
**UNIVERSITI PUTRA MALAYSIA**

***RECOVERY OF EXTRACELLULAR LIPASE FROM BURKHOLDERIA SP.ST8 IN AQUEOUS TWO-PHASE SYSTEMS***

**OOI CHIEN WEI**

# RECOVERY OF EXTRACELLULAR LIPASE FROM *BURKHOLDERIA* SP. ST8 IN AQUEOUS TWO-PHASE SYSTEMS

**By**

**OOI CHIEN WEI**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**February 2011**

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 EXTRACELLULAR LIPASE FROM *BURKHOLDERIA* SP. ST8 IN AQUEOUS TWO-PHASE SYSTEMS**

By

**OOI CHIEN WEI**

**February 2011**

**Chairman : Associate Professor Ling Tau Chuan, PhD**

**Faculty : Engineering**

Lipase has widespread applications in various industrial fields and high demand in enzyme market. However, conventional purification methods for lipase are tedious and require several rounds of purification steps. Therefore, development of a highly efficient and cost-effective protocol for the purification of lipase is essential. Aqueous two-phase system (ATPS) can be used as an attractive alternative for the purification of lipase from complex feedstock. Thus, this experimental investigation focused on the design of ATPS protocol as simplified and rapid purification technique for the microbial lipase of *Burkholderia* sp. ST8. Different approaches of ATPS were considered and each of the ATPS was evaluated independently.

Polyethylene glycol (PEG) 6000/potassium phosphate ATPS was employed for the purification of *Burkholderia* sp. ST8 lipase from fermentation broth. The simplicity and effectiveness of PEG/phosphate ATPS for the purification of lipase were proven in this study. Optimum condition for the purification of lipase was obtained in PEG 6000/potassium phosphate ATPS with TLL of 42.2% (w/w), $V_R$ of 2.70, pH 7,

addition of 1% (w/w) sodium chloride (NaCl) and 20% (w/w) feedstock load. Based on this ATPS, lipase was successfully purified to the PEG top phase and the purification fold of lipase was enhanced to 12.42, with a high yield of 93%.

The partitioning and purification of lipase was also explored by using temperature-induced aqueous micellar two-phase system (AMTPS) composed of single nonionic surfactant. AMTPS offers a convenient and efficient method for the purification of lipase with large loading capacity and the potential of recycling surfactant for the preparation of new AMTPS. Based on the AMTPS which consisted of 24% (w/w) Pluronic L81 and 0.5% (w/w) potassium chloride, the selectivity of lipase has been enhanced to 0.035 and the lipase was purified 7.2 fold in the bottom phase. In the second step of purification, addition of potassium thiocyanate salt solution to the bottom micellar phase has resulted in back-extraction of the surfactant to top phase. The lipase was then recovered in new aqueous bottom phase with the yield of 89% and the partition coefficients of 0.34 and 4.50 for lipase and surfactant, respectively.

Alcohol/salt ATPS was used to purify lipase. The high stability of *Burkholderia* sp. ST8 in the presence of organic solvents was exploited in alcohol/salt ATPS. Nine biphasic systems, comprised of alcohol-based top phase and salt-based bottom phase, were evaluated for their effectiveness in lipase purification. The optimum partition efficiency for the purification of lipase was obtained in an ATPS composed of 16% (w/w) 2-propanol and 16% (w/w) potassium phosphate in the presence of 4 % (w/w) NaCl. The purified lipase from the top phase had a purification fold of 13.5 and a yield of 99%. Furthermore, 2-propanol can be easily evaporated from the purified

lipase and recycled for the subsequent batch of ATPS. Thereby, alcohol/salt ATPS is a viable operation for the cost-effective and rapid purification of lipase.

Lastly, an extractive fermentation using ATPS was developed for the simultaneous cell cultivation and the downstream processing of extracellular lipase derived from *Burkholderia* sp. ST8. The cell growth and the lipase production in different types of ATPSs were investigated. An ATPS, which is composed of 9.6% (w/w) PEG 8000 and 1.0% (w/w) Dextran T500, provided the best condition for extractive lipase production. In this integrated process, biomass was accumulated in the bottom phase whereas the lipase was extracted to the top phase. High yield of lipase (92.1%) was recorded in the single batch operation. Repetitive batch of fermentation was progressively carried out by continuous replacement of the top phase every 24 h, which resulted in an average lipase concentration of 16.5 U/mL for seven extractive batch over the duration of 168 h. The extractive fermentation in ATPS is an attractive approach for the combination of the lipase production and the purification process, owing to the repeated use of the two-phase fermentation system and the ease of lipase recovery from fermentation culture.

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

**PEMULIHAN LIPASE EKSTRASEL DARIPADA *BURKHOLDERIA* SP. ST8 DENGAN SISTEM DUA-FASA AKUEUS**

Oleh

**OOI CHIEN WEI**

**Februari 2011**

**Pengerusi : Profesor Madya Ling Tau Chuan, PhD**

**Fakulti : Kejuruteraan**

Enzim lipase mempunyai aplikasi yang luas dalam pelbagai bidang industri dan permintaan yang tinggi dalam pasaran enzim. Namun, kaedah konvensional untuk penulenan enzim lipase adalah rumit dan lambat kerana memerlukan beberapa tahap dan langkah penulenan. Oleh demikian, pembangunan protokol yang efisien dan jimat kos untuk penulenan lipase adalah sangat penting. Penggunaan sistem dua-fasa akueus (SDFA) ialah satu alternatif yang menarik dan ekonomi untuk penulenan lipase daripada stok suapan yang kompleks. Dengan demikian, fokus penyelidikan ini ialah untuk mereka-bentuk protokol SDFA sebagai satu teknik yang senang serta pantas bagi penulenan lipase daripada mikroorganisma *Burkholderia* sp. ST8. Pelbagai jenis pendekatan untuk SDFA telah dinilai.

SDFA yang terdiri daripada polietilena glikol (PEG) 6000 dan kalium fosfat telah digunakan untuk penulenan lipase *Burkholderia* sp. ST8 daripada kaldu fermentasi. Kesederhanaan dan keberkesanan PEG/fosfat SDFA untuk penulenan lipase telah terbukti dalam kajian ini. Keadaan optimum untuk penulenan lipase telah diperolehi

dengan PEG 6000/kalium fosfat SDFA yang menggunakan kepanjangan garis ikatan 42.2 peratusan berat/berat (% b/b), nisbah isipadu 2.70, penambahan garam natrium klorida 1% (b/b), pH 7 dan muatan sampel yang sebanyak 20% (b/b). Berdasarkan SDFA ini, lipase telah ditulenkan ke fasa atas PEG dengan hasil yang setinggi 93% dan faktor penulenan lipase telah dipertingkatkan kepada 12.42.

Penulenan lipase juga dikaji dengan menggunakan sistem dua-fasa misel akueus (SDFMA) yang terdiri daripada surfaktan bukan ion tunggal dan diinduksi oleh suhu. SDFMA menawarkan kaedah yang mudah dan efisien untuk penulenan lipase dengan muatan beban yang besar dan potensi kitar semula surfaktan untuk penyediaan SDFMA yang baru. Berdasarkan SDFMA yang terdiri daripada 24% (b/b) Pluronic L81 dan 0.5% (b/b) kalium klorida, kepilihan lipase dalam SDFMA telah dipertingkatkan kepada 0.035 dan lipase telah ditulenkan 7.2 kali ganda di dalam fasa bawah. Dalam langkah penulenan yang kedua, penambahan larutan yang mengandungi kalium tiosianat ke fasa bawah misel akan menyebabkan surfaktan diekstrakan balik ke fasa atas yang baru. Kemudian, lipase akan dipulihkan ke dalam fasa bawah akueus yang baru, dengan pekali sekatan 0.34 dan 4.50 untuk lipase dan surfaktan masing-masing.

Selain itu, SDFA yang berasaskan alkohol dan garam telah digunakan untuk menulenkan lipase. Kestabilan lipase yang tinggi dalam pelarut organik telah dieksploit untuk kegunaan alkohol/garm SDFA. Sembilan SDFA yang terdiri daripada fasa atas yang berdasarkan alkohol dan fasa bawah yang berdasarkan garam telah dinilaikan untuk penulenan lipase. Sekatan kecekapan yang optimum untuk penulenan lipase telah diperolehi dengan SDFA yang terdiri daripada 16% (b/b) 2-

propanol, 16% (b/b) kalium fosfat dan 4% (b/b) natrium klorida. Lipase yang tulen dari fasa atas mempunyai lipatan penulenan yang sebanyak 13.5 dan hasil yang sebanyak 99%. Malahan, 2-propanol boleh disejatkan daripada lipase yang tulen dengan mudah dan dikitar semula untuk penyediaan SDFA yang baru. Keputusan kajian ini menunjukkan bahawa SDFA yang berasaskan alkohol dan garam boleh digunakan untuk operasi penulenan lipase secara pantas dan murah.

Fermentasi ekstraktif yang menggunakan SDFA telah dibangunkan dalam kajian ini untuk fermentasi sel serentak dengan pemprosesan hilir lipase ekstrasel *Burkholderia* sp. ST8. Pertumbuhan sel dan penghasilan lipase dalam pelbagai jenis SDFA telah diselidik. SDFA yang terdiri daripada 9.6% (b/b) PEG 8000 dan 1.0% (b/b) Dekstran T500 memberi keputusan yang terbaik untuk penghasilan dan penulenan lipase secara ekstraktif. Dalam proses yang terintegrasi ini, biojisim akan terkumpul di dalam fasa bawah manakala lipase akan diekstraks ke fasa atas. Hasil yang setinggi 92.1% tercatat dalam operasi fermentasi tunggal secara kelompok. Fermentasi secara berulang dan kelompok telah dilaksanakan dengan penggantian berterusan untuk fasa atas bagi setiap 24 jam dan keputusan kepekatan purata untuk lipase dalam tujuh fermentasi secara ekstraktif (jangka masa 168 jam) ialah 16.5 U/mL. Kombinasi penghasilan lipase dan proses penulenan lipase dalam SDFA ialah suatu pendekatan yang menarik kerana sistem fermentasi dua-fasa akueus boleh digunakan berulang kali dan penulenan lipase secara langsung daripada kaldu fermentasi adalah mudah.

## ACKNOWLEDGEMENTS

I wish to express my utmost gratitude to my main supervisor, Assoc. Prof. Dr. Ling Tau Chuan for his guidance and support during the course of this research. I would also like to thank my supervisory committee members, Assoc. Prof. Dr. Tey Beng Ti, Prof. Dr. Arbakariya Ariff, and Assoc. Prof. Dr. Siti Mazlina Mustapa Kamal for their encouragement and excellent advices.

Special thanks are due to all laboratory assistants of Department of Process and Food Engineering (KPM) and Laboratory of Immunotherapeutics and Vaccines (LIVES) for their consistent and kind assistance. Besides, I would like to acknowledge Dr. Hii Siew Ling from Universiti Tunku Abdul Rahman for providing the bacterial strain used in this research. This study was supported by the e-Science Fund (03-01-04-SF0785) from the Ministry of Science, Technology and Innovation of Malaysia (MOSTI).

I want to extend my sincere appreciations to my labmates and friends (Dr. Ramanan, Tam, Lo, Fatemeh, Fadzlie, Hor Shee, Joo Shun, Yu Kiat, Teck Kim, Pau Loke and Grrace Ng) for helping me all the time and sharing their invaluable knowledge with me. Last but not least, I would like to thank my family for their earnest love, tolerance and sacrifices. I could not have done this without all of them.

I certify that a Thesis Examination Committee has met on 25 February 2011 to conduct the final examination of Ooi Chien Wei on his thesis entitled "Recovery of Extracellular Lipase from *Burkholderia* sp. ST8 in Aqueous Two-Phase Systems" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Mohd Ali Hassan, PhD**
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

**Rosfarizan binti Mohamad, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Chin Nyuk Ling, PhD**
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

**Marco Antonio Rito-Palomares, PhD**
Professor
Tecnológico de Monterrey
Mexico
(External Examiner)

_____
**NORITAH OMAR, PhD**
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 April 2011

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:

**Ling Tau Chuan, PhD**
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

**Arbakariya Ariff, PhD**
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

**Tey Beng Ti, PhD**
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

**Siti Mazlina Mustapa Kamal, PhD**
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)



_____
**HASANAH MOHD GHAZALI, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

## DECLARATION

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

**OOI CHIEN WEI**

Date: 25 February 2011

## TABLE OF CONTENTS