Characterization of Waste Clay from Palm Oil Mill Effluent and Enzyme Immobilization Study for Cassava Saccharification Process

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Waste clay recovered from palm oil mill effluent (POME) was characterized and used as an enzyme-supporting material for the cassava saccharification process. The clay was treated by the Soxhlet extraction method to remove the residual oil and then characterized using a BET surface area analyser, XRF, XRD, FTIR, TGA, and FESEM. The chemical analysis showed that the sample had a high amount of CaO (93%) with a minor content of SiO_2 (1.378%) and Al_2O_3 (0.707%), with a surface area of 1.15 m^2/g. The XRD analysis revealed the major mineral presence to be calcite, as confirmed by FESEM analysis. The FTIR results also attested to the presence of a calcite phase and carbonate groups. To study the performance of the waste clay for enzyme immobilization application, the recovered waste clay was further used as an enzyme supporting material for enzyme immobilization in the cassava saccharification process. Results showed that the enzymes were successfully encapsulated and gave the highest immobilization yield of 70% with 2% clay concentration. In addition, the encapsulated enzymes also enhanced the reusability, where the enzyme retained 32% of its activity after seven cycles of saccharification processing.

Keywords: Waste clay; BET surface area; XRD; Supporting material; Enzyme immobilization

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INTRODUCTION

Enzymes (biocatalysts) have been a remarkable discovery in the field of bioprocess technology and have a range of biotechnological applications. Currently, there has been an increasing interest to enhance enzyme productivity and to increase the shelf life due to its unstable nature and sensitivity under certain conditions. In addition, using purified enzymes and discarding them after each use are costly and not economical; therefore, attempts have been made to immobilize enzymes in the bioprocessing field, especially in food and pharmaceutical technologies.

There are several advantages to immobilized enzymes, one being that they are easier to handle, which helps to prevent contamination of the substrate and enzymes in a reaction mixture. In addition, immobilized enzymes also facilitate the efficient recovery and reuse of costly enzymes with longer half-lives and less degradation (Sheldon 2007; Edama *et al.* 2014a).

Encapsulation is a process by which the enzymes are packaged or enclosed physically or chemically within a matrix or semi-permeable membrane layer (Abd. Rahim *et al.* 2013b). Through this method, the enzymes are restricted by the membrane walls (usually in a form of capsules), but free floating within the core space. The membrane itself is semi-permeable, allowing for free flow of substrates and nutrients, yet keeping the enzymes inside (Gorecka and Jastrzebska 2011); therefore, the selection of supporting material is one of the important factors that should be considered. There are several types of supporting material such as carrageenan, chitosan, and starch, but alginate is most commonly used because it has good biocompatibility, low cost, easy availability, and ease of separation; however, it has several disadvantages such as low mechanical strength and large pore size, which can cause high enzyme leakage from the alginate beads (Zhou *et al.* 2010; Duarte *et al.* 2013).

To increase the stability of the enzymes within the beads, alginate can be co-supported with an inorganic material such as clay (Edama *et al.* 2014c). Clay is a favorable material due to its porous structure and high mechanical strength. It is also chemically inert, thermally stable, and has a low cost of production. Several studies have been conducted using fresh clay for enzymes immobilization, as exhibited in Table 1.

Table 1. Recent Studies using Fresh Clay from Different Sources for Enzyme Immobilization

Types of Clay	Origin	Immobilization Method	References
Sayong kaolinite clay	Sungai Sayong, Perak, Malaysia	Encapsulation	Abd. Rahim *et al.* 2013c
Kankara kaolinite clay	Kankara village, Nigeria	Adsorption	Ajayi *et al.* 2012
Natural kaolin	Kuala Kangsar, Perak, Malaysia	Adsorption	Abdul Rahman *et al.* 2005

One of the sources of waste clay that can be obtained abundantly is palm oil mill effluent (POME). The POME is effluent generated from the processing of a type of oil palm fruits. The final POME is the combination of different sources within palm oil mill such as condensate from the sterilization process, sludge from the clarification process, and clay bath from the hydrocyclone process.

Clay bath is a technique to separate the kernel or the shell from the oil palm fruits. The disadvantage of this technique is that it results in high waste clay generation. Direct discharge of waste clay into the current POME treatment pond may lead to serious siltation problems and cause frequent dislodging of the ponds which may increase costs.

In the present work, waste clay was used as a raw material for the enzyme immobilization study for cassava saccharification process. Saccharification refers to the process of breaking a complex carbohydrate such as starch into small units of glucose (monosaccharide) by using enzyme. The clay (enzyme supporting material) was characterized using Brunauer Emmett Teller (BET) surface area analysis, X-Ray fluorescence (XRF), X-ray diffractometry (XRD), Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), and field emission scanning electron microscopy (FESEM). Then, the performance of the clay as an enzyme support material for glucose production was evaluated.

EXPERIMENTAL

Materials

The clay was obtained from clay bath palm oil mill effluent at the FELDA Serting Hilir Palm Oil Mill, Negeri Sembilan, Malaysia. All reagents and chemicals used were of analytical grade and obtained either from Sigma-Aldrich (USA) or Merck (USA).

Methods

Waste clay treatment

Soxhlet extraction using hexane as the solvent was conducted to remove the residual oil from the waste clay. First, 50 mL of fresh semi-dried waste clay was poured in a beaker and dried in an oven for 24 h. The dried clay was weighed and placed into an extraction thimble and then inserted into a 500-mL reflux flask. The extraction was carried out using 300 mL of hexane as a solvent at 150 °C. The extraction was terminated after 8 h, and the remaining hexane was removed by drying in an oven at 103 °C (Edama *et al.* 2014b).

Characterization techniques

Characterization of the clay was carried out to investigate the physicochemical characteristics of the material using various analytical methods. The specific surface area was measured and characterized using a Quantachrome Autosorb-1 analyzer (USA) through nitrogen adsorption at 77 K with a relative pressure (P/P_o) range of 0.035 to 0.35. The chemical analysis of the clay sample was carried out by a Philips X-Ray fluorescence spectrometer (Model PW-2400, USA) and the IR spectra were obtained using a Perkin Elmer Fourier transform infrared spectrometer (Model 2000, USA).

The mineralogical composition was identified by a Rigaku Ultima III X-ray diffractometer (Japan) with a 2θ angle ranging from 15° to 75°. To obtain the differential thermal analysis (DTA)/thermogravimetric analysis (TGA) curves, a Mettler Toledo TGA/SDTA851 differential calorimeter apparatus (Switzerland) was used. The sample was heated in a silica crucible at a constant heating rate of 20 °C/min operating in a stream of N_2 atmosphere with a flowrate of 40 mL/min from 35 to 1000 °C. The surface morphology of the clay sample was characterized by a Zeiss Supra 40VP FESEM (Germany) operating at an accelerating voltage to 5 kV. The sample was initially dusted on a double sided carbon tape that was placed on a metal stub and coated with a layer of gold to minimize the charging effects.

Encapsulation of enzymes

Exactly 2.5 g of waste clay (WC) was dissolved in 100 mL of a pH 6 buffer and stirred for 1 h at room temperature. Then, 2.5 g of sodium alginate was added to the clay solution and stirred for 1 h. Afterward, 2 mL of glycerol was added to the alginate-clay solution. A 2% w/v alginate-clay solution was produced by adding 3 mL of enzyme solution (1 mL of each enzyme: glucoamylase, cellulase, and alpha-amylase) into 12 mL of alginate-clay solution for a total mixture volume of 15 mL. The mixture was taken into a syringe, and beads were formed by dropping the mixture into a 0.2 M calcium chloride ($CaCl_2$) solution. The formed beads were allowed to harden for 3 h at room temperature, dried using filter paper, and washed several times with distilled water. The filtered calcium chloride solution was collected for loading efficiency determination, which was calculated by the following equation,

$$\text{Loading efficiency (\%)} = \left(\frac{C_i V_i - C_f V_f}{C_i V_i}\right) \times 100\% \quad (1)$$

where C_i is the initial protein concentration, V_i is the initial volume of enzyme solution, C_f is the protein concentration in total filtrate, and V_f is the total volume of the filtrate. The immobilization yield was defined as the yield for enzyme which was immobilized in the calcium alginate beads and expressed by the following equation,

$$\text{Immobilization yield (\%)} = \left(\frac{a_{imm}}{a_{free}}\right) \times 100\% \quad (2)$$

where a_{imm} is specific activity of immobilized enzyme (mmol/(min mg protein)) and a_{free} is the specific activity of free enzyme (mmol/(min mg protein)).

RESULTS AND DISCUSSION

Characterization Studies

BET surface area and chemical analysis

The data in Table 2 show the BET surface area and chemical compositions of the sample. The data reveal that the waste clay had relatively low surface area (1.15 m^2/g) compared to common porous materials such as activated clay (*e.g.*, kaolinite, montmorillonite), as reported in previous studies (Muthuvel *et al.* 2012; Edama *et al.* 2014c). The chemical composition of the clay sample is summarized in Table 2. The clay consists of SiO_2 (1.378%), Al_2O_3 (0.707%), MgO (2.269%), and CaO (93.048%). The high content of CaO in the sample indicates a high amount of carbonate. This result is comparable with a study by Skels (2011), which indicated that the sample known as quicklime had a high content of CaO (95%) but low SiO_2 (1.6%) and Al_2O_3 (0.25%) content, as well as some impurities.

Table 2. Chemical Composition and Specific Surface Area Analysis

Oxide	Composition (%)	Oxide	Composition (%)
SiO_2	1.378	K_2O	0.241
Al_2O_3	0.707	SO_3	0.127
Fe_2O_3	1.778	MgO	2.269
CaO	93.048	P_2O_5	0.164
Na_2O	0.053	**S_{BET}**	**1.15 m^2/g**
TiO_2	0.037		

X-ray diffraction

Figure 1 shows the XRD pattern of waste clay with a major peak at 29°, indicating that calcite is a major phase of the waste clay. It is also observed that most of the peaks correspond to h k l of 012, 104, 110, 113, 202, 024, 018, 116, 122, 1010, 208, 0012, 0210, and 1112, which demonstrated that the phase of the waste clay sample is calcite. The results were proven by comparing the h k l of the waste clay with the standard diffraction spectrum of calcite powder as reported in a previous study (Hoque *et al.* 2013). The strong and sharp peaks showed that the waste clay was substantially crystalline.

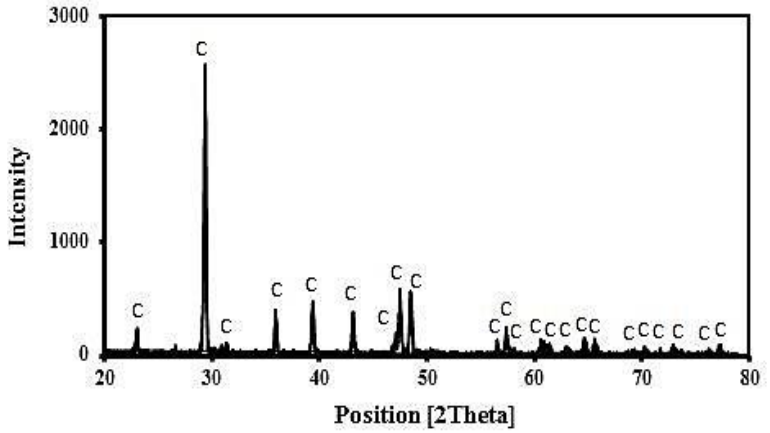

Fig. 1. X-ray diffraction (XRD) pattern of waste clay. C=calcite

Fourier transform infrared (FTIR) analysis

The usefulness of qualitative analysis such as FTIR from the characteristic frequencies provides information to identify the chemical compounds in the sample. The FTIR spectra of the sample were carried out in the range of 500 to 4000 cm^{-1}, as shown in Fig. 2. The weak band at 1794 cm^{-1} corresponds to the C=O bonds from carbonate. Meanwhile, the peaks at 1396 cm^{-1} and 873 cm^{-1} represent the characteristic of the C-O stretching and bending modes of calcium carbonate, respectively (Witoon 2011). The sharp band observed in Fig. 2 at 712 cm^{-1} represents the Ca-O bonds.

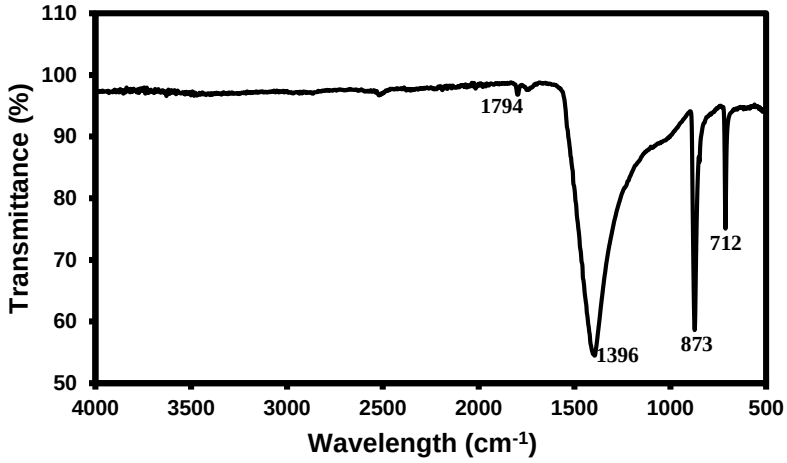

Fig. 2. FTIR spectra of waste clay

Thermal behavior

The thermal analysis results of the calcite-based clay sample are exhibited in Fig. 3. The TGA pattern of the sample shows one distinct stage of weight loss, where the total weight loss of about 34% occurred from 674 to 819 °C, which is in the thermal degradation temperature range for carbonates. The results are comparable with previous studies, as summarized in Table 3. The major weight loss at 785 °C corresponding to the 34% weight loss was due to the change of the $CaCO_3$ phase to the CaO phase (Witoon 2011). As the sample weight remained constant after 880 °C, the temperature of 900 °C was then suitable for use as the calcination temperature to ensure a complete conversion to CaO.

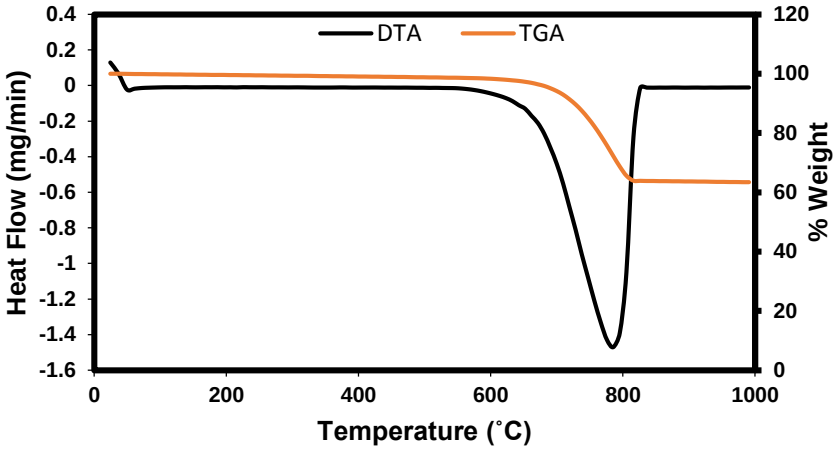

Fig. 3. Differential thermal and thermogravimetric analysis of waste clay sample

Table 3. Thermal Degradation Study of Waste Clay using TGA

	This Study	Witoon 2011	Trindade *et al.* 2009
Range of degradation temperature (°C)	674-819	680-850	700-850
Peak of degradation temperature (°C)	785	830	700
Weight loss (%)	34	42	28
Final degradation temperature (°C)	880	850	900

Morphology

The morphology of the waste clay was observed from the FESEM micrograph to visualize its textural and surface structure. Figure 4 clearly shows that the waste clay had an agglomerate structure and did not have a well-defined pore structure. This result is similar to the structure of calcite-based powder as reported by Tsai (2013). This finding is consistent with the results of the BET surface area. Furthermore, the image shown in Fig. 4b depicts the morphology and crystal structure of cube-like or hexagonal calcite crystals, which were confirmed by the XRD results. This cube-like crystal makes calcite stronger more and more stable (Hoque *et al.* 2013). Thus, due to this stability, this material is suitable for use as a supporting material for enzyme immobilization application.

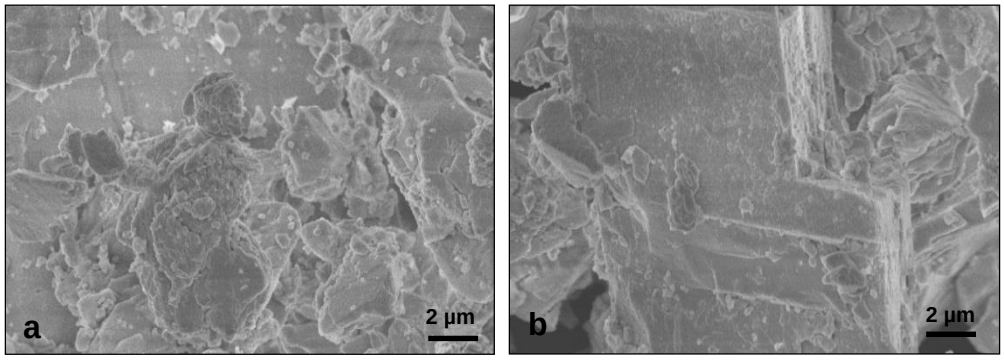

Fig. 4. FESEM micrograph of waste clay at 5000x magnification at two different spots

Enzyme Immobilization

Enzyme loading and immobilization yield

The effect of the waste clay concentration on the immobilization yield and enzyme loading for the tapioca starch saccharification process is shown in Fig. 5. It was observed that the highest yield was at 2% clay concentration with 70% activity. In general, the enzyme loadings were more than 90% for all clay concentrations and increased progressively as the clay concentration increased. The increase in loading efficiency of enzymes with high clay concentrations may be due to the formation of stronger and tighter beads; however, the immobilization yield slightly decreased as the clay concentration increased. The decrease in activity of the encapsulated enzymes in the clay beads is attributed to the diffusion limitation of the substrate to react with the enzymes (Gülay and Şanlı-Mohamed 2012). The enzymes encapsulated alginate-clay beads characterization have been thoroughly studied and published elsewhere (Edama *et al*. 2014b).

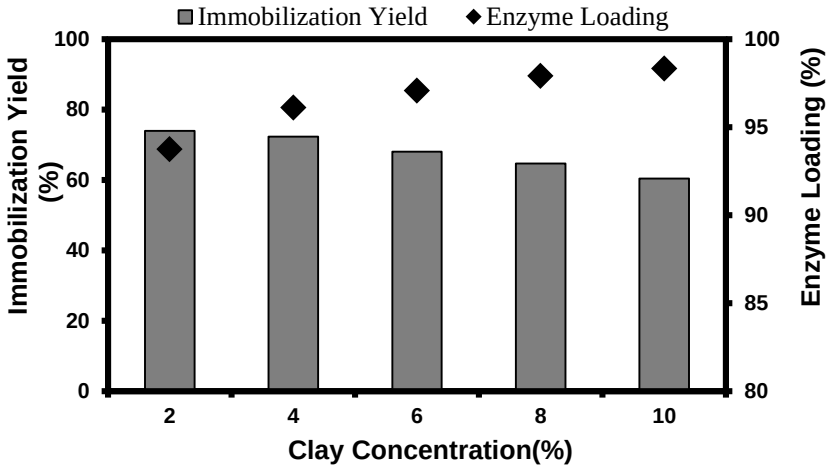

Fig. 5. Enzyme loading and immobilization yield at different concentrations of clay

Reusability

The reusability of the enzymes is the main advantage of the enzyme immobilization system, as it is a key factor for its cost-effective industrial use (Talekar and Chavare 2012). The reusability study of encapsulated enzymes was determined by using recovered enzymes from different cycles as proposed in a previous study (Abd Rahim *et al.* 2013a). The residual activity of each cycle was calculated by taking the enzyme activity of the first cycle as 100%. Figure 6 indicates the reusability patterns of the immobilized enzymes for the alginate beads and alginate-clay beads after several cycles. The encapsulated enzymes retained 65%, 56%, and 32% of their activities after the 5^{th}, 6^{th}, and 7^{th} cycles, respectively. The decrease in the enzyme activities may be due to enzyme leakage resulting from the soft surface of the alginate gel. Other reasons for the leakage may be damage to the beads during the repeated use and washing steps at the end of each cycle (Gülay and Şanlı-Mohamed 2012).

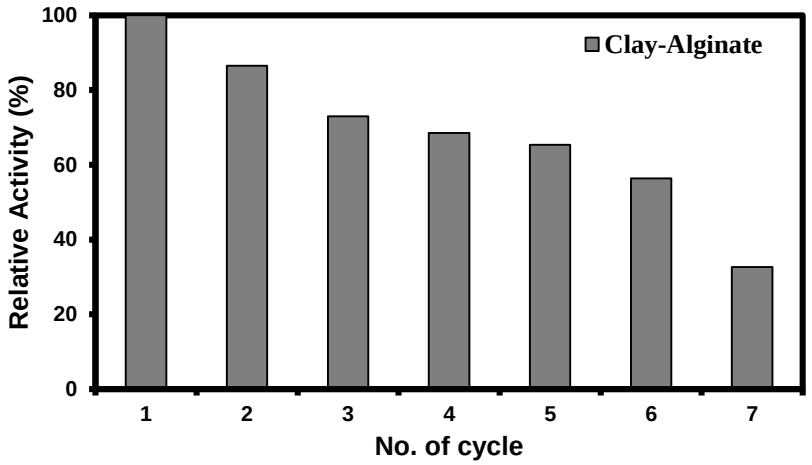

Fig. 6. Reusability of alginate-clay beads in tapioca slurry hydrolysis process

CONCLUSIONS

1. The major constituent of the palm oil mill effluent (POME) waste clay sample was CaO (93.048%), with minimal amounts of Fe_2O_3 (1.778%), SiO_2 (1.378%), and Al_2O_3 (0.707%), as confirmed by XRD and TGA analysis. The waste clay sample also had a relatively low surface area of 1.15 m^2/g.
2. The FESEM analysis showed that the waste clay recovered from POME had a strong and stable structure, which made it suitable as an enzyme supporting material.
3. The waste clay recovered from POME was successfully used as a supporting material in an enzyme immobilization study for glucose production. It was found that the highest yield obtained had a 2% waste clay concentration, and the encapsulated enzyme was able to be reused after up to seven cycles.

ACKNOWLEDGMENTS

This work was financially supported by University Teknologi MARA (UiTM) Cluster Grant (600-RMI/DANA 5/3/CG (16/2012) and Ministry of Higher Education (MOHE) Malaysia, Long-term Research Grant Scheme (600-RMI/LRGS 5/3 1/2012).

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Article submitted: August 4, 2014; Peer review completed: September 27, 2014; Revised version received and accepted: October 13, 2014; Published: October 20, 2014.