PRODUCTION OF ITACONIC ACID FROM PALM OIL MILL EFFLUENT (POME) USING *Aspergillus terreus* NRRL 1960 IMMOBILIZED IN PVA-ALGINATE SULFATE BEADS

QISTINA BINTI AHMAD KAMAL

UNIVERSITI TEKNOLOGI MALAYSIA

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QISTINA BINTI AHMAD KAMAL

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This thesis is specially dedicated to my husband En. Ishak bin Abdul Ghani and my beautiful daughter Iffah Qaireena binti Ishak, my beloved parents, Tn. Haji Ahmad Kamal bin Abdullah @ Chow Wai Chee and Pn. Hajah Kamariah binti Md. Yusoff, my brother Faruq bin Ahmad Kamal, my dearest friends and my respective supervisor, Dr Nor Azimah binti Mohd Zain

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ABSTRACT

Palm Oil Industry is one of the biggest and rapidly growing industries in Malaysia. However, this industry produced large amount of waste known as palm oil mill effluent (POME) which contributes to the pollution of river. It consists of 95 to 96% (v/v) water, 0.6 to 0.7% (v/v) oil and 4 to 5% (w/v) total solids. POME contains high nutrient including metal traces and could promote the growth of bacteria in the water. Consequently, it contributes to oxygen depletion and endangered the aquatic organisms. In this study, 44% (v/v) of glycerol was used as main substrate and 51% (v/v) POME was used as co-substrate and supplement for the growth and itaconic acid production by *Aspergillus terreus* NRRL 1960. The production medium chosen produced the highest yield based on literature study. To improvise the amount of yield, the *Aspergillus terreus* spore was immobilized in PVA-alginate-sulfate beads and the fermentation was carried out for 6 days. Fermentation process was done for 0% (w/v) beads for control, 5% (w/v) and 10% (w/v) of beads containing *Aspergillus terreus* NRRL 1960 spores and 10% (v/v) of free cell of *Aspergillus terreus* NRRL 1960 that contains 2.071 X 10^8 spore/mL. The itaconic acid production was highest on the 3rd day of the fermentation. Thus this study shows that immobilization system increased the yield up to 3 folds when the itaconic acid production of 10% (w/v) beads of 9.656 g/L been compared to production of free cells of 3.43 g/L on the 3rd day of fermentation. Besides producing the itaconic acid, immobilized *A. terreus* also could decolorized the POME. The decolorization process was highest on the 4th day of fermentation, which resulting 42.10% for the 5% beads, 44.15% for the 10% beads and the free cell produced was 65.30%. The reason of high decolorization in free cell is caused by the high growth indicated by biomass produced up to 17.46 g/L compared to only 11.31 g/L and 12.963 g/L for both 5% (w/v) and 10% (w/v) respectively. It can be conclude that as the fungus growth and producing mycelium, the colour causing compounds were bound to mycelium via adsorption that involves a combination of active and passive transport mechanism.

ABSTRAK

Industri Minyak Sawit adalah salah satu industri yang semakin meningkat yang terbesar dan pesat di Malaysia. Walau bagaimanapun, industri ini menghasilkan sejumlah besar sisa yang dikenali sebagai kilang minyak sawit (POME) yang menyumbang kepada pencemaran sungai. Ia terdiri daripada 95 kepada 96% (v/v) air, 0,6-0,7% (v/v) minyak dan 4 hingga 5% (w/v) jumlah pepejal. POME mengandungi nutrien yang tinggi termasuk unsur logam dan boleh menggalakkan pertumbuhan bakteria di dalam air. Oleh itu, ia menyumbang kepada kekurangan oksigen dan mengancam organisma akuatik. Dalam kajian ini, 44% (v/v) gliserol telah digunakan sebagai substrat utama dan 51% (v/v) POME telah digunakan sebagai substrat-bersama dan makanan tambahan untuk pertumbuhan dan pengeluaran asid itakonik oleh *Aspergillus terreus* NRRL 1960. Medium pengeluaran yang dipilih telah menghasilkan produk tertinggi berdasarkan kajian literatur. Untuk menambah jumlah hasil, spora *Aspergillus terreus* telah disekatgerak di dalam manik PVA-alginat-sulfat dan penapaian telah dijalankan selama 6 hari. Proses penapaian telah dilakukan untuk 0% (w/v) manik untuk kawalan, 5% (w/v) dan 10% (w/v) manik mengandungi spora *Aspergillus terreus* NRRL 1960 dan 10% (v/v) sel bebas *Aspergillus terreus* NRRL 1960 yang berkepekatan 2,071 X 108 spora/ mL. Pengeluaran asid itakonik adalah tertinggi pada hari ke-3 penapaian. Oleh itu, kajian ini menunjukkan bahawa sistem sekatgerak telah meningkatkan hasil sehingga 3 kali ganda apabila manik pengeluaran asid itakonik sebanyak 10% (w / v) daripada 9,656 g/L berbanding dengan pengeluaran sel-sel bebas sebanyak 3.43 g / L pada hari ke-3 penapaian. Selain menghasilkan asid itakonik, sel-sel bebas dari *A. terreus* juga boleh menyahwarnakan POME. Proses penyahwarnaan adalah tertinggi pada hari ke-4 penapaian, yang menyahwarnakan 42.10% untuk manik 5% (w/v) manik, 44.15% untuk manik 10% (w/v) dan sel bebas menyahwarnakan sehingga 65.30%. Diantara sebab penyahwarnaan yang tinggi dalam sel bebas adalah disebabkan oleh pertumbuhan yang tinggi ditunjukkan oleh biomass yang dihasilkan iaitu sebanyak 17,46 g/L berbanding dengan hanya 11.31 g/L dan 12.963 g/L bagi kedua-dua manik 5% (w/v) dan 10% (w/v). Ia juga boleh disimpulkan bahawa semasa proses pertumbuhan kulat dan penghasilan miselium, miselium melalui proses penjerapan telah menyerap komponen yang menyebabkan warna dengan proses pengikatan yang melibatkan gabungan mekanisme pengangkutan aktif dan pasif di dalam miselium tersebut.

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LIST OF ABBREVIATIONS

ABS	-	Absorbance
ADMI	-	American Dye Manufacturing Unit
BOD	-	Biological Oxygen Demand
COD	-	Chemical Oxygen Demand
et al.	-	and others
g	-	gram
L	-	Litre
μ	-	micro
μl	-	microlitre
mg	-	Milligram
mL	-	Milliliter
nm	-	Nanometer
pH	-	Hydrogen ion concentration
POME	-	Palm Oil Mill Effluent
Ppm	-	Parts per million
Rpm	-	Rotation per minute
v/v	-	Volume over volume
w/v	-	Weight over volume
%	-	Percent
°C	-	Degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Research Background

The palm oil industry in Malaysia has grown over the time these years and Malaysia has become one of the largest producers of palm oil and its by product in the world. It is estimated that more than 3.79 millions hectares of land has been used for the oil palm planting area in the year 2003, which means that one third of the total cultivated area in Malaysia has been used for this purpose (Yusoff and Hansen, 2007). The palm oil industry has contributes to the country income from the foreign exchange and increases the standard of living for the Malaysian people (Wu *et al.,* 2007). The industry also provides a source of income to poor families that attached to the government schemes and individual holder. In addition, it also provides the job opportunities to the agricultural worker. (Ma *et al.,* 1993; Khalid and Wan Mustafa, 1992).

According to Ahmad *et al.,* (2003) the process of palm oil milling can be divided to two categories that involved the wet and dry process. The wet process is the standard process that has been used commonly to extract the palm oil. It is estimated for each tonne of crude oil that been produced, the amount of water that been used for the extracting process is between 5 to 7.5 tonnes of water. From this amount, nearly half of

it will become the palm oil mill effluent (POME).

The raw POME is very unique since the oil extraction process does not require any chemical usage. Thus POME doesn't have any toxic material. POME has a very high concentration of minerals, proteins, carbohydrates, lipids and nitrogenous compounds. (Habib *et al.,* 1997). According to Wu *et al.,* (2007). POME has been considered as biphasic product. Means that even though it can be considered as a waste, it also can be used as a raw material in other process. There were technologies that being developed to convert POME into value added product. This will definitely gives a positive impact in solving environmental problems besides giving value added products.

One of the example is itaconic acid, it is a value product that can be produce from POME. This unsaturated dicarboxylic acid, also known as methylene-succinic acid are produced by the filamentous fungi *Aspergillus terreus* and *Aspergillus itaconicus*, Corma *et al.,* (2007). It uses carbohydrates from conventional substrate such as sucrose and glucose (Kautola, 1990; Reddy and Singh, 2002 and Willke and Vorlop 2001). The synthesis of itaconic acid from sucrose and glucose has proven to be uneconomical because of high substrate cost and relatively low yield, Berg and Hetzel (1978); (Blatt, 1943) and (Chiusoli, 1962) thus it cannot compete with fermentation processes.

One of way to increase the yield is using the immobilization method, in this case is fungal immobilization. Immobilization of fungal is an entrapment process of the fungal cells certain matrix. The fungal cells are enclosed or entrapped in a certain region for the retention of the catalytic activities of the fungus. The immobilization is intended for the repeated and continuous usage of the cell (Chibata, 1978). Commonly used for immobilization matrix is natural polymer such as alginates, chitosan, chitin and cellulose derivatives. Studies by Baldrian, (2003) and Valdman *et al.,* (2001) proved that fungal cell immobilization in these types of polymer can enhance the performance of these

fungal cell and the capability of adsorption in the biosorbent system for heavy metal ion.

1.2 Problem Statement

The conventional fermentation of Itaconic acid uses expensive conventional substrates such as glucose and sucrose (Kautola, 1990) and the yield is relatively low. Berg and Hetzel (1978); (Blatt, 1943) and (Chiusoli, 1962) This makes the fermentation of Itaconic acid less economical. By exploring new source of substrates and its supplement, will make the fermentation more profitable. In this study the substrate that will be used is Palm Oil Mill Effluent (POME). It will make it even more desirable since the process will treat waste and by the same time produce valuable byproduct. Treating the effluent itself will need large amount of land and money and this will decrease the profit of the company. If the company can utilize all the waste it produced, it will bring more profit to the company. Hence they will not hesitate to spend some amount for the treatment of Palm Oil Mill Effluent (POME) to the standard that will pass the DOE (Department of Environment) 2009 standard limit. In this study, the organism that will be use is *Aspergillus terreus* NRRL 1960. This fungus will produce itaconic acid and to emphasize the production of the itaconic acid, the immobilization or the fungal entrapment will be use. This is because the immobilization will lower the growth rate. Higher growth rate will make the itaconic production relatively lower

1.3 Research Objectives

The research objectives for this study are:

1 To immobilize *Aspergillus terreus* in PVA-alginate sulfate beads

2 To characterize the PVA-alginate sulfate beads and POME

3 To compare itaconic acid production from POME using immobilized *Aspergillus terreus* in PVA-alginate sulfate beads and free cell.

1.4 Scope of Research

In this study, the immobilization of the *Aspergillus terreus* NRRL 1960 in PVA-alginate sulfate beads is performed to produce Itaconic acid from the main substrate, which is glycerol and the co-substrate that has been used is Palm Oil Mill Effluent (POME) which at the same time also act as the supplement to the fungal growth. This experiment included the Itaconic acid fermentation using the free cell of the *Aspergillus terreus* NRRL 1960 to compare the production of the immobilized cells and the free cells. This study focus on the fungal growth in Optical Density (OD), the biomass formation (dry cell weight), pH of the medium, decolorization of the POME (ADMI), the itaconic acid production and the glycerol (substrate) concentration utilization of the fungus.

1.5 Research Significant

The raw POME has a high amount of organic compound and metal traces that could be useful for the fermentation process to produce value added product while treatment process happens. The itaconic acid fermentation from glucose and sucrose has a problem of high substrate cost and a very low yield. If utilization of the raw POME to produce itaconic acid is possible, it will reduce the cost to produce useful product of itaconic acid and thus lower the price. In addition, the immobilization process is predicted to increase the yield of this product. Immobilization will lower the growth rate and relatively increase the itaconic acid synthesis.

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