bhardwaj, A., Hartland, S. (1994). Dynamics of emulsification and demulsification of water in crude oil emulsions. *Industrial & Engineering Chemistry Research*. 33:1271–1279.

Cambiella, A., Ortea, E., Ríos, G., Benito, J.M., Pazos, C., Coca, J. (2006). Treatment of oil in water emulsions: performance of a sawdust bed filter. *Journal of Hazardous Material*. 131:195–199.

Cameotra SS, Makkar RS, Kaur J, Mehta SK (2010) Synthesis of biosurfactants and their advantages to microorganisms and mankind. *Advances in Experimental Medicine and Biology*. 672:261–280.

Chander, C. (2012). Production and characterization of biosurfactant from Bacillus subtilis MTCC441 and its evaluation to use as bioemulsifier for food bio-preservative. *Advance Applied Science. 3*(3):1827–1831.

Chen. S. Y., Y. H, Wei and J.S. Chang (2007). Repeated pH-stat fed-batch fermentation of rhamnolipid production with indigenous *Pseudomonas aeruginosa* S2. *Applied Microbial Biotechnology*. 76 (1): 67-74.

El-Sersy, N. (2012). Plackett-burman design to optimize biosurfactant production by marine *Bacillus subtilis* N10. *Romanian Biotechnological Letter*. *17*(2), 7049–7064

Feng, X., Mussone, P., Gao, S., Wang, S., Wu, S.Y., Masliyah, J.H., Xu, Z. (2009). Mechanistic study on demulsification of water-in-diluted bitumen emulsions by ethylcellulose. *Langmuir*. 26:3050–3057.

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.

Jarvis FG, Johnson MJ (1949). A glycolipide produced by *Pseudomonas aeruginosa*. *Journal of the American Chemical Society*. 71:4124–4126.

Lan, G., Fan, Q., Liu, Y., Chen, C., Li, G., Liu, Y., & Yin, X. (2015). Rhamnolipid production from waste cooking oil using *Pseudomonas* SWP-4. *Biochemical Engineering Journal*. 101: 44–54.

Lang, S., Wullbrandt, D. (1999). Rhamnose lipids – biosynthesis, microbial production and application potential. *Applied Microbiology and Biotechnology*. 51:22–32.

Li, A., Xu, M., Sun, W., & Sun, G. (2011). Rhamnolipid production by *Pseudomonas aeruginosa* GIM 32 using different substrates including molasses distillery wastewater. *Applied Biochemistry and Biotechnology*. *163*(5):600–11.

Liu, J., Huang, X.F., Lu, L.J., Xu, J.C., Wen, Y., Yang, D.H., Zhou, Q. (2010). Optimization of biodemulsifier production from *Alcaligenes* sp. S-XJ-1 and its application in breaking crude oil emulsion. *Journal of Hazardous Material*. 183:466–473.

Lyczak JB, Cannon CL, Pier GB (2000). Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Journal of Microbes Infection.* 2:1051–1060.

Makkar, R., Cameotra, S., & Banat, I. (2011). Advances in utilization of renewable substrates for biosurfactant production. *AMB Express*. 1–19.

Marchese, J., Ochoa, N., Pagliero, C., Almandoz, C. (2000). Pilot-scale ultrafiltration of an emulsified oil wastewater. *Environmental Science Technology*. 34:2990–2996.

Mutalik SR, Vaidya BK, Joshi RM, Desai KM, Nene SN (2008). Use of response surface optimization for the production of biosurfactant from *Rhodococcus* spp. MTCC 2574. *Bioresource Technology*. 99:7875–7880.

Nitschke M, Costa SGVAO (2007). Biosurfactants in food industry. *Food Science Technology*. 18:252-259.

Nitschke M, Costa SGVAO, Haddad R, Goncalves LAG, Eberlin MN, Contiero J (2005). Oil wastes as unconventional substrates for rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* LBI. *Biotechnology Progress*. 21:1562–6.

Nitschke M, Pastore GM (2006). Production and properties of a surfactant obtained from *Bacillus subtilis* grown on cassava wastewater. Bioresource Technology. 97:336-341.

Noparat, P., & Maneerat, S. (2014). Utilization of palm oil decanter cake as a novel substrate for biosurfactant production from a new and promising strain of *Ochrobactrum anthropic*. 2/3:865–877.

Norliza (2001). *Removal of suspended solid and residue oil from Palm oil mill effluent*. Unpublished Master’s Thesis, Universiti Sains Malaysia, Malaysia.

Oliveira, F. J. S., Vazquez, L., Campos, N. P. De, & Franc, F. P. De. (2009). Production of rhamnolipids by a *Pseudomonas alcaligenes* strain. *44*:383–389.

Omar, R., Idris, A., Yunus, R., Khalid, K., & Aida Isma, M. I. (2011). Fuel, 90:1536–1544.

Pinzon, N. M., & Ju, L. K. (2009). Improved detection of rhamnolipid production using agar plates containing methylene blue and cetyl trimethylammonium bromide. *Biotechnology Letters*. *31*(10), 1583–1588.

Rahman, R. N. Z. A., Geok, L. P., Basri, M., & Salleh, A. B. (2005). Physical factors affecting the production of organic solvent-tolerant protease by *Pseudomonas aeruginosa* strain K. *Bioresource Technology*. *96*(4):429–36.

Raza, Z. A., Khan, M. S., Khalid, Z. M., & Rehman, A. (2006). Production of Biosurfactant Using Different Hydrocarbons by *Pseudomonas aeruginosa* EBN-8 Mutant. *Zeitschrift Für Naturforschung C*. *61*(1-2).

Rosenberg E, Ron E (1999). High and low molecular mass microbial surfactants. *Applied Microbiology and Biotechnology*. 52:154-162.

Sánchez M, Aranda FJ, Espuny MJ et al. (2007). Aggregation behaviour of a dirhamnolipid biosurfactant secreted by *Pseudomonas aeruginosa* in aqueous media. *Journal of Colloid and Interface Science*. 307:246-253.

Savarino P, Montoneri E, Biasizzo M, Quagliotto P, Viscardi G, Boffa V (2007). Upgrading biomass wastes in chemical technology. Humic acid-like matter isolated from compost as chemical auxiliary for textile dyeing. *Journal of Chemical Technology and Biotechnology*. 82:939–948.

Siegmund I, Wagner F (1991). New method for detecting rhamnolipids excreted by *Pseudomonas* species during growth on mineral agar. *Journal of Chemical Technology and Biotechnology*. 5(4):265–268.

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.

Soberón-Chávez, G., Lépine, F., & Déziel, E. (2005). Production of rhamnolipids by *Pseudomonas aeruginosa*. *Applied Microbiology and Biotechnology*. 718–725.

Thanomsub B, Pumeechockchai W, Limtrakul A et al. (2006). Chemical structures and biological activities of rhamnolipids produced by *Pseudomonas aeruginosa* B189 isolated from milk factory waste. *Bioresource Technology*. 97:2457-2461.

Van Ginkel, C.G. (1989). Complete degradation of xenobiotic surfactant by consortium of microorganisms. *Biodegradation*. 7 (2): 151-164.

Wei YH, Chou CL, Chang JS (2005). Rhamnolipid production by indigeneous *Pseudomonas aeruginosa* J4 originating from petrochemical wastewater. *Journal of Biochemical Engineering*. 27:146-154.

Wei YH, Chou CL, Chang JS (2005). Rhamnolipid production by indigeneous *Pseudomonas aeruginosa* J4 originating from petrochemical wastewater. *Journal of Biochemical Engineering*. 27:146-154.

Wittgens, A., Tiso, T., Arndt, T. T., Wenk, P., Hemmerich, J., Müller, C., Blank, L. M. (2011). Growth independent rhamnolipid production from glucose using the non-pathogenic *Pseudomonas putida* KT2440. *Microbial Cell Factories*.

Xia, L., Gong, K., Wang, S., Li, J., Yang, D. (2010). Microwave assisted chemical demulsification of water-in-crude-oil emulsions. *Journal of Dispersion Science and Technology*. 31:1574–1578.

Yang, L., Nakhla, G., Bassi, A. (2005). Electro-kinetic dewatering of oily sludges. *Journal of Hazardous Material*. 125:130–140.

Yano, S., Murakami, K., Sawayama, S., Imou, K., & Yokoyama, S. (2009). *Journal of The Japan Institute.*

Zhang, J., Li, J., Thring, R.W., Hu, X., Song, X. (2012). Oil recovery from refinery oily sludge via ultrasound and freeze/thaw. *Journal of Hazardous Material*. 203–204:195–203.

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.