

**PRODUCTION OF MANNAN-DEGRADING ENZYMES BY *ASPERGILLUS NIGER* IN SHAKE FLASKS AND STIRRED TANK FERMENTER**

**By**

**SITI NORITA BINTI MOHAMAD**

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

**February 2005**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**Chairman: Professor Arbakariya Ariff, Ph.D.**

**Institute: Bioscience**

Optimization of medium composition and culture conditions for mannan-degrading enzymes production by *Aspergillus niger* was carried out in shake flasks and 2 L stirred tank fermenter. Preliminary, three potential strains (*Sclerotium rolfsii*, *Rhizopus oryzae* and *A. niger*) were screened, and *A. niger* was used for subsequent study. The mannan-degrading enzymes were purified partially and characterized with regard to pH optima and stability, temperature optima and stability, and  $K_m$  and  $V_{max}$  values. The influence of agitation speed, aeration rate and incubation temperature on the production of mannan-degrading enzyme in batch fermentation using 2 L stirred tank fermenter were also investigated.

Highest level of  $\beta$ -mannanase activity was obtained when guar gum ( $1495 \text{ nkat mL}^{-1}$ ) and bacteriological peptone ( $1744 \text{ nkat mL}^{-1}$ ) were compared to other carbon (locust bean gum, cellulose, carboxymethylcellulose and glucose) and nitrogen (peptone from meat,

yeast extract, ammonium sulphate, nitrate and citrate) sources used. The conditions predicted for the maximum production of  $\beta$ -mannanase through the use of response surface methodology were at pH 5.47, 57 g L<sup>-1</sup> bacteriological peptone and 21.3 g L<sup>-1</sup> guar gum. The maximal  $\beta$ -mannanase, endoglucanase,  $\beta$ -mannosidase and galactosidase activity obtained from the predicted equation was of 2010.8, 34.8, 1.6 and 39.0 nkat mL<sup>-1</sup>, respectively.

The optimal temperatures for  $\beta$ -mannanase activity were 50°C and 60°C, with half-life ( $t_{1/2}$ ) of 6 h at 60°C and 4 h at 70°C. The optimal temperature for endoglucanase activity was 60°C, with  $t_{1/2}$  of 6 h at 60°C and 45 min at 70°C. The optimal temperature for  $\beta$ -mannosidase was 70°C with  $t_{1/2}$  of 1.5 h at 70°C. While the optimal temperature for  $\alpha$ -galactosidase activity was 50 to 60°C with  $t_{1/2}$  of 2.5 h at 60°C. The  $\beta$ -mannanase, endoglucanase and  $\alpha$ -galactosidase had a pH optima at 3.5 while  $\beta$ -mannosidase at pH 3.0. The enzymes characterized in this study were defined as acidic proteins. The  $\beta$ -mannanase,  $\beta$ -mannosidase,  $\alpha$ -galactosidase and endoglucanase showed good stability at pH values of pH 3.5 – 7.0, pH 3.5 – 6.5, pH 3.5 – 5.0 and pH 4 – 7, respectively after a prolonged incubation (24 h at 50°C). High substrate specificity of crude culture filtrate, with low  $K_m$  value of  $\beta$ -mannanase (0.04 mg mL<sup>-1</sup>), endoglucanase (0.54 mg mL<sup>-1</sup>),  $\beta$ -mannosidase (1.67 mM) and  $\alpha$ -galactosidase (1.34 mM) indicates the synergistic effect of the enzyme mixture had occurred. The value of  $V_{max}$  for  $\beta$ -mannanase, endoglucanase,  $\beta$ -mannosidase and  $\alpha$ -galactosidase were 0.52, 0.12, 1.72 x 10<sup>-3</sup> and 4.68 x 10<sup>-3</sup> nmol mL<sup>-1</sup> min<sup>-1</sup>, respectively.

A fermentation in 2 L stirred tank fermenter using optimized medium yielded 678 nkat mL<sup>-1</sup> β-mannanase, associated with 1.25 nkat mL<sup>-1</sup> β-mannosidase, 18.46 nkat mL<sup>-1</sup> α-galactosidase and 40.15 nkat mL<sup>-1</sup> endoglucanase at impeller tip speed of 0.82 m s<sup>-1</sup>, aeration rate of 0.1 vvm and incubation temperature of 35°C. Higher degree of agitation speed and aeration rate had an inhibitory effect on the production of mannan-degrading enzymes.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

**PENGHASILAN ENZIM BAGI MENDEGRADASIKAN MANNAN OLEH  
*ASPERGILLUS NIGER* MENGGUNAKAN KELALANG PENGGONCANG DAN  
TANGKI FERMENTER PENGADUK**

Oleh

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Pengoptimaan komposisi media dan keadaan kultur untuk penghasilan enzim bagi mendegradasikan mannan oleh *A. niger* dijalankan di dalam kelalang penggoncang dan tangki fermenter pengaduk 2 L. Sebagai permulaan, tiga strain (*Sclerotium rolfsii*, *Aspergillus niger* and *Rhizopus oryzae*) yang mempunyai potensi telah dipilih dan *A. niger* telah digunakan untuk kajian yang seterusnya. Enzim utk mendegradasikan mannan telah dituliskan separa dan dicirikan untuk penentuan pH optima dan kestabilan, suhu optima dan kestabilan, dan nilai  $K_m$  and  $V_{max}$ . Kesan kelajuan putaran, kadar pengudaraan dan suhu inkubasi kepada penghasilan enzim bagi mendegradasikan mannan dalam fermentasi suapan sesekelompok menggunakan tangki fermenter pengaduk, 2 L juga dikaji.

Tahap tertinggi bagi aktiviti  $\beta$ -mannanase didapati apabila gum guar ( $1495 \text{ nkat mL}^{-1}$ ) dan pepton bakteriologikal ( $1744 \text{ nkat mL}^{-1}$ ) dibanding dengan sumber karbon (gum

kekacang locust, selulos, karboksimetilselulos and glukosa) and nitrogen (pepton daripada daging, yis ekstrak, ammonium sulfat, nitrat and sitrat) lain yang telah digunakan. Anggaran keadaan bagi penghasilan maksimum  $\beta$ -mannanase melalui penggunaan kaedah tindakbalas permukaan (response surface methodology) adalah pada pH 5.47,  $57 \text{ g L}^{-1}$  pepton bakteriologikal dan  $21.3 \text{ g L}^{-1}$  gum guar. Anggaran aktiviti maksimum bagi  $\beta$ -mannanase, endoglukanase,  $\beta$ -mannosidase and galaktosidase yang didapati dari persamaan, masing-masing adalah 2010.8, 34.8, 1.6 and  $39.0 \text{ nkat mL}^{-1}$ .

Suhu optima bagi aktiviti  $\beta$ -mannanase ialah  $50^{\circ}\text{C}$  dan  $60^{\circ}\text{C}$ , dengan jangka separa hayat ( $t_{1/2}$ ) selama 6 jam pada  $60^{\circ}\text{C}$  dan 4 jam pada  $70^{\circ}\text{C}$ . Suhu optima bagi aktiviti endoglukanase ialah  $60^{\circ}\text{C}$ , dengan  $t_{1/2}$  selama 6 jam pada  $60^{\circ}\text{C}$  dan 45 minit pada  $70^{\circ}\text{C}$ . Suhu optima bagi  $\beta$ -mannosidase adalah pada  $70^{\circ}\text{C}$  dengan  $t_{1/2}$  selama 1.5 jam pada  $70^{\circ}\text{C}$ . Sementara itu, suhu optima bagi aktiviti  $\alpha$ -galaktosidase ialah pada 50 ke  $60^{\circ}\text{C}$  dengan  $t_{1/2}$  selama 2.5 jam pada  $60^{\circ}\text{C}$ .  $\beta$ -mannanase, endoglukanase dan  $\alpha$ -galaktosidase mempunyai pH optima yang sama iaitu pada 3.5 sementara  $\beta$ -mannosidase pada pH 3.0. Enzim yang dicirikan di dalam kajian ini didefinisikan sebagai protein berasid. Kestabilan yang baik pada pH ditunjukkan oleh  $\beta$ -mannanase,  $\beta$ -mannosidase,  $\alpha$ -galaktosidase and endoglukanase, masing-masing pada pH 3.5 – 7.0, pH 3.5 – 6.5, pH 3.5 – 5.0 dan pH 4 – 7 selepas inkubasi (24 jam pada  $50^{\circ}\text{C}$ ). Kespesifikan substrat yang tinggi bagi filtrasi kultur kasar, dengan nilai  $K_m$  yang rendah bagi  $\beta$ -mannanase ( $0.04 \text{ mg mL}^{-1}$ ), endoglukanase ( $0.54 \text{ mg mL}^{-1}$ ),  $\beta$ -mannosidase (1.67 mM) and  $\alpha$ -galaktosidase (1.34 mM) menggambarkan yang kesan sinergistik telah berlaku pada campuran enzim.

Nilai  $V_{\max}$  untuk  $\beta$ -mannanase, endoglukanase,  $\beta$ -mannosidase and  $\alpha$ -galaktosidase masing-masing adalah 0.52, 0.12,  $1.72 \times 10^{-3}$  and  $4.68 \times 10^{-3}$  nmol mL<sup>-1</sup> min<sup>-1</sup>.

Fermentasi dalam tangki fermenter pengaduk 2 L menggunakan medium yang telah dioptimalkan menghasilkan 678 nkat mL<sup>-1</sup>  $\beta$ -mannanase, berserta dengan 1.25 nkat mL<sup>-1</sup>  $\beta$ -mannosidase, 18.46 nkat mL<sup>-1</sup>  $\alpha$ -galaktosidase dan 40.15 nkat mL<sup>-1</sup> endoglukanase pada kelajuan hujung pemutar 0.82 m s<sup>-1</sup>, kadar pengudaraan 0.1 vvm dan suhu inkubasi pada 35°C. Peningkatan darjah kelajuan putaran dan kadar pengudaraan mempunyai kesan perencatan pada penghasilan enzim untuk mendegradasikan mannan.

## ACKNOWLEDGEMENTS

All praise to Allah S.W.T. who has guide my safely, through every mile, grant me wealth, give me health and most of all give me care and love well. I thank Allah S.W.T. for giving me the strength to finish my study.

I would like to express my sincere appreciation and deepest gratitude to my supervisor, Professor Dr. Arbakariya Ariff for his invaluable guidance, kind and suggestions during the course of this study. My deep appreciation is also extent to the members of my supervisory committee, Dr. Hirzun Mohd Yusof and Dr. Rosfarizan Mohamad for their constructive criticism, guidance and suggestions that have been a great help.

I would also like to express my gratitude to the Director General and Director of Research, of Fisheries Department to for their permission to pursue the study. Thanks also extended to the former and current Head of the Centre of Fisheries Department in Batu Berendam, Mr. Hambal Hj. Hanafi and Mr. Hj Rosly Hassan for supporting me to continue the study. Sincere appreciations also go to staff of Fermentation Unit, Mr Sobri, Ms. Lyana and Mr. Rizal for their assistance throughout my study.

Special thanks goes to my friends, Kak Noorull, Julia, Fidh, Musa, Bazli, Mai, Ina, Siew Ling, Yan Peng, Chin Ming, Xiao Cui, and Mr. Naza for their kind, patience and support during my study. I also wish to express my thanks to all my friends in Freshwater Fisheries Research Center, Batu Berendam especially to Ayong, Kahar, Reha, Zie and



Kak Nab for helping me during the period of my study. A great appreciation also dedicated to Khiriyah, for spending her precious time commenting this thesis.

Finally, I would like to express my highest gratitude to Hjh Rokiah Md. Diah, Hj. Mohamad Hj. Abu, Hasan Ali, all my brothers, sisters in-law, niece and nephew; thank you for your understanding, caring and moral support given during the period of my study.

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## **DECLARATION**

I hereby declare that the thesis is based on my original work expect for quotation and citation which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other at UPM or other institutions.

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Date:

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## LIST OF ABBREVIATIONS

nkat mL <sup>-1</sup>	:	nanokatal/ millilitre
U mL <sup>-1</sup>	:	Unit/millilitre
mg mL <sup>-1</sup>	:	milligram/millilitre
PDA	:	Potato Dextrose Agar
<i>p</i> NP- $\alpha$ Gal	:	<i>p</i> -nitrophenyl- $\alpha$ -D-galactoside
<i>p</i> NP- $\alpha$ Man	:	<i>p</i> -nitrophenyl- $\beta$ -D-mannoside
h	:	hour
min	:	Minute
L	:	litre
mM	:	millimolar
$\mu$ L	:	microlitre
GG	:	Guar gum
LBG	:	Locust bean gum
CMC	:	Carboxymethylcellulose
$^{\circ}$ C	:	Degree Celsius
DNS	:	Dinitrosalicylic acid
DOT	:	Dissolved oxygen tension
rpm	:	Rotation per minute
<i>Re</i>	:	Reynolds number based on diameter of rotation
vvm	:	Volume of air per min/ volume of fermentation media