

OPTIMISATION OF ENZYMATIC SLUDGE PALM OIL RECOVERY FROM PALM OIL MILL EFFLUENT USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Various operational variables affecting the extraction of sludge palm oil (SPO) from palm oil mill effluent (POME) by enzymatic treatment were investigated. The enzyme used in the study was cellulase from *Trichoderma reesei*, commercially known as Celluclast 1.5L FG (Novozymes). A software application of the Central Composite Face-centred Design (CCFD) known as Response Surface Methodology (RSM) was used to study the effects of independent variables generated by the Design Expert Software, namely incubation time, X_1 (12.73-147.27 hr), enzyme concentration, X_2 (0.08%-0.92%) and rotation speed, X_3 (65.91-234.09 rpm) on the oil recovered from POME. In this study, a second-order polynomial regression model was used to interpret the experimental data with a coefficient of determination (R^2) value of 0.852. From the RSM-generated model, the optimum conditions for extraction of oil from POME were identified to be at an enzyme concentration value of 0.25% in 81.46 hr reaction time, with a rotation speed of 111.05 rpm. Predicted oil yield was 93.56% while the experimental oil yield was 81.95% as revealed by the One Sample T-Test of confirmatory studies.

Keywords: palm oil mill effluent, sludge palm oil, enzymatic treatment, cellulase enzyme, oil extraction.

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INTRODUCTION

The palm oil industry has grown exponentially in the past few decades, and concurrently the technology to process the fresh fruit bunch (FFB) has also developed in tandem. A more efficient oil extraction rate (OER) from FFB has been identified as one of the criteria to increase the yield of palm

oil (Vijaya *et al.*, 2013; Noorshamsiana *et al.*, 2013). This is crucially needed to increase the earning of the palm oil industry. Even when all machinery related to processing are at peak performances, oil losses are inevitable in the mechanical extraction process. Oil losses occur at various stages of milling, particularly in the sludge water, empty fruit bunch (EFB) and mesocarp fibre that contains minor components in residual oil which can be further extracted for production of nutraceutical products (Rusnani *et al.*, 2013; 2012). Steriliser condensate and sludge water streams are mixed in the sludge pit for oil recovery before they are released into the effluent treatment plant. The mixed sludge is referred to as mixed raw effluent (MRE) or known as palm oil mill effluent (POME).

Recently, attention has been focused on organic effluents which cause environmental pollution.

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As such, many technologies have been introduced to provide more efficient effluent treatment to meet the standards imposed by the Department of Environment (DOE). The rapid development of the palm oil industry in Malaysia over the years has produced a large amount of POME. During palm oil extraction on average, about 1.5 t of POME are produced for every tonne of FFB processed (Mahzad *et al.*, 2009; Ahmad *et al.*, 2003). Some researchers have reported that about 600-700 kg of POME are generated for every 1000 kg of processed FFB (Muzaffar *et al.*, 2007; Hassan *et al.*, 1997; 2002). The palm oil mills in Malaysia processed a total of 92.9 million tonnes of FFB to produce about 18.9 million tonnes of crude palm oil (CPO) in 2011 (Noorshamsiana *et al.*, 2013). By assuming that the ratio of FFB processed to POME generated is 1 to 1.5, the total POME generated in 2011 was about 139.35 million tonnes. This much POME would contain almost 1.67 million tonnes of oil. The recovery of the oil losses from the POME will help to enhance the OER as well as to contribute towards additional income for the palm oil mills (Noorshamsiana *et al.*, 2010).

The POME consists mainly of cellulosic material, fat, oil and grease (Parveen *et al.*, 2010; Agamuthu, 1995). The POME also contains substantial quantities of solids namely suspended solids and dissolved solids, commonly known as palm oil mill sludge (POMS). The amount of POMS generated is increasing every year, proportional to the POME generated arising from increased FFB processing (Jin Suk, 2009). The POMS has a higher nutrient value than the slurry (Zakaria *et al.*, 1994). It has a high amount of moisture content with a pH of about 8-9 and enriched with nutrients. The POMS emits unpleasant odour and as a result is considered as a source of surface and ground pollution. Therefore, industries are looking for cost-effective sustainable technology for the disposal of this sludge (Sivasothy and Ramachandran, 2013)

The POME contains about 1.0% - 1.2% residual oil that could not be extracted because of the limitation imposed when the oil is extracted by mechanical means. This residual oil will eventually end up in the effluent treatment ponds (Susanto, 1981). The oil recovered from POME is termed sludge palm oil (SPO), and is considered low quality sludge oil (Ainie *et al.*, 1995) mainly because of its high free fatty acid content which usually exceeds 10%. The SPO consists of free oil (1.0%) and encapsulated oil (0.2%). Encapsulated oil is found within the plant cell inside the fibre, and is linked to proteins and a wide variety of carbohydrates such as cellulose, hemicellulose, pectin and starch. The extracted SPO can be used for non-edible applications such as in the production of laundry soap, fatty acids, candles and other items. The recovery of oil from sludge is one of the options to fully extract the oil from milling

processes before the sludge is sent to the effluent treatment plant. In a conventional palm oil mill, oil recovery from sludge is done by using the physical process of centrifugation. It is estimated that about 1% (w/v) of oil is still contained in the centrifuged sludge (Ho *et al.*, 1992). Another alternative method to extract oil from sludge is by Soxhlet extraction that uses hexane or petroleum spirit as a solvent but the sludge needs to be dried first to remove the water content in the sludge (Chow, 1996). However, to facilitate the extraction of the encapsulated oil in the cell, it is necessary to degrade the cell walls to increase the secretion of the oil. The use of enzymes as a biocatalyst for cell wall degradation will cater for this purpose, thus facilitating the release of oil globules. Cellulase enzyme, namely Celluclast (0.5% w/v) has been studied and was reported to be capable of obtaining the oil from the sludge (Ho *et al.*, 1992). The use of enzymes as a biological means to recover oil from the sludge is believed to be more benign to the environment.

Enzymes are natural protein molecules that act as highly efficient catalysts in biochemical reactions. They can be recycled and bio-degraded. In this study, the cellulase enzyme was used to extract the entrapped oil within the cellulosic cell wall of the suspended solids in the POME. The enzyme reacted selectively to hydrolyse the cellulose into glucose, cellobiose or cello oligosaccharides.

In order to optimise the experimental conditions, the Response Surface Methodology (RSM) was used in this study. The RSM is a combination of mathematical and statistical techniques for developing, improving and optimising the processes and it was used to evaluate the relative significance of various process parameters even in the presence of complex interactions.

This article reports the combined effect of three process parameters; incubation time (hr), enzyme concentration (%) and impeller speed (rpm) on enzyme-assisted extraction of SPO from POME using the Central Composite Face-centred Design (CCFD) in the RSM. Results of this study will provide valuable information on the interrelation of oil yield and operating variables of the enzymatic extraction process.

MATERIALS AND METHODS

Enzyme

The cellulase enzyme, supplied in 100 ml bottle, used in this study was produced from *Trichoderma reesei* by Novo Nordisk, Denmark and commercially named Celluclast[®] 1.5 litre FG, has a declared activity of 700 EGU g⁻¹ (EGU = Endo-glucanase Units). Celluclast[®] 1.5 litre FG is a brown liquid with a density of approximately 1.2 g ml⁻¹. For practical

applications, the optimum temperature is about 50°C -60°C and pH of 4.5-6.0. This enzyme catalyses the breakdown of cellulose into glucose, cellobiose and higher glucose polymers, and has a pronounced viscosity-reducing effect on soluble cellulosic substrates (Grujić, 1998).

Chemicals

Citric acid is a weak organic acid with the chemical name 2-hydroxypropane-1, 2, 3-tricarboxylic acid (C₆H₈O₇). It is a white crystalline powder at room temperature and it can exist either in an anhydrous (water-free) form or as a monohydrate.

Trisodium citrate occurs in two forms of which are the crystal form (dihydrate) called trisodium citrate (crystal) and the anhydrous form called trisodium citrate (anhydrous). Trisodium citrate has a molecular weight of 258.07 (C₆H₅Na₃O₇). Trisodium citrate occurs as colourless crystals or as a white powder and it is odourless and having cool and salty taste. Both chemicals are to be used as buffer agents for the enzymatic reactions.

Batch Experiments

This study was performed in a batch scale and the experiment parameters were performed as proposed by the design expert software. The experiment parameters focused on the effects of incubation time, enzyme concentration and impeller speed on the enzymatic hydrolysis of cellulose. For the purpose of optimising the amount of enzyme concentration, the experiment was performed by adding different dosages of enzyme concentration in the reactor which consisted of 2 litres samples of POME sludge.

Samples of fresh POME sludge were taken from the MPOB Palm Oil Mill Technology Centre in Nilai, Negeri Sembilan, Malaysia. The experiments were carried out by varying the incubation time, hr (X_A), enzyme concentration, % (X_B) and impeller speed, rpm (X_C). All the experiments were carried out at 55°C with the added buffer solution (44 ml of 0.1M citric acid + 56 ml of 0.05M Tri-sodium citrate) at pH 4.8. For the calculation of oil recovery, the sludge sample was put into a drying oven at 60°C overnight and then weighed to determine the moisture content. The residual oil was then extracted from the cooled sample for the determination of oil content. The percentage of oil recovery at anytime (t) was calculated as:

$$\text{Oil recovery (\%)} = \frac{\text{Total oil} - \text{average oil at time (t)}}{\text{Total oil}} \times 100 \quad (1)$$

where total oil is an average of fresh sludge oil before treatment.

Experimental Design

RSM is a statistical technique used for multiple regression analysis of quantitative data obtained from statistically designed experiments by solving the multivariable equations simultaneously (Shreela *et al.*, 2009). The graphical representation of these equations is known as response surfaces, which determine the mutual interaction between the test variable and their subsequent effect on the response (Shreela *et al.*, 2009; Montgomery, 1997).

The main objective of RSM is to determine the optimum operational conditions of the process. The RSM usually contains three steps: (a) design and experiments, (b) response surface modeling through regression, and (c) optimisation. The sequential process and procedure of the RSM is given in the flow chart (Figure 1).

CCFD is one type of RSM consisting of three parts namely, factorial, centre-point and axial points. A second-order model can be constructed efficiently with central composite designs (CCD) (Montgomery, 1997).

The incubation time (X_A, hr), enzyme concentration (X_B, %) and impeller speed, (X_C, rpm) were chosen as the independent variables using 5³ factorial experimental design with six star points (a=1) and six replicates at centre-points, according to CCFD. The ranges and the levels of the variables investigated in the research are given in Table 1. The percentage of oil recovery (Y) was taken as the response of the design experiments. The quadratic equation model for predicting the optimal point was expressed according to Equation (2).

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k \beta_{ij} x_i x_j + \epsilon, i = 1, 2, 3, \dots, k \quad (2)$$

where x_i is a dimensionless value of an independent variable, X_i the real value of an independent variable, x_i is the real value of the independent variable at the centre-point and Δ x_j is the step change (Veera *et al.*, 2006).

Three factors were studied and their low and high levels are given in Table 1. Twenty experiments were conducted according to the scheme mentioned in Table 2. Design Expert Version 8.0.4 (Stat Ease, USA) was used for regression and graphical analysis of the data obtained.

RESULTS AND DISCUSSION

Enzymatic Hydrolysis

There are three steps of enzymatic hydrolysis of cellulose which are: the adsorption of cellulase enzymes onto the surface of the cellulose, the biodegradation of cellulose to fermentable sugars, and the desorption of cellulose.

TABLE 1. INDEPENDENT VARIABLES IN THE EXPERIMENTAL PLAN

Levels	Factor 1	Factor 2	Factor 3
	Incubation time (hr), X_A	Enzyme concentration (%), X_B	Impeller speed (rpm), X_C
-1.682 (min)	12.73	0.08	65.91
-1 (low)	40	0.25	100
0 (mid)	80	0.50	150
+1 (high)	120	0.75	200
+1.682 (max)	147.27	0.92	234.09

TABLE 2. THE CENTRAL COMPOSITE FACE-CENTRED DESIGN'S EXPERIMENTAL DESIGN MATRIX EMPLOYED FOR THREE INDEPENDENT VARIABLES (actual values given in *Table 1*)

Standard order	X_A (hr)	X_B (%)	X_C (rpm)	Y (% oil recovery)
1	-1	-1	-1	95.8023
2	1	-1	-1	92.0786
3	-1	1	-1	0.9943
4	1	1	-1	5.5298
5	-1	-1	1	21.9713
6	1	-1	1	45.0934
7	-1	1	1	83.958
8	1	1	1	85.5146
9	-1.682	0	0	35.5345
10	1.682	0	0	40.7465
11	0	-1.682	0	39.3963
12	0	1.682	0	27.4635
13	0	0	-1.682	10.8456
14	0	0	1.682	21.7288
15	0	0	0	96.7314
16	0	0	0	98.8958
17	0	0	0	72.5118
18	0	0	0	82.3101
19	0	0	0	80.2341
20	0	0	0	98.8737

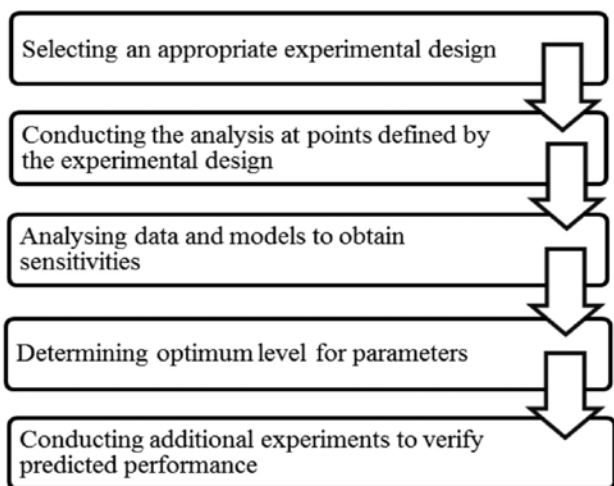


Figure 1. Block diagram showing sequential process of conducting response surface methodology (RSM).

The application of the enzymatic hydrolysis treatment leads to a slightly higher fraction of easily extractable oil because the enzyme action on the cells causes additional breakage of the cell wall structure. The breakage will increase the permeability of the oil through the cell wall. Hence, the enzymatic hydrolysis pre-treatment will enhance the yield of the extracted oil (Sanisah, 2008).

Figure 2 shows the microscopic evaluation of the sludge with and without enzymatic treatment. Figure 2a shows that the entrapped oil is visible inside the sludge cell wall. With the enzymatic reaction, it was observed that the oil globules were released due to the degradation of the sludge cell wall (Figure 2b). This observation is in agreement with previous studies which reported that the enzyme system can be used to degrade the insoluble cell wall components and thus releasing the oil globules in aqueous extraction (Dominguez *et al.*, 1996; Rosenthal *et al.*, 1996; Sharma *et al.*, 2002).

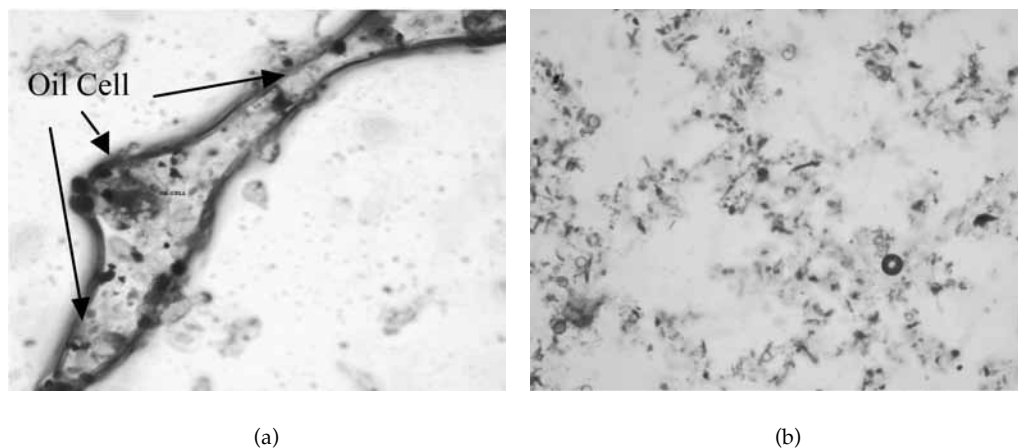


Figure 2. Microscopic comparison between (a) fresh sludge and (b) sludge after enzymatic treatment (10X magnification).

Response Surface Methodology (RSM)

The RSM via CCFD analysis provides important information regarding the optimum level of each variable along with its interactions with other variables and their effects on extraction of SPO from POME using enzymatic treatment. The most important physical factors that affect the enzymatic treatment; which are the incubation time (hr), enzyme concentration (%) and impeller speed (rpm) were determined using the CCFD. The results for the percentage of oil recovery, *Y* (response) were measured according to design matrix and the measured responses are listed in Table 2.

Using the results of the experiments, the following second order polynomial equation as a function of incubation time, hr (X_A), enzyme concentration, % (X_B), and speed, rpm (X_C) were obtained (in terms of coded factors):

$$Y = 87.35 + 2.51 X_A - 7.25 X_B + 4.43 X_C - 1.66 X_A X_B + 2.98 X_A X_C - 35.47 X_B X_C - 11.78 X_A^2 - 13.44 X_B^2 - 19.50 X_C^2 \quad (3)$$

The coefficients of the regression model (Equation 3) which contains three linear, three quadratics and three interactions where X_A is $X_{\text{Incubation time}}$, X_B is $X_{\text{Enzyme concentration}}$ and X_C is $X_{\text{Impeller Speed}}$.

Analysis of variance (ANOVA) is a statistical technique to test the statistical significance of the ratio of mean square variation due to regression and mean square residual error as shown in Table 3 (second-order equation).

The ANOVA table indicates that the equation adequately represents the actual relationship between the response which is the percentage recovered oil and the significant variables. The associate p-value is used to estimate whether *F* is large enough to indicate statistical significance. Values of 'Prob > *F*' less than 0.05 indicates that

TABLE 3. ANALYSIS OF VARIANCE (ANOVA) FOR QUADRATIC MODEL FOR PERCENTAGE RECOVERY OF OIL

Source	Sum of squares	df	Mean square	F value	Probability>F
Model	19 775.69	9	2 197.3	6.38	0.0039
Residual	3 443.95	10	344.39	-	-
Lack of fit	2 798.59	5	559.72	4.34	0.0666
Pure error	645.36	5	129.07	-	-
Total	23 219.64	19	-	-	-

the model terms are significant. The probability of less than 0.05 indicates that the model terms are significant at 95% of probability level.

Effect of Incubation Time (hr) and Enzyme Concentration (%)

Figure 3 shows variation of the percentage of oil recovery with respect to incubation time and enzyme concentration keeping the impeller speed constant. Experiments were carried out by varying incubation time from 12.73 to 147.27 hr and enzyme concentration from 0.08%-0.92%. It was observed that with the increase in incubation time to 81.46 hr and enzyme concentration at 0.25%, the percentage of oil recovery reached maximum at 93.56%. At a short period of incubation time and lower enzyme concentration, the amount of oil obtained was more than that of at a higher enzyme concentration. As indicated in Figure 3, the increase in oil recovery caused by the increase of incubation time at a constant enzyme concentration was greater than that of the increase in enzyme concentration at a constant incubation time.

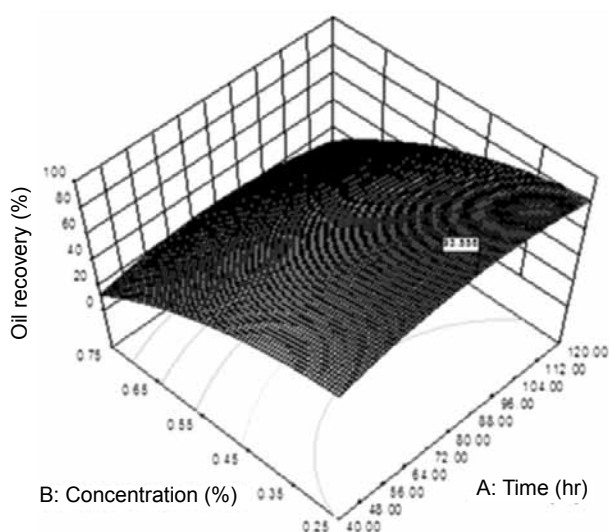


Figure 3. Surface plot of sludge palm oil recovery as a function of incubation time (hr) and enzyme concentration (%) depicted at fixed impeller speed.

Effect of Incubation Time (hr) and Speed (rpm)

The effect of different incubation time and impeller speed on the SPO yield from POME during the enzymatic treatment is shown in Figure 4. The graph shows a response surface for the oil recovery as a function of incubation time and impeller speed performed at constant cellulase concentration. The oil recovery increased gradually with incubation time whereas it decreased slowly with the increase of impeller speed. In enzymatic treatment, the speed of impeller plays an important role to ensure a proper mixing of enzyme, sludge and the buffer solution. Thus, the enzyme can degrade the cell wall to increase the oil extractability with its maximum activity. As can be observed, when the impeller speed was less than 100 rpm, the amount of oil obtained was low. This may be due to the insufficient enzyme mixing which later hindered the hydrolysis of the cell wall in an effective way. On the other hand, the higher speed of more than 150 rpm could attribute to the formation of foam causing most of the sludge to assemble at the centre of the reactor. This situation might interfere with the mixing of the reaction mixture, resulting in the lower oil recovery. This result is consistent with previous reports (Nurdiyana

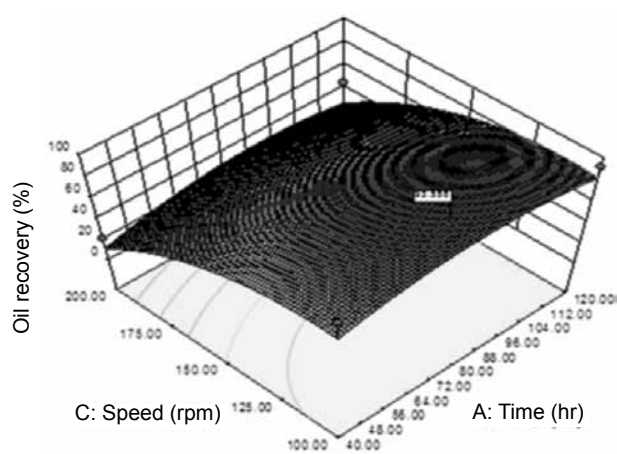


Figure 4. Surface plot of sludge palm oil recovery as a function of incubation time (hr) and impeller speed (rpm) depicted at fixed enzyme concentration (%).

and Siti Mazlina, 2009; Sharma *et al.*, 2002; Mukataka *et al.*, 1983). Mukataka *et al.* (1983) have reported that excessively high mixing speeds (>200 rpm) could reduce the extent of cellulose conversion, while a moderate mixing speed ranging from 100-200 rpm provided a good combination for the fast hydrolysis rate and high conversion yield. Nurdiyana and Siti Mazlina (2009) used a rotation speed ranging from 100-300 rpm in the enzymatic protein extraction from fish waste and observed that the rotation speed of 171 rpm was ideal for optimum protein recovery. In other study, increasing the shaking speed from 25 to 150 rpm enhanced the interaction between the substrate and had no appreciable adverse impact on the activity of enzymes, as reflected by the slightly higher conversion yields of the 150 rpm runs. As a result, the continuous and high speed shaking produced the highest conversion yield, whereas the intermittent and low speed shaking regimes resulted in lower conversion (Hanna *et al.*, 2001). Sharma *et al.* (2002) used a sequence of increasing shaking speed of 50, 80, 100 and 200 rpm for peanut oil extraction by aqueous enzymatic extraction and concluded that the decrease in shaking speed led to a decrease in oil recovery, whilst increasing the speed led to emulsification and reduced the amount of clear oil obtained at the top. In this study, the speed is the most influential parameter to be controlled in order to get a higher percentage of oil recovery.

Effect of Enzyme Concentration (%) and Speed (rpm)

Figure 5 shows the response surface plot for the SPO recovery as a function of enzyme concentration (0.08%-0.92%) and impeller speed (65.91-234.09 rpm) during enzymatic treatment performed at constant incubation time. The figure reveals that the oil yield increased reaching a maximum (93.56%) with 0.25% of enzyme concentration and 111.05 rpm of impeller speed. As can be observed, the percentage of oil recovery increased with the increase of enzyme concentration and moderate impeller speed. Enzyme concentration and impeller speed have a significant effect on enzymatic reaction. For a constant amount of substrate present in the solution, a higher concentration of enzyme will increase enzymatic reaction. In the enzymatic oil extraction process, the increase of enzyme concentration, gives a high oil yield. The increase in the yield of the oil due to the action of enzyme on cell wall would result in more release of oil from it (Mishra *et al.*, 2005). The higher extraction rate via enzymatic hydrolysis occurred at the highest level of enzyme to sample ratio (Nurhidayah and Siti Mazlina, 2009). Moreover, in another study, Beatriz *et al.* (2003) reported that higher enzyme activity led to a high yield of protein and oil extraction from coconut.

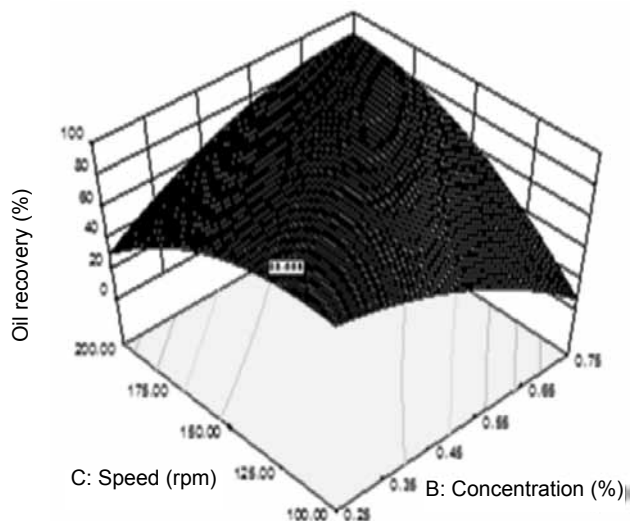


Figure 5. Surface plot of sludge palm oil recovery as a function of enzyme concentration (%) and impeller speed (rpm) depicted at fixed incubation time (hr).

Selection of Optimal Levels and Estimation of Optimum Response Characteristic

The objective of this study is to determine the optimum conditions in order to get a higher percentage of SPO recovery from the POME. Table 4 summarises the optimal levels of various parameters obtained after examining the response curves. The table indicates that the optimal level for incubation time is 81.46 hr when the speed is 111.05 rpm and the enzyme concentration is 0.25%, with the maximum percentage of oil recovery being 93.56%.

One Sample T-test

Three confirmation experiments were conducted at selected optimal levels of the process parameters and the results are shown in Table 5. One sample T-test was used to run the confirmation experiment's results. As can be seen in Table 6, the P-value is 0.114 which is greater than 0.05. Due to that reason, it can be concluded that the experimental results and the results from the model are almost similar and the model is adequate.

TABLE 4. OPTIMUM AND CONFIRMATIVE VALUES OF PROCESS PARAMETERS FOR MAXIMUM PERCENTAGE OF OIL RECOVERY FROM PALM OIL MILL EFFLUENT USING CELLULASE ENZYME

Optimal levels of process parameters
Incubation time (hr) = 81.46
Enzyme concentration (%) = 0.25
Speed (rpm) = 111.05

TABLE 5. DATA OF CONFIRMATION RUN

Run	Incubation time (hr)	Enzyme concentration (%)	Speed (rpm)	Y, SPO recovery (%)
1	81.46	0.25	111.05	74.1569
2	81.46	0.25	111.05	81.9528
3	81.46	0.25	111.05	52.0684

TABLE 6. RESULT FOR ONE SAMPLE T-TEST FOR DIFFERENCE

95 % CI	T-value	P-value
(30.8852 < 93.558 < 107.9002)	-2.7	0.114

CONCLUSION

This project investigated the percentage of SPO recovery from POME sludge using cellulase enzyme treatment and at the same time also determined the variables that have a significant effect on the percentage of SPO recovered. Experiments were carried out to explore the three process factors *viz.*: optimum incubation time (12.73-147.27 hr), enzyme concentration (0.08% - 0.92%) and impeller speed (65.91 – 234.09 rpm) for maximum SPO recovery. The entire experiments and assessment-measured data were evaluated based on a CCFD and were analysed to RSM for the purpose of examining the roles of the three process factors on the percentage of oil recovery.

It was found that a second-order polynomial regression model was properly interpreted in the experimental data with coefficient of determination (R^2) value of 0.852. The optimum conditions for maximum oil recovery of 93.56% was computed at the following parameters; incubation time, $X_A = 81.46$ hr, enzyme concentration, $X_B = 0.25\%$ and impeller speed, $X_C = 111.05$ rpm. Whereas, an experimental oil yield was 81.95% as revealed by confirmatory study. This study has proven that the CCFD model is suitable to evaluate the optimum conditions for the cellulase enzyme-assisted oil recovery from POME.

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