
**UNIVERSITI PUTRA MALAYSIA**

# OPTIMIZATION OF CULTURE CONDITION FOR PRODUCTION OF EXTRACELLULAR INULINASE AND INVERTASE FROM *ASPERGILLUS NIGER* ATCC 20611

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# OPTIMIZATION OF CULTURE CONDITION FOR PRODUCTION OF EXTRACELLULAR INULINASE AND INVERTASE FROM *ASPERGILLUS NIGER* ATCC 20611

**By**

**MOJDEH DINARVAND**

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

**December 2011**

**DEDICATION**

*Dedicated*

*To my beloved my family, for their help and support*

*To my best friends Madineh, Leila, Athar, Nasrin and Azadeh for their encourage and ...*

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

# OPTIMIZATION OF MEDIUM AND PROCESS PARAMETERS FOR THE PRODUCTION OF EXTRACELLULAR INULINASE AND INVERTASE FROM *ASPERGILLUS NIGER* ATCC 20611

By

**MOJDEH DINARVAND**

**December 2011**

**Chairman: Associate Professor Shuhaimi bin Mustafa, PhD**

**Faculty: Biotechnology and Biomolecular Sciences**

In industry, filamentous fungus like *Aspergillus niger* is generally used as an enzyme source. Amongst the enzymes that are industrially produced by this fungus are inulinase and invertase. Inulinase and invertase constitute a significant class of enzymes for production of fructooligosaccharide and fructose, which are commonly used in food industry and pharmaceutical. Inulinase and invertase production are affected by medium composition in fermentation and optimum medium formulate is usually strain dependent. Therefore, optimization studies were conducted to determine the best parameters for inulinase and invertase production.

Different sources and concentrations of carbon, and nitrogen sources, metal ions and surfactants were examined in this study. It was found that, *A. niger* ATCC 20611

produced high amount of inulinase after 96h of incubation with 6% (v/v) inoculum, pH 6.5, temperature 35°C and 100 rpm shaking rate in the presence of 25% (w/v) sucrose as a carbon source, 0.5% (w/v) meat extract as an organic nitrogen source, 1.5% (w/v) $NaNO_3$ as the best inorganic nitrogen sources and 2 mM (v/v) $Zn^{2+}$ as metal ion. Under this optimum condition, inulinase enzyme was produced at 3199 U/ml. However, it was also observed that surfactant (Triton X-100) played inhibitory effect on enzyme production by this fungus. The activity of the inulinase exposed at 50$^{o}$C were maintained at 92.0%, 89.0% and 53.0% for 30 min, 60 min and 120 min, respectively.

Meanwhile, the production of invertase was improved using different nutrient and physical factors. Medium containing 10% (w/v) sucrose, 1% (w/v) yeast extract, 1.5% (w/v) $NaNO_3$, 1mM (v/v) $Ca^{2+}$ and 1% (v/v) Triton X-100 was found to be optimal for invertase production. The optimal physical factors were at temperature 30˚C, pH 6.0, inoculum size of 6% (v/v) and agitation rate of 200 rpm. Under this optimum condition, invertase was produced at 3072 U/ml. The activity of the invertase exposed at 50$^{o}$C were maintained at 90%, 49% and 15% for 60 min, 120 min and 180 min, respectively.

The potential of these two media for the production of inulinase and invertase could be explored in a large scale fermentation to produce enzymes of industrially feasible and economical.

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

**OPTIMUM MEDIUM DAN PARAMETER PROSES BAGI PENGHASILAN SELLUAR INULINASE DAN INVERTASE DARIPADA *ASPERGILLUS NIGER* ATCC 20611**

Oleh

**MOJDEH DINARVAND**

**Disember 2011**

**Pengerusi: Profesor Madya Shuhaimi bin Mustafa, PhD**

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*Kulat berfilamen seperti Aspergillus niger selalunya diguna di dalam industri sebagai sumber enzim. Kebanyakkan enzim yang dihasilkan dalam industri oleh kulat ini adalah inulinase dan invertase. Inulinase dan invertase adalah enzim yang penting dalam penghasilan fruktooligosakarida dan fruktosa, yang mana kebiasaannya diguna pakai di dalam industri makanan dan farmasi. Penghasilan inulinase dan invertase dipengaruhi oleh kandungan medium fermentasi dan selalunya formulasi medium yang optimum adalah bergantung kepada jenis strain. Oleh sebab itu, kajian untuk mengoptimumkan enzimdijalankan bagi mengenal pasti parameter yang terbaik dalam penghasilan inulinase dan invertase.*

*Pelbagai sumber dan kepekatan karbon, nitrogen, ion logam dan surfactants yang berbeza diuji di dalam kajian ini. Di dapati, A. niger ATCC 20611 menghasilkan jumlah inulinase yang tertinggi selepas 96 jam pengeraman dengan 6%*

*(isipadu/isipadu), pH 6.5, suhu 35$^0$C dan 100 rpm kadar goncangan dengan adanya 25% (berat/isipadu) sukrosa sebagai sumber karbon, 0.5% (berat/isipadu) ekstrak daging sebagai sumber organik nitrogen, 1.5% (berat/isipadu) $NaNO_3$ merupakan sumber inorganik nitrogen yang terbaik dan 2mM (isipadu/isipadu) sebagai ion logam. Dengan keadaan yang optimum ini, sebanyak 3199 U/ml inulinase telah dihasilkan. Walaubagaimanapun, didapati surfactant (Triton X-100) berperanan sebagai perencat terhadap penghasilan enzim oleh fungus ini. Aktiviti inulinase yang dibiarkan pada suhu 50$^o$C didapati stabil pada 91.75%, 89.30% dan 53.00% selama 30 min, 60 min and 120 min, masing-masing.*

*Sementara itu, penghasilan enzim invertase dapat ditingkatkan menggunakan nutrisi dan faktor fizikal yang berbeza. Medium yang mengandungi 10% (berat/isipadu) sukrosa, 1% (berat/isipadu) ekstrak yis, 1.5% (berat/isipadu) $NaNO_3$, 1mM (isipadu/isipadu) $Ca^{+2}$ dan 1% (isipadu/isipadu) Triton X-100 dikenal pasti sebagai medium yang optimum bagi penghasilan enzim invertase. Faktor-faktor fizikal yang optimum adalah pada suhu 30ºC, pH 6.0, saiz inokulum sebanyak 6% (v/v) dan kadar emparan 200 rpm. Pada keadaan yang optimum, enzim invertase dihasilkan sebanyak 3072 U/ml. Aktiviti invertase yang dibiarkan pada suhu 50$^o$C didapati stabil pada 90%, 49% dan 15% selama 60 min, 120 min dan 180 min, masing-masing.*

*Potensi kedua-dua medium bagi tujuan penghasilan inulinase dan invertase boleh dikaji dengan lebih menyeluruh di dalam fermentasi yang berskala besar bagi menghasilkan enzim yang lebih berkualiti dan ekonomi.*

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I certify that an Examination Committee has met on ……. to conduct the final examination of Mojdeh Dinarvand on her Master of Science thesis entitled "Optimization of medium and process parameters for the production of extracellular inulinase and invertase from *Aspergillus niger* ATCC 20611 " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree**)** Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are follows:

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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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## DECLARATION

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

**MOJDEH DINARVAND**
Date: 15 December 2011

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