# Laccase Enzyme Immobilization on Ferromagnetic Support Material for Oil Palm Empty Fruit Bunches (OPEFB) Pretreatment

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# Laccase Enzyme Immobilization on Ferromagnetic Support Material for Oil Palm Empty Fruit Bunches (OPEFB) Pretreatment

**Shah Samiur Rashid[1,*], Hu Shian Huan[1], Mohd Hasbi Bin Ab. Rahim[1], Md. Belal Hossain Sikder[1,2] and Ahmed Abdulsalam H Sasi[1]**

[1]Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Gambang, Kuantan 26300, Pahang Darul Makmur, Malaysia
[2]Department of Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Bangladesh

*Corresponding author: samiur@ump.edu.my

**Abstract.** Rapid development of oil palm industry causes a lot of environmental issues due to the large number of waste products such as OPEFB. OPEFB is rich in lignocellulose which can be hydrolyzed to product sustainable biofuel, an alternative sources of energy. The production of biofuel from lignocellulose waste requires pretreatment process to enhance efficiency of saccharification of the sugar content of the lignocellulose waste. Enzymatic pretreatment technique is more desirable as this technique does not generate greenhouse gas or any inhibitor that can interferes the saccharification process. Enzyme immobilization technique also involved to increase the stability of the enzyme and promote reutilization of the enzyme. In this study, laccase enzyme was immobilized on ferromagnetic particles to promote easy separation of the immobilized laccase enzymes from the reaction media. The result shows that laccase enzyme immobilized on the nickel particles which encapsulated with silica. 0.235 IU/g of immobilized laccase degraded 58 % of the lignin content in 0.5 g of OPEFB after 24 hours. Pretreated OPEFB increase the efficiency of saccharification by 161% in compare with the untreated OPEFB.

## 1. Introduction

Climate change due to global warming is considered one of the major negative impacts of the modern industrial civilization that believed to be contributed through emission of anthropogenic greenhouse gas (GHG) because of excessive consumption of fossil fuel as the main source of energy [1]. Therefore, intense urge has been perceived to find an alternative sustainable renewable energy. Natural biomass is considered as an alternative source of renewable energy due to its higher content of convertible sugar polymer, cellulose. There are a lot of biomasses such as waste paper, different fruit peels (banana, orange and pomegranate etc.), sugarcane bagasse, different natural fibers (jute, kenaf, OPEFB etc.) [2] have been explored as they are abundant in nature. However, due to many reasons there is no sustainable technology for alternative renewable energy production yet to be reported.

More than 17 million tons of OPEFB is being produced yearly in Malaysia alone [3]. As OPEFB contains more than 50 % (w/w) of convertible sugar polymers (cellulose), it can be a promising biomass for the production of alternative energy carrier such as bioethanol/biobutanol. [4]. But the sugar will not be readily available before breaking down the recalcitrant lignin layers of OPEFB.

Therefore, it is necessary to undergo pretreatment process before hydrolysis of the OPEFB to get the fermentable sugars. Rashid et al [5], Ishmael et al [6] and Amani et al [7] already optimized the physico-chemical, enzymatic and biological processes for the pretreatment of OPEFB. Therefore, journey is going on to look for a sustainable process condition for pretreatment of OPEFB as none of these processes are proven to be either environmentally sustainable.
Enzymes catalyzes many biochemical and chemical reactions and are universally present in plants and animals. Other than the different industrial applications it is highly potential in waste management especially for solid wastes treatment and waste water purification [8]. However, all these desirable characteristics of enzymes and their widespread industrial applications are often obstructing by their lack of long-term operational stability, shelf life and by their recovery & reusability. Enzyme immobilization is one of the strategies to overcome these problems [9, 10]. Therefore, this study is aimed to provide an alternative process for pretreatment of biomasses. Thus in the current research, feasibility study of lignin hydrolytic enzyme immobilization on ferromagnetic particle was conducted to enhance the fermentable sugar recovery rate from OPEFB.

## 2. Materials and Methods

### *2.1. Ferromagnetic particles preparation and Encapsulation*

Nickel and iron were collected from commercial sources. Then the size of the nickel and iron were determined by using machine Zetasizer Nano S90. The size of the nickel and iron was 2.42 μm, 1.64 μm respectively and laccase enzyme was immobilized on those nanoparticles. Ferromagnetic particles were encapsulated with TEOS using ethanol-water solution at room temperature.

### *2.2. Characterization of encapsulated ferromagnetic particles*

The ferromagnetic particles and encapsulated ferromagnetic particles were characterized using XRD and FTIR. The samples were characterized for the X-ray diffraction patterns, using a Cu K $\alpha$ radiation ($\lambda$ = 0.154 nm) at 100 mA and 50 Kv. To perform the FTIR, Perkin Elmer spectrum 100 model was used in the transmission mode with wavenumber range of 4000-700 $cm^{-1}$.

### *2.3 Immobilization of laccase enzyme on activated ferromagnetic particles and Characterization*

The laccase enzyme was brought from sigm-aldrich (Novozym 51003). This laccase enzyme was immobilized on the ferromagentic nanoparticles using the method described by Wang et al [11] with slightly modification. The total protein content of the immobilized enzymes on the ferromagnetic particles was estimated using the Bradford protein assay. The laccase enzyme activity was estimated by the method described by Shah et al [4]. The immobilized laccase enzyme on the ferromagnetic particles was characterized using FTIR.

### *2.4 Efficacy of immobilized laccase enzyme on OPEFB*

The OPEFB was pretreated according to the method described by Shah et al [4] with slightly modification and the degree of pretreatment was assessed using weight loss method. Morphology of the pretreated OPEFB was determined by Scanning Electron Microscope (SEM). The reducing sugar content was determined by Dinitrosalicylic acid (DNS) method of Miller.

## 3. Results and Discusion

The biomass of OPEFB was collected from Dominion Square Sdn. Bhd, Gambang, Pahang, Malaysia and cellulose, hemicellulose, lignin and ash content was found 43%, 39%, 17% and 1% respectively. Several researchers also reported the similar composition of OPEFB [6, 12].

### *3.1 Encapsulation of Ferromagnetic Particles*

Ferromagnetic particles (Fe/Ni) encapsulation was carried out using mixture of TEOS and NaOH/$NH_4OH$. It was observed that white hollow particles solid were produced by mixture TEOS and $NH_4OH$ while a dense thin white layer was produced by mixture of TEOS and NaOH. Yamashita et al. [13] also reported that no silica particles were formed when using NaOH concentration of 1 to 3M.

Formation of white hollow particles indicates a successful silica encapsulation. Therefore, 5% of NH4OH was selected for encapsulation process, in this study.

*3.2 Characterization of Encapsulated Ferromagnetic Particles by XRD*

Presence of silica and iron can be detected through the peak at 2-theta values of 44.97 º and 65.24 º (Figure 1 (a)). Besides, the presence of hematite or iron (III) oxide ($Fe_2O_3$) contributes to the peak at 2-theta values of 22.86 º. Presence of silica can be detected through the peak at 2-theta values of 37.52 º, 43.44 º while nickel contributes to the peaks at 2-theta values of 44.66 º and 51.99 º figure 1 (b). Therefore, it can be assumed from this XRD analysis that iron and nickel particles were coated/ encapsulated by silica.

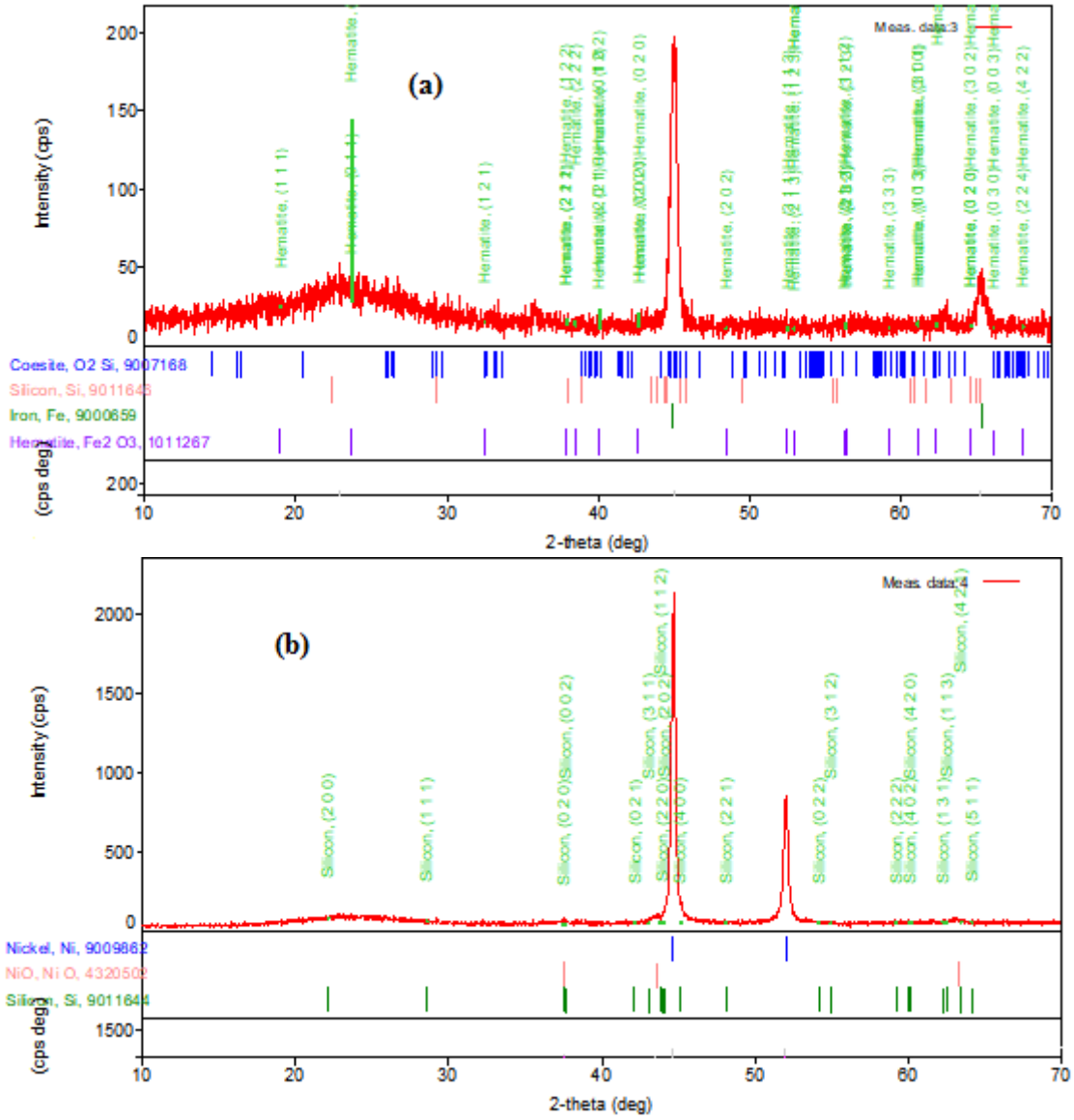


**Figure 1.** XRD analysis of encapsulated (a) iron particles and (b) nickel particles

*3.3 Characterization of Activated Encapsulated Ferromagnetic Particles by FTIR*

After functionalization and activation of the encapsulated ferromagnetic particles (Fe/Ni), it was characterized by FTIR and the results were presented by figure 2 (a-b). Silica coating on the iron particles can be recognized by the peak at 1644 $cm^{-1}$ corresponds to Si-OH group and the peak at 1091 $cm^{-1}$ corresponds to Si-O-Si group (data not shown). Cristiano et al. [14] also reported that silica

coated magnetic nanoparticles gave a peak at 1616 $cm^{-1}$ corresponds to Si-OH groups and another peak at 1037 $cm^{-1}$ corresponds to Si-O-Si group. FTIR spectrum of immobilized laccase on the activated iron particles (Figure 2 (a)) shows a large increase in the intensity for the peak at around 3444 $cm^{-1}$ which corresponds to -OH group and -NH group contributed by laccase enzyme. Also, there is an increase of intensity for the peak at around 1600 $cm^{-1}$ which corresponds to carbonyl group (C=O) of carboxylic acid. El-Batal et al. [15] also reported the FTIR spectrum for laccase has a peak at 3089 $cm^{-1}$ corresponding to -OH and -NH functional groups and another peak at 1635$cm^{-1}$ which corresponds to carbonyl group. Hence, it could be assumed that laccase enzymes immobilized on the activated iron particles.

Figure 2 (b) represents the FTIR spectrum of activated nickel particles and Si-Ni particle with immobilized laccase enzyme. The characteristic peak at 1634 cm-1 corresponds to Si-OH group and the peak at 1098 cm-1 corresponds to Si-O-Si group. Also, C=N group is present which contributes to the peak at 1400 cm-1. FTIR spectrum of immobilized laccase on the activated nickel particles (Figure 2 (b)) shows that there is a large increase in the intensity for the peak at around 3433 cm-1 which corresponds to -OH group and -NH group contributed by laccase enzyme. Also, there is an increase of the intensity for the peak at around 1598 cm-1 which corresponds to carbonyl group (C=O) of carboxylic acid. Hence, it could be assumed that laccase enzymes immobilized on the activated Si-Ni particles.

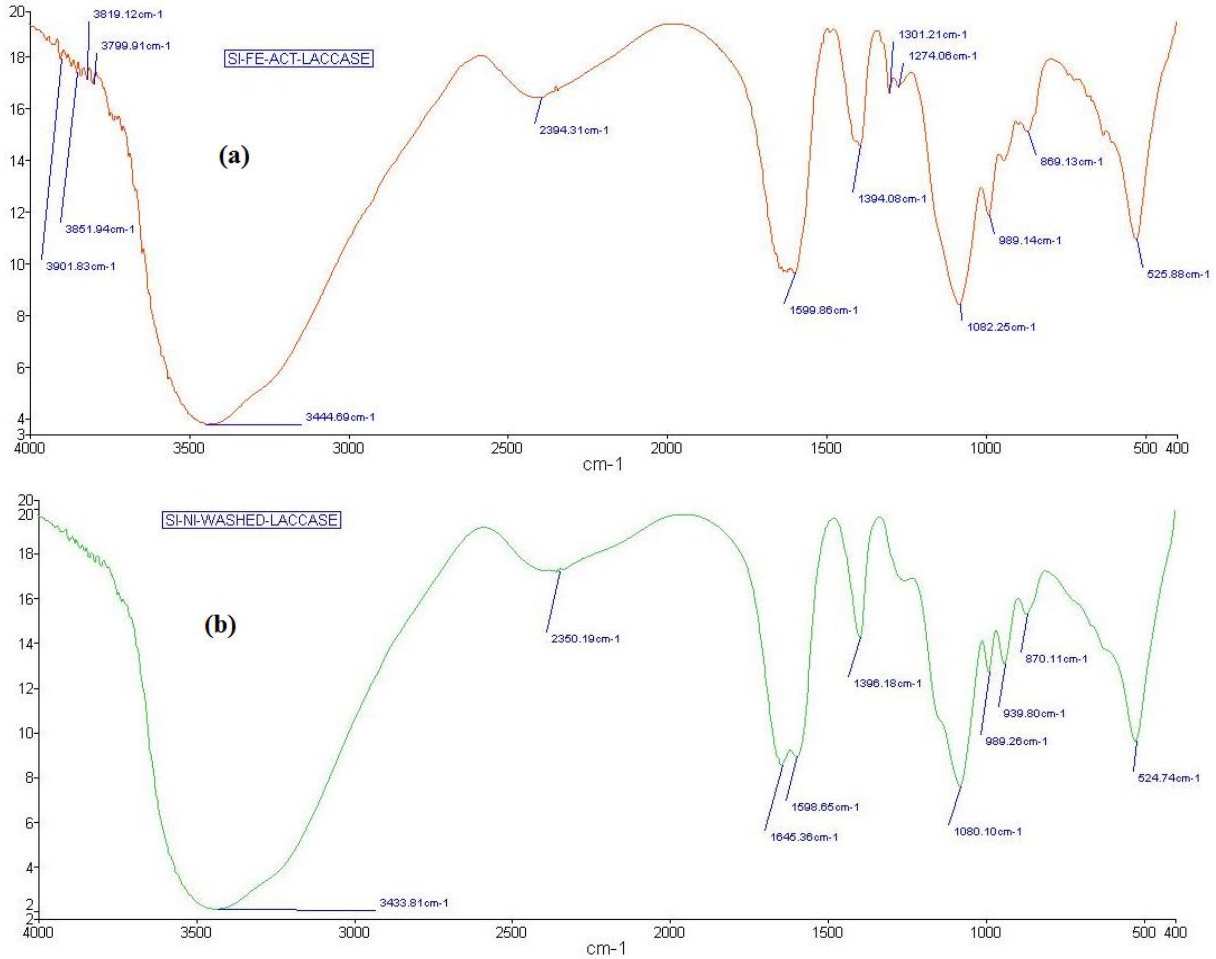


**Figure 2** FTIR spectrum of activated (a) Si-Fe and (b) Si-Ni particles with immobilized laccase enzyme

Saccharification of the OPEFB pretreated with the immobilized laccase enzyme was carried out and the efficacy was evaluated based on the released reducing sugar after saccharification. It was found

from the current study that saccharification efficiency was doubled when the OPEFB was pretreated with the immobilized laccase enzyme in compare with the un-pretreated OPEFB.

## 4. Conclusion

Based on the current investigation it could be concluded that pretreatment of OPEFB with laccase enzyme immobilized on the Si-Fe/Ni particle can enhance the saccharification process of OPEFB sharply.

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