UNIVERSITI PUTRA MALAYSIA

RECOVERY OF LIPASE FROM BURKHOLDERIA SP. USING AQUEOUS TWO-PHASE SYSTEMS

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FK 2012 15

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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 Doctor of Philosophy

July 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

RECOVERY OF LIPASE FROM *BURKHOLDERIA* SP. USING AQUEOUS TWO-PHASE SYSTEMS

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July 2012

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Conventional recovery methods for lipase are tedious and require several rounds of recovery steps. Therefore, the development of a cost-effective and recyclable protocol for the recovery of lipase is essential. Aqueous two-phase system (ATPS) can be used as an attractive alternative for the recovery of lipase from complex feedstock. This research is focused on the design of an ATPS protocol as a simplified and rapid recovery technique for the microbial lipase of *Burkholderia* sp. ST8. A multifactor experimental design based on a 'change-one-factor-at-a-time' approach was employed to study the effects of lipase production. A high lipase activity was achieved at a 250 rpm agitation speed for 36 hours of fermentation time in a cultivation medium containing 0.1 % (v/v) of Tween 80, 0.3 % (w/v) of nutrient broth and 0.1 % (w/v) of $CaCl_2$ at a pH of 9 with a volume ratio of 20:80 of medium volume to free volume of the flask which resulted in a high average production of 48.3 U/mL.

Three different novel techniques for the direct recovery of lipase based on sustainable ATPS have been developed. The recycling concept in the system is based on the principals of green chemistry, with good efficiency and economic viability. The first novel method is a recycling hydrophilic organic solvent/inorganic salt aqueous two-phase flotation (ATPF) system, which integrates solvent sublation (SS) and aqueous two-phase extraction (ATPE) in the recovery of lipase from fermentation broth. A purification factor of 14 and a lipase yield of 99 % were achieved in the optimized ATPF system with recovery of alcohol and salt are 70% and 61 %, respectively. The second and third lipase recovery techniques were explored by using a recyclable temperature-induced polymer, an ethylene oxide-propylene oxide (EOPO) polymer in an ATPF system as well as an ATPS. As for the second method, a recycling EOPO/ammonium sulphateATPF was developed for the recovery of lipase from fermentation broth. Under the optimal conditions, the average yield and purification factor were 98.5 % and 15, respectively. It was also demonstrated that EOPO and salt was recovered up to 91 % and 75 % in the ATPF system. Direct lipase recovery using recycling an EOPO/potassium phosphate ATPS was studied as the third protocol in this thesis. The average purification factor of lipase and the yield obtained from four successive purifications were 15.3 and 99.1 %, respectively. There was no significant difference in using fresh or recycled chemicals in these three novel methods based on ATPS. The choice of method in lipase recovery based on ATPS is subjected to the requirement of the purity and cost of the system. Hence, the simplicity and effectiveness of these three lipase recovery methods based on sustainable ATPS were proven in this study.

Lastly, an extractive fermentation technique was employed using a thermoseparating reagent to form a two-phase system for simultaneous cell cultivation and downstream processing. A 10 % (w/w) solution of EOPO with a molecular mass of 3900 g/mol and a pH of 8.5, a 200 rpm speed, and 30 °C were selected as the optimal conditions for lipase production (55 U/ml). Repetitive batch fermentation was performed by continuous replacement of the top phase every 24 hours, which resulted in an average cell growth mass of 4.8 g/L for 10 extractive batches over 240 hours. The dry cell mass in the bioreactor was 22 % higher than that in the flasks. *Burkholderia* sp. ST8 lipase was successfully produced and recovered by using thermoseparating reagents in a single step - extractive fermentation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMULIHANLIPASE DARIPADA *BURKHOLDERIA* SP. BERDASARKAN SISTEM DUA-FASA AKUEUS

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Kaedah-kaedah konvensional untuk pemulihan lipase adalah memenatkan dan memerlukan beberapa langkah pemulihan yang rumit. Oleh sebab itu, pembangunan protokol pemulihan lipase yang berkos efektif serta berupaya dikitar semula adalah amat mustahak. Sistem dua-fasa akueus (SDFA) boleh digunakan sebagai kaedah alternatif untuk pemulihan lipase daripada stok suapan yang kompleks. Dengan itu, kajian penyelidikan ini memfokuskan kepada reka-bentuk protokol SDFA sebagai teknik pemulihan lipase daripada mikroorganisma *Burkholderia* sp. ST8. Eksperimen yang berdasarkan pendekatan 'perubahan-satu-faktor-pada-satu-masa' telah diapplikasikan untuk mengoptimumkan penghasilan lipase. Pada keadaan optimum iaitu 250 putaran per minit pergolakan kelajuan selama 36 jam masa penapaian dalam medium yang mengandungi 1 % (i/i) Tween 80, 0.3 % (i/i) nutrien bubur dan 0.1 % (b/i) $CaCl_2$ pada pH 9 dengan nisbah kapasiti 20:80 medium kepada kelantangan kelalang, menghasilkan lipase dengan purata sebanyak 48.3 U/mL dalam kajian ini.

Tiga teknik pemulihan terus lipase yang berbeza berdasarkan lestari SDFA telah dikajikan. Konsep kitar semula adalah berprinsipkan kimia hijau, baik dari segi kecekapan dan ekonomi. Kaedah pertama pemulihan lipase yang novel adalah pengitaran semula pelarut organik hirofilik/garam bukan organik pengapungan dua-fasa akueus (PDFA) yang mengintegrasikan kaedah sublation pelarut (SP) dan pengekstrakan dua-fasa akueus dalam pemulihan lipase daripada media penapaian. PDFA telah dioptimumkan pada keadaan 40 mL isipadu 50 % (b/b) 2-propanol, 1.0 L isipadu 250 g/L kalium fosfat, pH 8.5, 100 % (i/ i) stok suapan, 30 mL/min kadar aliran N_2 selama 30 minit dalam tiub kolorimeter berjejari 8 cm dengan keliangan G4 (5-15 μm) kaca cakera tersinter. Faktor penulenan pada 14 dan hasil lipase sebanyak 99 % telah dicapai dalam PDFA ini. Teknik pemulihan lipase yang kedua dan ketiga telah dieksplorasi dengan menggunakan induksi suhu polimer, etilena oksida-propilena oksida (EOPO) polimer dalam PDFA dan SDFA. Bagi kaedah kedua, EOPO/ammonium sulfat PDFA untuk pemulihan lipase daripada media penapaian telah dihasilkan. Pada keadaan optimum, purata kecekapan pemisahan dan faktor penulenan masing-masing adalah sebanyak 76 % dan 15. Keadaan ini juga menunjukkan bahawa EOPO dalam PDFA telah dipulihkan sebanyak 91 %. Pemulihan terus lipase dengan penggunaan kitaran semula EOPO/kalium fosfat SDFA telah dikaji sebagai protokol ketiga dalam tesis ini. Pada SDFA ini, lipase yang tulen telah dipindahkan kepada fasa polimer yang terdiri daripada EOPO 3900 dengan kepanjangan talian garis ikatan 48.5 % (b/b), nisbah isipadu sebanyak 2.3 dan stok suapan sebanyak 20 % (b/b) pada pH 7. Purata faktor penulenan lipase dan hasilan yang diperoleh dari keempat-empat pemulihan yang berturutan adalah masing-masing 15.3 dan 99.1 %. Di samping itu, didapati bahawa tiada perbezaan yang ketara dengan menggunakan bahan kimia yang segar atau dikitar semula dalam

tiga teknik pemulihan lipase berdasarkan SDFA. Pemilihan teknik adalah berdasar keperluan darjah ketelunan and cost yang mampu. Oleh yang demikian, kecekapan dan keberkesanan dalam tiga kaedah pemulihan lipase berdasarkan SDFA telah terbukti dalam kajian ini.

Akhir sekali, teknik penapaian ekstraktif telah diaplikasi dengan menggunakan reagen induksi suhu untuk membentuk satu sistem yang mempunyai kebaikan penghasilan sel dan pemulihan lipase serentak. Satu pelarut 10 % (b/b) EOPO dengan jisim molekul 3900 g/mol dan pH 8.5, kelajuan 200 putaran per minit dan 30 °C telah dipilih sebagai syarat-syarat optimum untuk penghasilan lipase (55 U/ml). Kelompok fermentasi yang berulangan telah dikaji dengan penggantian fasa pada setiap 24 jam, yang menyebabkan pertumbuhan sel purata jisim sebanyak 4.8 g/L untuk 10 kelompok ekstraktif iaitu, 240 jam. Berat kereing sel di dalam bioreaktor adalah 22 % lebih tinggi daripada dalam kelalang. *Burkholderia* sp. ST8 lipase telah berjaya dihasilkan dan ditulenkan dengan menggunakan reagen indusi suhu polimer dengan hanya satu langkah - penapaian ekstraktif.

ACKNOWLEDGEMENTS

I wish to express my utmost gratitude to my main supervisor, Dr. Mohd Shamsul Anuar for his guidance and support during the course of this research. I would also like to thank my supervisory committee members, Prof. Dr. Ling Tau Chuan, Prof. Dr. Arbakariya Ariff, and Assoc. Prof. Dr. Yus Aniza Yusof for their encouragement and excellent advice.

Special thanks to all the laboratory officers of the Department of Process and Food Engineering (KPM) and the Laboratory of Immuno Therapeutics and Vaccines (LIVES) for their consistent and kind assistance. Further, I would like to acknowledge Dr. Hii Siew Ling from the Universiti Tunku Abdul Rahman for providing the bacterial strain used in this research.

I wish to extend my sincere appreciation to my labmates and friends (Dr. Ooi Chien Wei, Dr. Ramanan, Dr. Tan Joo Shun, Tam, Lo, Fatemeh, Fadzlie, Yu Kiat, Teck Kim, Yin Hui and Grrace Ng) for frequently helping me and sharing their invaluable knowledge with me. Last but not least, I would like to thank my family for their earnest love, tolerance and sacrifice. I could not have completed this thesis without all of them.

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

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DECLARATION

I declare that this thesis is my original work except for quotations and citations whichhave been duly acknowledged. I also declare that it has not been previously, and isnot concurrently, submitted for any other degree at Universiti Putra Malaysia or atany other institution.

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SHOW PAU LOKE

Date: 19th July 2012

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