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**IMMOBILIZATION OF ALKALINE PROTEASE ON POLYACRYLAMIDE
AND POLY(ACRYLAMIDE-CO-METHACRYLIC ACID) BEADS BY
INVERSE SUSPENSION POLYMERIZATION**

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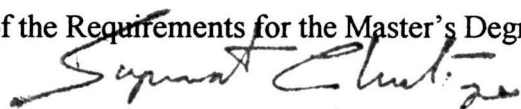
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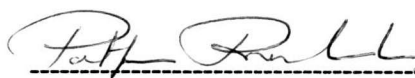
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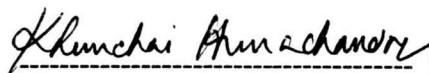
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พิมพ์ต้นฉบับบทความวิทยานิพนธ์ภายในกรอบสี่เหลี่ยมนี้เพียงแผ่นเดียว

อารีรัตน์ งามบุญศรี : การตรึงแอลคาไลน์โปรตีเอสบนบีดโพลีอะคริลาไมด์และโพลีอะคริลาไมด์-โค-กรดเมทาคริลิกโดยวิธีอินเวิร์สซัสเพนชันโพลีเมอไรเซชัน (IMMOBILIZATION OF ALKALINE PROTEASE ON POLYACRYLAMIDE AND POLY(ACRYLAMIDE-CO-METHACRYLIC ACID) BEADS BY INVERSE SUSPENSION POLYMERIZATION) อ.ที่ปรึกษา: รศ.ดร.สุดา เกียรติกำจรวงศ์, อ.ที่ปรึกษาร่วม: ผศ.นภา ศิวรังสรรค์, 181 หน้า, ISBN 974-635-918-5

แอลคาไลน์โปรตีเอสได้รับการตรึงบนบีดโพลีอะคริลาไมด์ระหว่างการโพลีเมอไรเซชันของโมโนเมอร์อะคริลาไมด์โดยวิธีอินเวิร์สซัสเพนชันโพลีเมอไรเซชัน, N,N'-methylene-bis-acrylamide(MBA), Pluronic PE 8100, และพาราฟินเหลว ใช้เป็นตัวเชื่อมโยง เซอร์เฟคแทนท์ และวัฏภาคต่อเนื่อง ตามลำดับ แอมโมเนียมเปอร์ซัลเฟตและ N,N,N',N'-tetraethylmethylenediamine (TEMED) ใช้เป็นตัวเริ่มปฏิกิริยาแบบรีดอกซ์ ได้ศึกษาผลของการเปลี่ยนแปลงความเข้มข้นของโมโนเมอร์ ตัวเชื่อมโยง เอนไซม์ ตัวเริ่มปฏิกิริยา ตัวเร่งปฏิกิริยา และเซอร์เฟคแทนท์ต่อฤทธิ์ของเอนไซม์ นอกจากนี้ยังได้ศึกษาผลของการเปลี่ยนแปลงอัตราการกวน เวลา และอุณหภูมิที่ใช้สำหรับการโพลีเมอไรเซชันต่อฤทธิ์ของเอนไซม์อีกด้วย ทดสอบฤทธิ์ของเอนไซม์ที่ได้รับการตรึงบนบีดโดยใช้เคซีนเป็นสับสเตรต การทดลองได้สรุปผลของพารามิเตอร์แต่ละตัวเพื่อเลือกภาวะที่ดีที่สุดสำหรับการโพลีเมอไรเซชันเพื่อตรึงแอลคาไลน์โปรตีเอสบนบีดโพลีอะคริลาไมด์ให้มีฤทธิ์สูงสุด ภาวะที่สามารถตรึงเอนไซม์แอลคาไลน์โปรตีเอสให้มีฤทธิ์สูงสุดประกอบด้วยอะคริลาไมด์ (3.14 มิลลิโมลาร์), MBA (15 มิลลิโมลาร์), แอลคาไลน์โปรตีเอส (1.5 มิลลิกรัมต่อ 5 ลูกบาศก์เซนติเมตร), APS (6.5 มิลลิโมลาร์), TEMED (47.75 มิลลิโมลาร์), อัตราการกวน 300 รอบต่อนาที เวลาสำหรับการโพลีเมอไรเซชัน 2 ชั่วโมง และอุณหภูมิ 30°C. ฤทธิ์ของเอนไซม์มีค่าเท่ากับ 178 ยูนิต สามารถตรึงเอนไซม์ได้ร้อยละ 42 และมีค่าคอนเวอร์ชันร้อยละ 92 ได้ศึกษาผลของการเปลี่ยนแปลงสัดส่วนของอะคริลาไมด์และกรดเมทาคริลิกที่ 100/0, 97.5/2.5, 95/5, 90/10 ร้อยละโดยน้ำหนักต่อฤทธิ์ของเอนไซม์ ฤทธิ์ของเอนไซม์ลดลงเมื่อความเข้มข้นของกรดเมทาคริลิกเพิ่มขึ้น เปรียบเทียบการดูดซึมน้ำและในสารละลายเกลือของโพลีอะคริลาไมด์และโพลี(อะคริลาไมด์-โค-กรดเมทาคริลิก) การดูดซึมน้ำเพิ่มขึ้นเมื่อเพิ่มความเข้มข้นของกรดเมทาคริลิก การดูดซึมน้ำในสารละลายเกลือต่ำกว่าการดูดซึมน้ำ เปรียบเทียบปรากฏการณ์ของความคงทนต่อความเป็นกรด-เบสและอุณหภูมิต่อฤทธิ์ของเอนไซม์ที่ได้รับและไม่ได้รับการตรึง เอนไซม์ที่ไม่ได้รับและไม่ได้รับการตรึงให้ฤทธิ์ของเอนไซม์สูงสุดที่ความเป็นกรด-เบส 10 และ 10.5 ที่อุณหภูมิเดียวกัน 45°C. ตามลำดับ เอนไซม์ที่ไม่ได้รับและที่ได้รับการตรึงสามารถเก็บได้ที่อุณหภูมิ -20 ถึง 4°C. เป็นเวลาหนึ่งเดือนโดยไม่มีการสูญเสียฤทธิ์ของเอนไซม์ ฤทธิ์ของเอนไซม์ที่ไม่ได้รับการตรึงลดลงร้อยละ 51 โดยที่ฤทธิ์ของเอนไซม์ที่ได้รับการตรึงบนบีดโพลีอะคริลาไมด์และโพลี(อะคริลาไมด์-โค-กรดเมทาคริลิก)ลดลงร้อยละ 37 และร้อยละ 42 ตามลำดับเมื่อเก็บเอนไซม์ทั้งสองที่อุณหภูมิ 60°C. เป็นเวลาหนึ่งเดือนที่อุณหภูมิสูงเอนไซม์ที่ได้รับการตรึงมีความเสถียรสูงกว่าเอนไซม์ที่ไม่ได้รับการตรึงและเก็บได้นานกว่า

ภาควิชา.....สหสาขาวิชาปิโตรเคมี-โพลีเมอร์.....
สาขาวิชา.....วิทยาศาสตร์โพลีเมอร์.....
ปีการศึกษา.....2539.....

ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสี่เหลี่ยมนี้เพียงแผ่นเดียว

C785229 : MAJOR POLYMER SCIENCE
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AREERAT NGANBUNSRI: IMMOBILIZATION OF ALKALINE PROTEASE ON POLYACRYLAMIDE AND POLY(ACRYLAMIDE-CO-METHACRYLIC ACID) BEADS BY INVERSE SUSPENSION POLYMERIZATION. THESIS ADVISOR: ASSOC. PROF. SUDA KIATKAMJORNWONG, Ph.D. THESIS CO-ADVISOR: ASSIST. PROF. NAPA SIWARUNGSON, M.S. 181 pp. ISBN 974-635-918-5

The alkaline protease was entrapped during inverse suspension polymerization of acrylamide monomer. N,N'-methylene-bis-acrylamide (MBA), Pluronic PE 8100, and paraffin wax were utilized as a crosslinker, surfactant, and continuous phase, respectively. Ammonium persulfate (APS) and N,N,N',N'-tetraethylmethylenediamine (TEMED) were used as the redox initiator. The effects of concentration of monomer, crosslinker, enzyme, initiator, accelerator, and surfactant on enzymatic activity were investigated. The effects of stirring rate, polymerization time, and temperature on enzymatic activity of the product were also carried out. The effects of each parameter were established for the best polymerization conditions for entrapment of the alkaline protease for the optimum enzymatic activity. The enzymatic activity was determined using casein as a substrate. Conditions that showed the optimum enzymatic activity were: acrylamide (3.14 mM), MBA (15 mM), alkaline protease (1.5 mg/5 cm³), APS (6.5 mM), TEMED (47.75 mM), at stirring rate of 300 rpm, polymerization time 2 h, and temperature 30°C. The enzymatic activity was 178 units, with 42% immobilization and 92% conversion. The effect of acrylamide/methacrylic acid ratios (100/0, 97.5/2.5, 95/5, 90/10% W/W) on the enzymatic activity were investigated. The enzymatic activity was decreased with increasing the methacrylic acid concentration. The water absorption of polyacrylamide and poly(acrylamide-co-methacrylic acid) in deionized water and saline solutions was also carried out for comparison. The water absorption was increased with increasing methacrylic acid concentration while the absorption in saline solutions was less than that in deionized water. The effects of pH and temperature on enzymatic activity of free- and immobilized enzyme were compared. The maximum enzymatic activity of free- and immobilized enzymes was shown at pH 10 and 10.5 at the same temperature of 45°C, respectively. The free- and immobilized enzymes kept at temperature -20 to 4°C for one month were stable and without loss of enzymatic activity. The enzymatic activity of the free enzyme was decreased to 51%, while the enzymatic activities of immobilized enzyme on polyacrylamide and poly(acrylamide-co-methacrylic acid) were decreased to 37% and 42%, respectively after an one month storage at 60°C. At higher temperatures, the immobilized enzyme was thermally stable for a longer shelf life than the free enzyme.

ภาควิชา.....สหสาขาวิชาปิโตรเคมี-โพลีเมอร์

สาขาวิชา.....วิทยาศาสตร์โพลีเมอร์

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LIST OF ABBREVIATIONS

AM	acrylamide
MAA	methacrylic acid
PAM	polyacrylamide
poly(AM-co-MAA)	poly(acrylamide-co-methacrylic acid)
MBA	N,N'-methylene -bis-acrylamide
APS	ammonium persulfate
DMAPN	3-dimethylaminopropionitrile
TEMED	N,N,N',N'-tetraethylmethylenediamine
EDTA	ethylenediamine tetraacetic acid
PMSF	phenylmethylsulfonyl fluoride
TCA	trichloroacetic acid
BSA	bovine serum albumin
FT-IR	Fourier Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
SEM	Scanning Electron Microscopy
°C	degrees Celsius
°ศ.	องศาเซลเซียส
g	gram
%	percent
W/W	weight by weight

μm	micrometer
mM	millimolar
nm	nanometer
cm^{-1}	wavenumber
h	hour
min	minute
%RA	% Relative activity
N	normality
cm^3	cubic centimeter
Polym. Plast. Technol. Eng.	Polymer Plastic Technology and Engineering
Prog. Polym. Sci.	Progress in Polymer Science
Biotechnol. Appl. Biochem.	Biotechnology and Applied Biochemistry
Biotechnol. Bioeng.	Biotechnology and Bioengineering
Polym. Bull.	Polymer Bulletin
Appl. Microbiol.	Applied Microbiology
Makromol. Chem.	Makromolekulare Chemie
Arch. Biochem. Biophys	Archives of Biochemistry and Biophysics
J. Appl. Polym. Sci.	Journal of Applied Polymer Science
J. Appl. Biochem.	Journal of Applied Biochemistry
J. Biol. Chem.	Journal of Biological Chemistry
J. Biotechnol	Journal of Biotechnology
Anal. Chem.	Analytical Chemistry
J. Polym. Sci.	Journal of Polymer Science