

**UNIVERSITI TEKNOLOGI MARA**

**CHARACTERIZATION AND  
PERFORMANCE EVALUATION OF  
ENZYMES SUPPORTING MATERIAL  
DEVELOPED FROM SG. SAYONG  
CLAY AND WASTE CLAY FOR  
TAPIOCA SACCHARIFICATION  
PROCESS**

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Thesis submitted in fulfillment  
of the requirements for the degree of  
**Master of Science**


**Faculty of Plantation and Agrotechnology**

September 2015

## AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my work, unless otherwise indicated or acknowledge as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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## ABSTRACT

Immobilization of the enzymes on inorganic clay mineral is very useful in practical applications to overcome limitations of immobilized enzyme such as low long term stability and poor reusability of enzyme. Inorganic clay mineral proved to be an excellent supporting material for immobilization of enzymes by encapsulation technique. In this research, two types of clay material with different characteristics were evaluated for the enzyme immobilization process. The clay that used in this research was obtained from natural and palm oil processing waste clay sources. Characterization study has been done for both clay material using BET Surface Area, X-Ray Fluorescence (XRF), Fourier Transform Infrared (FTIR), X-Ray Diffraction (XRD) and Field Emission Scanning Electron Microscopy (FESEM) analysis. The enzymes were encapsulated on the alg-clay beads where the alginate was used as a medium to be mixed with clay to encapsulate enzymes in the form of beads. The enzymes were successfully encapsulated within alg-clay beads with enzyme loading efficiency was more than 90%. The result also revealed that the highest immobilization yield was obtained at 2% (w/v) of the clay concentration for both types of clay material. From the reusability study, the results proved that immobilization of the enzymes onto the clay supporting material showed a high stability and availability for multiple uses as it could be reused up to seven cycles. The relative activity of enzyme immobilized on treated clay retained about 50% activity, meanwhile, the immobilized enzyme on untreated and waste clay retained 23% and 32% of activity respectively. Overall, the use of clay from natural and waste sources as enzymes supporting materials was successfully done in this research. Different sources and characteristic of clay could result in the different performance of tapioca saccharification process. This method could have significant economic impact on the industrial of bioconversion of starch into glucose as it has low cost of the materials and its ability to retained the enzyme for many times and can be a good potential of enzymes supporting material in immobilization technology.

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# **CHAPTER ONE**

## **INTRODUCTION**

### **1.1 BACKGROUND OF STUDY**

In the last decades, the applications of enzymes in large scale industries such as chemical and pharmaceutical industry have become increasingly as well as in many other innovating areas of technology such as sensing applications, biotechnology and organic synthesis [1]. The use of enzymes offers a distinct advantage due to their high catalytic efficiency, substrate specificity and selectivity under mild reaction condition with low energy requirements [2]. However, there are also limitation in using enzymes in large scale production such as high cost and lack of long-term operational stability. It is difficult to separate them from the reaction system, which limit the recovery of the enzyme and may lead to contamination of the final product [3].

Alternative way to overcome this problem is by immobilization of the enzyme. Immobilized enzymes are often more stable and more easy to recover than enzymes in free solution as well as can improve the catalytic activity [4, 5]. It also could be reused for multiple times on the same reaction and it prevents contamination of the substrate with enzyme/protein or other compounds, which would then lower the purification costs [6, 7]. There are several immobilization methods available including physical adsorption, entrapment, covalent binding, encapsulation etc. Among of all this methods, encapsulation is the cheapest and the simplest immobilization method, and involves minimal modification of the native structure of the enzymes [8].

Natural polymer is usually used as a structured carrier for encapsulation especially polysaccharides group such as carrageenan, chitosan and starch, but alginate is the most common [9]. Alginate has a good biocompatibility, low in cost, easy availability, non-toxicity and offers easy separation [10]. However, there are few problems of using alginate beads such as distorted size, gel degradation, severe mass transfer limitations, poor mechanical strength and large pore size which can cause high enzymes leakage from the beads [11]. These defects may influence the stability and viability of the encapsulated