FERMENTATION AND PURIFICATION OF LIPASE BY *BURKHOLDERIA METALLICA* USING COLUMN REACTOR

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Sekian, terima kasih.

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FERMENTATION AND PURIFICATION OF LIPASE BY *BURKHOLDERIA METALLICA* USING COLUMN REACTOR

by

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DECLARATION BY AUTHOR

This dissertation is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

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Nur Ezzreen Zafhrina Binti Mohamad Redza

Date: June 2020

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Caption
ATPS	Aqueous Two Phase System
ATPE	Aqueous Two Phase Extraction
AIRS	Aqueous Impregnated Resin System
TAPPIR	Tunable Aqueous Polymer-Phase Impregnated Resin
Mw	Molecular Weight
Ml	Milliliter
Mm	Milimeter
Min	Minute
Nm	Nanometer
S	Second
Sp	Species
R-P HPLC	Reverse-Phase High Performance Liquid Chromatography
Pf	Purification Factor
PEG	Polyethylene Glycol
pI	Isoelectric Point
pH	Potential of hydrogen
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight

μmol	Micromole
μl	Microliter

Symbol	**Caption**
° C	Degree of celcius
-	Minus
÷	Divide
=	Equal to
>	Greater than
<	Less than
%	Percentage

FERMENTASI DAN PEMURNIAN LIPASE OLEH BURKHOLDERIA METALLICA MENGGUNAKAN CAIRAN REAKTOR COLUMN YANG DIRESAPI YANG DITETAPKAN

ABSTRAK

Dalam hasil kerja ini, fermentasi dilakukan untuk bahan makanan dan pemurnian lipase oleh *Burkholderia metallica* menggunakan reaktor lajur. Parameter yang disasarkan untuk penyelidikan ini seperti kestabilan garam, jenis dan kepekatan Polietiena glikol (PEG) dan garam, pH larutan pengekstrakan dan kadar aliran ml/s un tuk melihat prestasi sistem pemurnian ekstraktif. Sistem resin berair impregnated (AIRS) digunakan menggunakan manik-manik kaca berliang yang dicangkokkan dengan polietilena glikol (PEG). Kajiaan terhadap AIRS dilakukan dengan pelbagai berat molekul polietilena glikol (PEG) (PEG 2,000, PEG 4,000, PEG 6,000) dan pelbagai jenis garam (natrium sitrat, kalium sitrat dan natrium asetat) sebagai komponen fasa. Pengekstrak cecair PEG tidak digerakkan pada penyokong lengai (manik kaca berliang) dan sesuai dengan keadaan fasa berair untuk mengurangkan masalah pengekstrakan dua fasa berair (ATPE). Teknik ini menggabungkan pengekstrakan cecair-cecair dengan mudah dengan operasi kromatografi lajur. Kemudian sasarkan biomolekul yang diserap pada keadaan berair (fasa pegun) dan kotoran dikeluarkan dari aliran. Hasil daripada eksperimen One-factor-at-a-time (OFAT) menunjukkan bahawa komposisi lipase optimum 20% (w/w) PEG 6,000 resin yang diresapi dengan larutan pengekstrakan 5% natrium sitrat pada pH 7,7, 4% natrium klorida (NaCl) dan 20% (w/w) tetap dari pemuatan kasar pemisahan lipase yang lebih baik. Kesimpulannya hasil kajian menunjukkan kaedah satu kali pemurnian, kaedah pemisahan secara tidak membahayakan tubuh badan manusia dan ringan, langkah menghilangkan pemisahan fasadan juga pengurangan jejak ATPE yang menghasilkan faktor pemurnian peningkatan tinggi AIRS

yang merupakan Resin yang Diterapkan Fasa Polimer Berair yang ditingkatkan (TAPPIR), digunakan untuk pemurnian lipase dari *Burkholderia metallica*.

FERMENTATION AND PURIFICATION OF LIPASEBY *BURKHOLDERIA METALLICA*USING AQUEOUS IMPREGNATED COLUMN REACTOR

ABSTRACT

In this work, fermentation was conducted for the feedstock and purification of lipase by *Burkholderia metallica* using a column reactor. The targeted parameter for this research such as salt stability, types and concentrations of polyethylene glycol(PEG) and salt, pH of extraction solution and flowrate ml/s to see the performance of extractive purification system. Aqueous impregnated resin system(AIRS) is applied using porous glass beads grafted with polyethylene glycol (PEG). An evaluation studies on AIRS were performed with various molecular weight of polyethylene glycol (PEG) (PEG 2,000, PEG 4,000, PEG 6,000) and different type of salts (sodium citrate, potassium citrate and sodium acetate) as phase component. Liquid extractant of PEG was immobilized on the inert support (porous glass beads) and correspond to the aqueous state phase to surmount the drawback of aqueous two phase extraction (ATPE). The technique combines liquid-liquid extraction with ease if operation of column chromatography. Then target biomolecules absorbed on aqueous (stationary phase) and impurities removed from the flow through. One-factor-at-a-time (OFAT) experimental result showed that the optimum composition of lipase purification was 20% (w/w) PEG 6,000 impregnated resin with extraction solution of 5% sodium citrate at pH 7.7, 4% sodium chloride (NaCl) and 20% (w/w) fixed of crude loading have a better separation of lipase. In conclusion the outcome of the studied showed single step purification, biocompatible and mild separation condition, eliminated phase separation step and thus reduced ATPE footprint that results the purification factor of high value reveals AIRS which

is the improved Tunable Aqueous Polymer Phase Impregnated Resins (TAPPIR), could be successfully used to purify lipase from *B.metallica*.