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EXTRACTION, REIMMOBILIZATION AND CHARACTERIZATION OF SPENT IMMOBILIZED LIPASE

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By

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EXTRACTION, REIMMOBILIZATION AND CHARACTERIZATION OF SPENT IMMOBILIZED LIPASE

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Chairman : Associate Professor Mohd Shukuri Mohamad Ali, PhD
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Currently, lipases are considered as one of the important catalysts in substituting the use of chemical reactions in a wide variety of processes. In 2015, the report estimated that the U.S. enzyme's market had a high demand for processed foods and synthesis reactions, which generated more than USD 1 billion in sales. However, the reusability of the industrial immobilized lipase was limited only after several cycles of reactions. After that, the spent immobilized lipase will be replaced with the new immobilized lipase. The inability to reuse the spent immobilized lipase leads to an increased cost required for the new uptake of the enzyme. Practically in industry, the spent immobilized lipase is a waste from industrial users. The spent immobilized lipase still has potential use even though the activity was lower than the new immobilized lipase. There was no study done on the extraction and reimmobilization of the enzyme from the spent immobilized lipase. Therefore, the objective of this research is to study the feasibility of the spent lipase to be extracted, reimmobilized, and characterized.

General methodology involved the recovery of the spent immobilized lipase via chemical and mechanical extraction. The chemical extraction approach via Reverse Micelles Extraction (RME) showed the highest lipase recovery, which was 66% compared to the 34% of lipase yield obtained from the mechanical extraction method. The extracted lipase was reimmobilized via simple adsorption into the ethanol pretreated carrier. The characterization of the reimmobilized lipase at different pH and temperature was conducted. The optimum conditions of immobilization resulted in 96% of the extracted lipase being immobilized. The reimmobilized lipase optimum activity was at 50°C and pH 6. The reimmobilized lipase was incubated for 20 h in pH 6 buffer at 50°C of water bath shaker. The reimmobilized lipase still had 27% residual activity after 18 h of incubation, which indicated higher thermal stability compared to the free lipase.

The Scanning Electron Microscope (SEM) was used to study the morphology of the reimmobilized lipase. The morphological of immobilized lipase was analyzed based on the pore and the particle sizes of the support. SEM also showed oil on the surface of immobilized lipase before and after the solvent treatment. The structural analysis of free lipase and reimmobilized lipase was determined by Fourier-transform infrared spectroscopy (FTIR). The structures of the amide group I (CO stretch) and amide group II (NH bend), which formed the functional group of the free commercial lipase, extracted lipase and reimmobilized lipase, had been identified. In conclusion, the free lipase was successfully extracted from the spent immobilized lipase and reimmobilized into Accurel MP1008 carrier. It exhibited high thermal stability, and the reusability of the spent enzyme will promote continued use of industrial lipase and reduce the cost of the manufacturing process.

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

PENGEKSTRAKAN, PENYEKATGERAKAN DAN PENCIRIAN LIPASE TERSEKATGERAK YANG TELAH DIGUNAKAN

Oleh

NUR FATHIAH BINTI MOKHTAR

Jun 2020

Pengerusi : Profesor Madya Mohd Shukuri Mohamad Ali, PhD
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Pada masa ini, lipase dianggap sebagai salah satu pemangkin penting dalam menggantikan penggunaan tindak balas kimia di dalam pelbagai proses. Pada tahun 2015, berdasarkan laporan pasaran enzim, A.S dianggarkan mempunyai permintaan enzim yang tinggi untuk pemprosesan makanan dan tindak balas sintesis yang menjana lebih daripada 1 bilion USD jualan. Walau bagaimanapun, kebolehgunaan semula lipase industri hanya terhad selepas beberapa kitaran tindak balas. Kemudian, lipase tersekatgerak lama akan digantikan dengan lipase tersekatgerak yang baru. Ketidakupayaan untuk menggunakan lipase tersekatgerak yang lama akan meningkatkan kos yang diperlukan untuk lipase tersekatgerak yang baru. Secara praktikal, lipase tersekatgerak yang telah digunakan merupakan sisa buangan dari penggunaan industri. Lipase tersekatgerak yang telah digunakan masih mempunyai potensi untuk digunakan semula walaupun aktivitinya lebih rendah dari lipase tersekatgerak yang baru. Tiada kajian dilakukan terhadap pengekstrakan dan penyekatgerakan semula enzim daripada lipase tersekatgerak yang telah digunakan. Oleh itu, objektif kajian ini adalah untuk mengkaji kemungkinan lipase yang telah digunakan untuk diekstrak, disekatgerak semula, dan dicirikan.

Metodologi umum melibatkan pemulihan lipase tersekatgerak yang telah digunakan melalui pengekstrakan kimia dan mekanikal. Pendekatan pengekstrakan kimia melalui ekstraksi misel terbalik (RME) menunjukkan pemulihan lipase tertinggi, iaitu 66% berbanding dengan 34% hasil lipase diperolehi dari kaedah pengekstrakan mekanikal. Lipase yang diekstrak telah disekatgerakan semula melalui penjerapan mudah ke dalam pembawa yang telah dirawat oleh ethanol. Keadaan penyekatgerakan yang optimum menyebabkan 96% lipase yang diekstrak telah disekatgerak. Pencirian lipase tersekatgerak telah dijalankan pada pH dan suhu yang berbeza. Aktiviti optimum untuk lipase tersekatgerak adalah pada 50˚C dan pH 6. Lipase tersekatgerak

telah diinkubasi selama 20 jam di dalam penimbal pH 6 dan 50˚C penggoncang rendaman air. Lipase tersekatgerak masih mempunyai lebihan aktiviti sebanyak 27% selepas diinkubasi selama 18 jam yang menunjukkan kestabilan haba yang lebih tinggi berbanding dengan lipase bebas.

Mikroskop elektron pengimbas (SEM) telah digunakan untuk mengkaji morfologi lipase tersekatgerak. Morfologi lipase tersekatgerak dianalisis berasaskan liang pori dan saiz pembawa enzim. SEM juga menunjukkan kehadiran minyak pada permukaan lipase tersekatgerak sebelum dan selepas dirawat oleh pelarut. Analisis struktur lipase bebas dan lipase tersekatgerak ditentukan oleh spektroskopi inframerah transformasi Fourier (FTIR). Struktur amide group I (CO stretch) dan amide group II (NH bend) yang membentuk kumpulan berfungsi dalam lipase komersial bebas, lipase yang diekstrak dan lipase tersekatgerak, telah dikenalpasti.

Kesimpulannya, lipase bebas telah berjaya diekstrak dari lipase tersekatgerak yang telah digunakan dan disekatgerak semula ke dalam pembawa Accurel MP1008. Ia mempamerkan kestabilan haba yang tinggi, dan kebolehgunaan semula enzim buangan akan menggalakkan penggunaan lipase industri secara terus dan mengurangkan kos untuk proses pembuatan.

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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:

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This is to confirm that:

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LIST OF ABBREVIATIONS

Å	Angstrom
µL	Microliter
µm	Micrometer
µmoles	Micromoles
AOT	Bis(2-ethylhexyl) sulfosuccinate sodium salt
BSA	Bovine serum albumin
CaCl2	Calcium chloride
g	Gram
h	Hour
HCl	Hydrochloric acid
HEA	Hen egg albumin
KCl	Potassium chloride
K_2HPO_4	Dipotassium phosphate
KH_2PO_4	Potassium dihydrogen phosphate
L	Liter
M	Molar
mM	Milimolar
mg	Miligram
min	Minute
mL	Mililiter
nm	Nanometer
pH	Negative logarithm of hydrogen ion concentration
RME	Reverse Micelles Extraction

RMs	Reverse Micelles
rpm	Rotation per minute
sec	Second
U/mL	Unit per milliliter

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Commercial lipase D2 is used in the food processing industry, especially in the production of structured lipids as dietary ingredients derived from fats and oils (Flood & Kondo, 2003; Tecelão et al., 2012). In industrial use, immobilized enzymes had better performance in terms of operational, storage, and thermal stability (Katchalski-Katzir & Kraemer, 2000; Tecelão et al., 2012; Talekar & Chavare, 2012). The study on the reusability of the carrier was conducted for papain enzymes before. The papain enzyme was reimmobilized on the regenerated carrier recovered from spent bound papain by using chelating metal ions to improve enzyme activity (Iqbal & Afaq, 2001). However, the immobilized lipase consists of free lipase and carrier (Accurel MP1008) can only be reused after several cycles (Bosley & Pelow., 1997; Rehm et al., 2016).The simple adsorption technique and extreme condition in the bioreactor system caused the inconsistency of enzyme activity (Rehman et al., 2016). After several usages, the immobilized lipase was considered waste from the industry (Iqbal & Afaq, 2001; Rehman et al., 2016). The waste-immobilized lipase classified into spent and denatured immobilized lipase. The spent immobilized lipase had low activity than the new immobilized lipase but higher activity than the denatured immobilized lipase. Practically, the spent immobilized lipase was treated with boiling water until denatured before being discharged from the bioreactor. The spent immobilized lipase, which potentially had residual activity was retrieved from the bioreactor and used for further analysis. This study is the first attempt to recover the lipase from spent immobilized lipase.

1.2 Problem statement

Spent immobilized lipase was abundantly present in the reaction after the synthesis process, and once the productivity lost to almost 50% after several cycles of reaction, the spent immobilized lipase will become a waste. The recovery of support or enzyme from the wasted immobilized lipase can significantly reduce the cost required to purchase a new fresh immobilized lipase, especially on both support and enzyme. The price for lipase is very expensive than that carrier. MEMBRANA GMBH Company stated that 1 g of Accurel MP1008 support only cost RM 0.60 per gram. Meanwhile, the price for 1 g of lipase (ROL) is RM 555.00 (Sigma-Aldrich). However, there is a lack of discoveries on the recovery, reimmobilization, and characterization of lipase from spent immobilized lipase.

1.3 Significance of the study

The lipase can be recovered from the spent immobilized lipase, which will significantly reduce the cost needed for the new enzyme. The reimmobilization of extracted lipase was more cost-effective. RME is the most suitable recovery method due the time-saving and easily upscale (Basheer & Thenmozhi, 2010; Gangadharappa et al., 2017; Jalil et al., 2018). No study on the extraction of lipases from spent immobilized lipase was conducted before. After lipases extraction, the optimization of lipase immobilization techniques can be used to enhance the stability of the reimmobilized enzyme using the previous immobilization method (Zhang et al., 2013; Datta et al., 2013). Therefore, the study on lipase extraction from spent immobilized lipase via Reverse Micellar Extraction (RME) can be beneficial. The RME method can be used to upscale the lipase recovery from the spent immobilized lipase.

1.4 Research objectives

1.4.1 General objective:

To extract, reimmobilize, and characterize the lipase from spent immobilized lipase into industrial use macroporous support (Accurel MP1008). The specifications of the support are listed in Appendix iv.

1.4.2 Specific objectives:

1. To examine the remaining activity of the spent immobilized lipase
2. To recover the lipase through mechanical and chemical methods
3. To optimize the enzyme reimmobilization
4. To perform biochemical, morphological and structural characterization of the reimmobilized lipase

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