STUDIES ON THE EFFECT OF DIFFERENT BIOPROCESS PARAMETERS ON PECTINASE PRODUCTION BY *Aspergillus niger*

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To my beloved mother and father

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In the name of Allah, the Beneficent, the Merciful.

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ABSTRACT

Pectinase is heterogeneous group of enzymes that have a polysaccharides substrate and breaks down the pectin component, which found in the plant cell wall, into simple sugar and galacturonic acid. The breaks down of the pectin will cause the plant tissues to undergo some modification on its cell wall, or other activities such as maceration or cell lysis. The pectinases or usually known as pectinolytic enzymes were extensively used in food industries that involve degradation of plant materials to fasten the fruit juice extraction process. Among different biofactories of pectinases, filamentous fungi such as *Aspergillus niger* were the best known for the production and secretion of pectinase. Therefore, the objective of this research was to develop industrial culture medium and a cultivation strategy for the production and secretion of pectinases in a semi-industrial scale by *A. niger*. In this study, the effect of medium composition on the production and secretion of pectinase was studied through the classical method where the medium screenings were done initially to find the best medium for the production of pectinase. The optimized medium through classical method were pectin industrial (30 g L^{-1}), ammonium sulphate (3.33 g L^{-1}), di-potassium hydrogen phosphate (1 g L^{-1}), magnesium sulfate heptahydrate (0.05 g L^{-1}), potassium chloride (0.05 g L^{-1}) and iron sulphate heptahydrate (0.1 g L^{-1}). Following this step, medium optimization was carried out using statistical approach and the optimized medium were pectin industrial (32.22 g L^{-1}), ammonium sulphate (4.33 g L^{-1}), di-potassium hydrogen phosphate (1.36 g L^{-1}), magnesium sulphate heptahydrate (0.05 g L^{-1}), potassium chloride (0.05 g L^{-1}) and iron sulphate heptahydrate (0.1 g L^{-1}). Therefore, the result of pectinase production for optimized medium was 64.83 % higher compared to unoptimized medium. Next, the effect of processing parameters which was pH condition (controlled pH at 5.5 and uncontrolled pH) was studied in the batch fermentation. The pectinase production of controlled pH was 51.35 % higher compared to the uncontrolled pH. However, the selection of the best condition for fed-batch fermentation was uncontrolled pH due to the low costing during the cultivation. Finally, the fed-batch strategies for full media and monocorbon were studied and the best feeding strategies was from the monocarbon feeding due to the higher prectinase yield with 429.95 U mL^{-1} compared to the full media feeding with 138.27 U mL^{-1}. Thus, these all together lead to the development of industrial process for pectinase production in semi-industrial scale.

ABSTRAK

Pektinase adalah kumpulan heterogen enzim yang mempunyai substrat polisakarida dan bertanggungjawab untuk memecahkan komponen pektin, yang ditemui di dinding sel tumbuhan, ke dalam gula ringkas dan asid galacturonik . Pemecahan komponen pektin akan menyebabkan tisu tumbuhan menjalani beberapa pengubahsuaian pada dinding sel, atau aktiviti-aktiviti lain seperti kehabisan tenaga atau sel lysis. Enzim pektinase atau biasanya dikenali sebagai enzim pektinolitik telah digunakan dengan meluas dalam industri makanan yang melibatkan degradasi bahan tumbuhan untuk mengikat proses pengekstrakan jus buah-buahan. Antara pengilangan berbeza pektinase, kulat berfilamen seperti *Aspergillus niger* adalah yang terbaik dikenali untuk pengeluaran dan rembesan pektinase. Oleh itu, objektif kajian ini adalah untuk membangunkan penghidupan media didalam industri dan strategi menghidupkan *Aspergillus niger* untuk pengeluaran dan rembesan pektinase dalam skala semi- industri oleh *A. niger*. Dalam kajian ini, kesan komposisi medium kepada pengeluaran dan rembesan pektinase akan dikaji menggunakan kaedah klasik di mana pemilihan media telah dilakukan pada mulanya untuk mencari medium terbaik untuk pengeluaran pektinase. Medium yang optimum adalah melalui kaedah klasik terdiri daripada pektin perindustrian (30 g L^{-1}), ammonium sulfat (3.33 g L^{-1}), di-kalium hidrogen fosfat (1g L^{-1}), magnesium sulfat heptahydrate (0.05 g L^{-1}) , kalium klorida (0.05 g L^{-1}) dan besi sulfat heptahydrate (0.1 g L^{-1}). Berikutan langkah ini, pengoptimuman medium telah dijalankan dengan menggunakan pendekatan statistik dan medium yang telah dioptimumkan terdiri daripada pektin industri (32.22 g L^{-1}), ammonium sulfat (4.33 g L^{-1}), di-kalium hidrogen fosfat (1.36 g L^{-1}), magnesium sulfat heptahydrate (0.05 g L^{-1}), kalium klorida (0.05 g L^{-1}) dan magnesium sulfat heptahydrate (0.1 g L^{-1}). Oleh itu, hasil daripada pengeluaran pektinase daripada medium yang telah dioptimumkan adalah 64.83% lebih tinggi berbanding dengan media dioptimumkan melalui kaedah klasik. Seterusnya, kesan parameter pemprosesan seperti pH (pH dikawal pada 5.5 dan tidak terkawal) telah dikaji didalam fermentasi sesekelompok. Pengeluaran pektinase yang dikawal oleh pH adalah 51.35% lebih tinggi berbanding dengan pH yang tidak terkawal. Walau bagaimanapun, pemilihan keadaan yang terbaik untuk suapan sesekelompok fermentasi adalah pH yang tidak terkawal kerana kerana pengekosan yang rendah semasa penanaman. Akhir sekali, strategi makan-kumpulan untuk media penuh dan monokarbon dikaji dan strategi pemberian makanan terbaik adalah daripada makanan monokarbon yang disebabkan oleh hasil pektinase yang tinggi dengan 429.95 U mL^{-1} berbanding dengan media penuh makan dengan 138,27 U mL^{-1}. Oleh itu, ini semua bersama-sama membawa kepada pembangunan proses industri untuk pengeluaran pectinase dalam skala semi-industri.

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A. niger	-	*Aspergillus niger*
$Y_{P/x}$	-	Yield coefficient
$U_{enzyme}\, g_{cells}^{-1}$	-	Yield coefficient unit
$g\ L^{-1}$	-	Cell biomass unit
$U\ mL^{-1}$	-	Pectinase unit
μ	-	Specific growth rate
d_i	-	Impeller diameter
d_t	-	Tank diameter
CDW	-	Cell dry weight
$FeSO_4 \cdot 7H_2O$	-	Ferrous sulphate heptahydrate
HCl	-	Hydrochloric acid
$H_2PO_4^-$	-	Orthophosphate
K_2HPO_4	-	Di-potassium hydrogen phosphate
KNO_3	-	Potassium nitrate
KCl	-	Potassium chloride
$MgSO_4.7H_2O$	-	Magnesium sulphate heptahydrate
NaCl	-	Sodium chloride
$NaNO_3$	-	Sodium nitrate
$(NH_4)_2SO_4$	-	Ammonium sulphate

CHAPTER 1

INTRODUCTION

1.1 Introduction of Research

Nowadays, the pectinolytic enzyme by filamentous fungi from industrial food waste like orange peels was used in the food industry for juice and wine production and is extensively done in order to utilize the abundant waste and commercialize in industries (Mantovani *et al.*, 2005). As a result, pectinolytic enzyme becomes one of the upcoming enzymes of the commercial sector (Kashyap *et al.*, 2001).

Since 1968 until now, many studies done on the pectinolytic enzyme or pectinase enzyme for commercial and widely used in industrial processing either in fruit or vegetables (Solis-Pereyra *et al.*, 1993). Whereas, filamentous fungus is used for commercial enzyme production in food and beverage industries as genera of *Aspergillus* is granted as GRAS (generally regarded as safe) (Iwashita, 2002). Therefore, many studies are focusing on the increasing of the yield of production and secretion of pectinase from *Aspergillus niger* especially in bioprocessing parameter indirectly will help the industrial sector in establishing its productivity.

However, there are other application of pectinase enzyme instead of used in the food industry which are basically used as retting and degumming of fiber crops. Retting is a fermentation process where certain bacteria and fungi decompose the pectin of the bark and release the fiber. The treatment of pectic wastewater also used pectinase enzyme in their process in order to remove pectin substances from wastewater. Next, it is also used in paper making as it can depolymerise polymers of galacturonic acid and will lower the cationic demand of pectin solutions and will filtrate it from peroxide bleaching. Furthermore, oil extraction is basically used pectinase enzyme to extract the oil in aqueous process by liquefying the structural cell wall component. Whereas, in tea fermentation; it helps to improve the foam forming property of instant tea powders by destroying tea pectin (Kashyap *et al.,* 2001).

The advantages of the use of pectinase in the beverage industries include allowing the producer to diversify the type of product in term of its cloudy, clearer juice and concentrates. The enzymes also help to produce the juices and concentrate in a very stable and have a good taste. Interestingly, pectinase can help in reducing production cost in term of higher yield, less equipment and labour especially in a concentration process (Kashyap *et al.*, 2001).

1.2 Problem Statement

The optimization of production medium for pectinase production towards low cost and suitable media composition for industrial purpose was crucially important in order to meet the increasing demand of this enzyme. Therefore, in this study; a filamentous fungus of *Aspergillus niger* was used for the production and secretion of pectinase in a semi industrial scale. In order to optimize the production and secretion of pectinases by *A. niger*, the different bioprocess parameters were studied. The parameters were including the cultivation media at shake flasks level and bioprocessing condition (pH condition) in stirred tank 16-L bioreactor. Furthermore, the limitations studies on feeding strategist with different feeding solution in order to increase the yields of pectinase production in the semi-industrial scale 16-L bioreactor application. Hence, these all together will lead to the development of industrial process for pectinase production in semi-industrial scale.

1.3 Research Objective

The objective of the research was to develop industrial culture medium and a cultivation strategy for the production of pectinase in semi-industrial scale by *A. niger*.

1.4 Research Scope

To accomplish the objective, there are five research scopes were applied:

1) Medium screening and optimization for shake flask cultivation using classical method.
2) Medium optimization for shake flask cultivation using statistical method.
3) Comparison between classical media optimization method and statistical medium optimization method.
4) Batch cultivation of *A. niger* in a stirred tank 16-L bioreactor for high production of pectinase.
5) Fed-batch cultivation of *A. niger* in a stirred tank 16-L bioreactor for high production of pectinase.

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