
UNIVERSITI PUTRA MALAYSIA

# OPTIMIZATION OF CELLULASE PRODUCTION BY CHAETOMIUM GLOBOSUM STRAIN 414 USING OIL PALM EMPTY FRUIT BUNCH FIBRE AS SUBSTRATE

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By

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| m | Growth associated rate constant for cellulase production (U/g cell) |
| n | Non-growth associated rate constant for cellulase production (U/g cell.h) |
| $P_o$ | Initial cellulase concentration (U/l) |
| $P$ | Cellulase concentration (U/l) |
| t | Time of fermentation (h) |
| $X$ | Cell concentration (g/l) |
| $X_o$ | Initial cell concentration (g/l) |
| $X_m$ | Maximum cell concentration (g/l) |
| $\mu_x$ | Specific growth rate ($h^{-1}$) |

Abstract of the Dissertation Submitted to the Senate of Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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**May 1997**

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Faculty : Food Science and Biotechnology

The production of three major components of cellulase (FPase, CMCase and ß-glucosidase) by *Chaetomium globosum* strain 414 was studied in a shake flask experiment. The effects of physical and chemical treatments on the oil palm empty fruit bunch (OPEFB) fibre for subsequent use as substrate for cellulase production were investigated. The effects of different types and concentrations of nitrogen sources on cellulase production were also examined. The optimized medium composition obtained from the shake flask experiment was used for cellulase production in a 2 L stirred tank fermenter (impeller tip speed = 1.64 m/s) where the effect of different levels of dissolved oxygen tension (DOT) at a fixed agitation speed on cellulase production was investigated. The experimental data obtained from batch fermentations in a shake flask and the fermenter using the optimized medium were analysed to form the basis for a kinetic model of the process. The partially purified cellulase preparation from this fungus was used for the saccharification of OPEFB fibre. The effect of different methods of treatment of OPEFB fibre on the rate and degree of hydrolysis was investigated.

The use of 2-mm OPEFB fibre increased cellulase production about two fold compared to 10-mm fibre. Chemical treatment significantly increased the cellulose and reduced the lignin contents. Cellulase activities, obtained from fermentation using OPEFB fibre treated with 0.5% $HNO_3$ followed by autoclaving were about three times higher than those obtained in fermentation using pure celluloses. The cellulase of *C. globosum* strain 414 contained a high proportion of ß-glucosidase with the ratio of specific activity of ß-glucosidase to FPase of about 8. Peptone gave the highest cellulase production followed by yeast extract, urea, $KNO_3$ and $(NH_4)_2SO_4$. A good agreement between the calculated data and the experimental data for both cell growth and cellulase production were observed, suggesting that the proposed model based on logistic and Luedeking-Piret equations is sufficient to describe the growth of *C. globosum* strain 414 and cellulase production. The maximum activities of FPase, CMCase and ß-glucosidase obtained from fermentation with 50% DOT were 2.5, 59.5 and 12.8 U/ml, and these gave the overall productivities of 20.8, 495 and 53.3 U/L.h, respectively. Cellulase production in stirred tank fermenter were significantly higher than that obtained in shake flask. The yield and overall productivity of the saccharification of the autoclaved OPEFB fibre treated with 2% NaOH were 0.7600 g reducing sugar/g OPEFB and 0.0178 g reducing sugar/g cellulose.h, respectively.

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# PENGOPTIMUMAN PENGHASILAN SELULASE DARIPADA KULAT *CHAETOMIUM GLOBOSUM* STRAIN 414 MENGGUNAKAN SERABUT TANDAN KELAPA SAWIT KOSONG SEBAGAI SUBSTRAT

**Oleh**

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Penghasilan tiga komponen utama selulase (selobiohidrolase, endoglukanase dan ß-glukosidase) oleh kulat *Chaetomium globosum* strain 414 telah dikaji dalam percubaan kelalang bergoncang. Kesan rawatan fizikal dan kimia terhadap penyediaan serabut tandan kelapa sawit kosong (TKSK) untuk digunakan sebagai substrat bagi penghasilan selulase telah dikaji. Kesan lima jenis sumber nitrogen dengan kepekatan yang berbeza terhadap penghasilan selulase juga telah dikaji. Komposisi medium optimum untuk penghasilan selulase yang diperoleh dari percubaan kelalang bergoncang telah digunakan untuk penghasilan selulase dalam tangki fermenter berkacau bersaiz 2 L (had laju hujung impeler = 1.64 m/s) dengan mengkaji kesan paras oksigen terlarut (OT) yang berbeza-beza dengan kelajuan pengacau yang tetap, terhadap penghasilan selulase. Data kajian yang diperoleh dari fermentasi sesekumpul dalam kelalang bergoncang dan fermenter yang menggunakan medium optimum telah dianalisis bagi membentuk model kinetik asas untuk proses tersebut. Persediaan selulase separa tulen daripada kulat ini telah

digunakan untuk sakarifikasi serabut TKSK. Dalam percubaan sakarifikasi ke atas serabut TKSK, kesan rawatan yang berbeza ke atas serabut TKSK terhadap kadar dan darjah hidrolisis telah dikaji.

Penggunaan serabut TKSK 2-mm tanpa rawatan telah meningkatkan penghasilan selulase sebanyak dua kali ganda berbanding dengan serabut TKSK 10-mm. Rawatan kimia telah meningkatkan kandungan selulosa dan menurunkan kandungan lignin dengan bermakna. Penghasilan selulase dari fermentasi menggunakan serabut TKSK yang dirawat dengan $HNO_3$ (0.5%) dan diikuti dengan pengautoklafan adalah hampir tiga kali ganda lebih tinggi daripada penghasilan selulase dari fermentasi menggunakan selulosa tulen. Selulase dari kulat *C. globosum* strain 414 menggandungi paras ß-glukosidase yang tinggi dengan nisbah aktivi khusus ß-glukosidase : FPase sebanyak 8. Pepton telah memberi hasil selulase yang maksimum, diikuti dengan ekstrak yis, urea, $KNO_3$ dan $(NH_4)_2SO_4$. Satu keputusan yang hampir sama antara nilai yang dikira dengan data percubaan bagi pertumbuhan sel dan juga penghasilan ketiga-tiga komponen selulase telah dicerapkan, menunjukkan bahawa model yang dicadangkan berasaskan persamaan logistic dan Luedeking-Piret adalah mencukupi bagi menerangkan pertumbuhan sel *C. globosum* strain 414 dan penghasilan selulase. Fermentasi dengan OT ketepuan udara 50%, kepekatan maksima bagi aktiviti-aktiviti FPase, CMCase dan ß-glukosidase yang diperolehi adalah masing-masing, 2.5, 59.5 dan 12.8 U/ml, dan ini memberikan produktiviti keseluruhan masing-masing, 20.8. 495 dan 53.3 U/L.j. Penghasilan selulase dari fermenter adalah lebih tinggi jika dibandingkan dengan penghasilan selulase dari kultur kelalang bergoncang. Hasil dan produktiviti keseluruhan

daripada sakarifikasi serabut TKSK yang dirawat dengan 2% NaOH dan diautoklaf masing-masing adalah 0.76 g gula penurun/g TKSK dan 0.0178 g gula penurun/g selulosa.j.

# CHAPTER 1

# INTRODUCTION

Four major factors that lead to the renewed interest in lignocellulose research are (i) the increase in demand from the third world countries for modern materials, (ii) changes in the economics of competing materials, (iii) a renewed concern for our environment, and (iv) global interest in recycling. After a thousand years, lignocellulose is once again emerging as a resource of precursors for the production of polymeric materials, organic chemicals and fuel. Several researchers reported that much of our current demand for energy and feedstock could be met by exploitating the potential of lignocellulosic wastes (Brown and Gritzali, 1984; Lloyd, 1984).

The bioconversion process for converting lignocellulose into fermentable sugars involves three steps, namely, (i) pretreatment of the substrate, (ii) production of the cellulase enzymes and (iii) hydrolysis of the pretreated substrate. Much efforts in cellulase research have been directed toward cellulolytic microorganisms, as well as to the structure, function and synergistic activity of these enzymes. Cellulase is a mixture of enzymes that act in concert to hydrolyze crystalline cellulose to glucose. There are at least three major groups of cellulase involved in the hydrolysis of cellulose, namely, (i) endo-1,4-ß-D-glucanase, (ii) exo-1,4-ß-D-glucanase, and (iii) ß-glucosidase.

The application of industrial enzymes has grown substantially within the industrial and institutional sector. Worldwide consumption of industrial enzymes amounted to approximately US$1.3 billion in 1994 and about one third was accounted for by the US market (Hansen, 1995). The estimated worldwide enzyme consumption by product type is given in Table 1 and the global market for industrial enzyme is shown in Figure 1. It is found that protease is the largest amount of enzyme consumed by the food and detergent industries. Other types of enzymes are amylase, cellulase and lipase.

**Table 1**

Estimated Worldwide Enzyme Consumption by Product Type, 1990

| Enzyme | Consumption ($x10^6$ t) |
|---|---|
| Protease | 309 |
| Rennet (animal and microbial) | 74 |
| Glucose isomerase | 41 |
| Glucoamylase | 75 |
| Amylase | 112 |
| Cellulase | 55 |
| Lipase | 11 |
| Papain | 8 |
| Invertase | 8 |
| Pectinase | 7 |
| Other | 20 |
| Total | 720.00 |

Source: Nielsen et al. (1994)

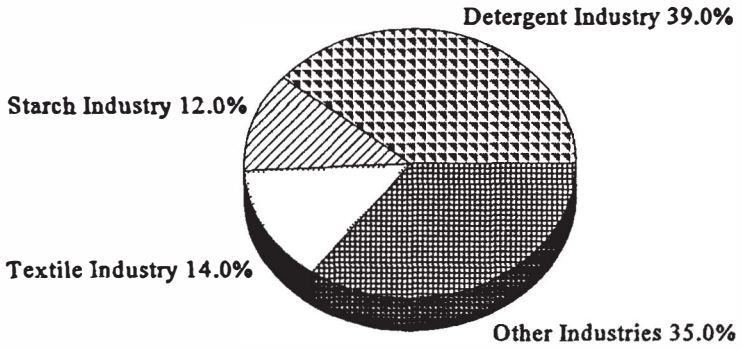


Figure1: Global Market For Industrial Enzymes in 1994
Source: Nielsen et al. (1994)

Although the major application of cellulase is for the conversion of lignocellulosic materials into fermentable sugars, cellulase also has many applications in various industries, such as, detergent, animal feed, paper, and fruit industries. The detergent mechanism using cellulase revealed by the changes in physico-chemical properties of cellulose has also been proposed by Murata et al. (1993). Cellulase cleaves ß-1,4-glycosidic bonds in cellulose and acts on natural textile fibres containing cotton. Cleaning by removal of particulate soils, softening and improving colour brightness are the three basic benefits obtained from cellulase (Nielsen et al., 1994). The direct addition of enzymes to animal feed can enhance the digestibility of feed components. A wide range of enzyme products, such as, Bio-Feed Beta, Bio-

Feed Plus, Energex, Bio-Feed Pro, Bio-Feed Alpha and Alpha-Gal has proven to be a successful tool in allowing feed compounders to develop feeds with inexpensive formulations (Hansen, 1995).

Another application of cellulase enzyme is in wastepaper recycling. Before wastepaper can be recycled, the ink has to be removed. Various cellulases have been tested at the USDA Forest Products Laboratory in Wisconsin, U.S. (Hansen, 1995). Their results showed that it is possible to de-ink wastepaper using only low doses of alkaline cellulase without adding sodium hydroxide. The enzymes are believed to remove the tiny strands of fluff protruding from the surface of the paper fibres. Another industrial application of cellulase is in the citrus industries. The citrus industry requires a large amount of water to wash off the essential oils from the peel of citrus fruits. The result is a thick emulsion. Citrozym CEO, which has specific enzyme activities of hemicellulases, arabanases, pectinases and cellulases, is highly suitable for the oil-water phase separation. This is a new application of cellulase and is already established in the citrus industries in Argentina, Brazil, Italy, Mexico, and the USA (Normann, 1993). The demand for cellulase has increased enormously with increase in industries related to its application.

Although *Trichoderma reesei* and its mutants produced high level of cellulase activities (Persson et al., 1991), the economic saccharification of raw materials has not yet been achieved because cellulases from this source has low specific activity, low thermostability and high sensitivity to product inhibition (Mandels, 1985). In addition, the ratio of ß-glucosidase to FPase for cellulase from *T. reesei* was very low (Ryu and