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THE STUDY ON BIOLOGICAL pH TREATMENT OF ACIDIC PALM OIL MILL EFFLUENT

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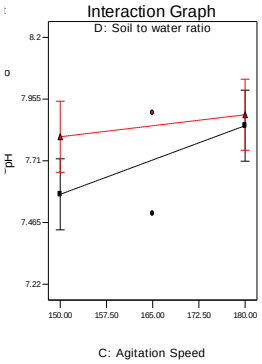
Graphical abstract



Abstract

This study investigated the biological pH treatment of acidic palm oil mill effluent (POME). In this study soil mixed culture (SMC) was acclimatized for 10 days (30°C and 150 rpm) with POME and used as inoculum. Selected factors used in this study were reaction time (3-5 days), temperature (25-30 °C), agitation speed (150-180 rpm), soil to water ratio (1:1 and 1:3) and inoculum types (peat and alluvium inoculum). Response surface method (RSM) was used to design and analyzed experimental data. In this study reaction time gave highest contribution which was at 29.81%. Reaction time was important for microbial growth in biological pH treatment. Interaction between reaction time and agitation speed gave highest contribution which was at 17.21%. Agitation provides a proper mixing on acidic POME and SMC thus increased the microbial activities. In this study, analysis of variance (ANOVA) was used to analyze the experimental data and the coefficient of determination (R2) value of 0.8301 was obtained. This study had proven the application of RSM was useful in experimental data analysis and increased the pH value from 4 to 8.

Keywords: Palm oil mill effluent, response surface method, soil mixed culture, analysis of variance

Abstrak

Kajian ini berkenaan rawatan pH secara biologikal bagi sisa kilang kelapa sawit (POME) yang berasid. Dalam kajian ini kultur tanah campuran (SMC) telah diaklimitasi selama 10 hari (30°C dan 150 rpm) dengan POME dan digunakan sebagai inokulum. Faktor yang digunakan dalam kajian ini adalah masa tindak balas (3-5 hari), suhu (25-30 °C), kelajuan pengadukan (150-180 rpm), nisbah tanah kepada air (1: 1 dan 1: 3) dan jenis inokulum (inokulum gambut dan aluvium). Kaedah permukaan sambutan (RSM) telah digunakan untuk mereka bentuk dan menganalisa data eksperimen. Dalam kajian ini masa tindak balas memberi sumbangan tertinggi iaitu 29.81%. Masa tindak balas adalah penting bagi pertumbuhan mikrob dalam rawatan pH secara biologikal. Interaksi antara masa tindak balas dan kelajuan pengadukan memberikan sumbangan tertinggi iaitu 17.21%. Pengadukan memastikan campuran di antara POME berasid dan SMC berlaku, seterusnya meningkatkan aktiviti mikrob. Dalam kajian ini, analisis varians (ANOVA) telah digunakan untuk menganalisa data eksperimen dan nilai pekali penentuan (R2) 0.8301 diperolehi. Kajian ini telah membuktikan penggunaan RSM adalah sesuai dalam menganalisa data eksperimen dan meningkatkan nilai pH dari 4 kepada 8.

Kata kunci: Sisa kilang kelapa sawit, kaedah permukaan sambutan, kultur tanah campuran, analisis varians

1.0 INTRODUCTION

In Malaysia, palm oil extraction generates about 50 million tons of palm oil mill effluent (POME) annually [1]. This situation leads to the production of highly pollutant waste from palm oil mill. POME consists of 95-96% of water, 0.6-0.7% of oil and 4-5% of total solids where half of it was a suspended solid consisting of debris from the palm oil fruit [2]. Freshly discharged POME was acidic with pH ranging from 4 to 5 and temperature around 80 to 90°C with addition of appreciable amounts of plant nutrient [3]. Microorganisms such as bacteria were responsible for decomposing organic waste. When organic matter such as dead plants, leaves, grass clippings, manure, sewage, or even food waste was present in a water supply, the bacteria began the process of breaking down this waste.

Considering the high organic content in acidic POME, anaerobic process was the most common treatment but time constraint method. The most commonly used anaerobic process was facultative ponds and open digesting tank. However, this process required extensive land area and long retention time before it can reach the environmental requirement. It also produced large quantity of greenhouse gases including methane and carbon dioxide resulted from open ponds and tanks activities. In order to overcome this issue, a biological treatment studied was being raised up.

The biological treatment using mixed culture had gained much interest due to the low operating cost. Mixed culture was a microbial culture contains two or more different strains of organisms. The use of mixed culture provides several advantages over a pure culture. The mixed culture can better adapt to changing conditions during growth [4]. Natural occurring mixed cultures are particularly efficient means for utilization of substrate mixtures in the context of wastewater treatment [5]. In wastewater treatment, soil can acts as a filter, exchanger and absorber. Microbes that exist in soil help to degrade the organic matter in the wastewater and increasing the wastewater treatment capacity.

There were several factors considered in performing biological pH treatment of acidic POME. The pH value can be affected due to the microbial growth during the reaction time. Substrate and inoculums concentration also had been reported as important factors that affecting microbial growth [6]. The relationship between applied substrates and pH was studied by other researchers [7-8]. Agitation was used to perform high rate of fermentation and proper mixing of substrate. Temperature was used for biological pH treatment of acidic POME as microorganism react in certain temperature range. Different types of soil gave different properties in moisture content, organic content and soil structure. This lead to the different amount of microorganism exists in the soil that contributes to waste properties break down. Application of soil in biological treatment has low environmental impact and less cost compared to the chemical and physical treatment.

Therefore, this study was conducted to study the interaction effect between the factors using response surface method (RSM). Selected factors which were reaction time, temperature, agitation speed, soil water ratio and inoculum types were studied by using RSM. RSM was a collection of mathematical and statistical techniques for empirical model building [9]. Design Expert software (version 8.06) was used to construct experimental design table and analyze experimental data. Results of this study were analyzed using analysis of variance (ANOVA). The relatively high value of coefficient of determination (R^2) showed the model can represent the experimental data.

2.0 EXPERIMENTAL

2.1 Sample Collection

Acidic POME was collected at mixed raw effluent (MRE) point of a palm oil mill in Kuantan, Pahang and was kept in a freezer at 4°C to avoid degradation. Soil sample was collected 15cm from the ground of peat soil. The same procedure was done for alluvium soil. Peat soil was collected at palm oil mill and alluvium soil was collected near the palm oil tree root system.

2.2 Characterization of Soil

The characterization of peat and alluvium soil involve the determination of pH, texture, moisture content, conductivity, nitrogen content, organic carbon content, available phosphorus and cation-exchange capacity. The characterization of soil was performed according to the standard method on soil analysis.

2.3 Inoculum Preparation

Soil mixed culture (SMC) was prepared by mixing soil sample with water. The peat soil and water were mixed together to give the soil to water (s/w) ratio of 1:1 (100 g soil and 100 mL water) and 1:3 (100 g soil and 300mL water). The same procedure was done for alluvium soil. Soil mixed culture (SMC) was mixed with palm oil mill effluent (POME) in ratio of 1:3 (50 mL SMC and 150 mL POME). The mixture was acclimatized for 10 days (30°C and 150 rpm) and used as inoculum. Acclimatization process in a biological process enhanced the ability of the microbes to degrade organics [10].

2.4 Experimental Setup

In this study, inoculum was mixed with palm oil mill effluent (POME) in ratio of 1:3 (50 mL inoculum and 150 mL POME). Then the mixture was placed in incubator shaker. The experiments were carried out under anaerobic condition. The experimental table was designed and constructed using response surface method (RSM). The experiments were carried out by varying the factors according to the given ranges (Table 1). Initial pH value for palm oil mill effluent

(POME) was 4. The final pH value of POME was determined after experiment completed.

Table 1 Experiment factors and ranges

Factors	Unit	Type	Low	High
Reaction time	day	Numeric	3	5
Temperature	°C	Numeric	25	30
Agitation speed	rpm	Numeric	150	180
Soil to water ratio	-	Categoric	1:3	1:1
Inoculum types	-	Categoric	Peat	Alluvium

3.0 RESULTS AND DISCUSSION

3.1 Soil Characterization

Table 2 shows the result on soil characterization. The peat and alluvium soil were analyzed to determine their pH, texture, moisture content, conductivity, nitrogen content, organic carbon content, available phosphorus and cation-exchange capacity. Soil characterization was important to determine the soil properties and its behavior. Bacteria that exist in soil were used as a source of inoculum.

From the result, alluvium soil had higher pH value compared to the peat soil. Soil with lower pH value tends to release magnesium and ferum ions. This situation leads to the production of phosphorus in soil [11]. This can be shown by higher available phosphorus exist in the peat soil (Table 2).

Soil moisture content depends on soil type. Results of this moisture content also depend on soil texture. Percentage of coarse sand, fine sand, silt and clay contributes towards its moisture content. Salt concentration that exists in the soil was directly proportional with soil conductivity. Salt concentration restricts the water intake in the soil thus increase its moisture content. This can be shown by low moisture content exist in the alluvium soil that have low conductivity [12].

Table 2 Soil Characterization

Soil type	Alluvium	Peat
pH	4.3	3.5
Nitrogen (%)	0.05	0.37
Moisture content (%)	17.18	46.16
Organic Carbon (%)	0.55	11.40
Conductivity	45.65	1039
Avail Phosphorus (ppm)	7.59	2747
Coarse sand (%)	12	51
Fine Sand (%)	37	20
Silt (%)	18	6
Clay (%)	38	18
Cation-exchange capacity (cmol/kg)	4.54	14.35

3.2 Analysis on Biological pH treatment

The experimental results are shown in Table 3. From the data, the pH value was found within the range of 7.22 to 8.20. The significant effect of each factor on the pH value was evaluated by analysis of variance (ANOVA). Results from analysis of variance (ANOVA) in Table 4 shows that the regression model for biological pH treatment was significant. The coefficient of determination (R^2) value of the pH model was 0.8301. This showed that the model could represent the experimental data. From ANOVA, reaction time gives the highest contribution which was at 29.84%. This followed by agitation speed (9.29%), soil to water ratio (6.62%), inoculum types (2.29%) and temperature (0.46%). Interaction between reaction time and agitation speed give the highest contribution which was at 17.21%. Figure 1 shows the predicted versus actual plot for biological pH treatment. The plot shows that the actual values were distributed near to the straight line. It also shows a good convergence between predicted and actual values. The equation for the pH model was shows in Equation 1 to 4. Factors D (soil to water ratio) and E (inoculum types) were categoric factor and were not included in the equation.

Soil to water ratio: 1:3
Inoculum types: Peat

$$pH = 8.6565 + 0.88375A - 0.3135B + 4.16667x10^{-3}C + 0.024AB - 8.16667x10^{-3}AC + 1.36667x10^{-3}BC \quad (1)$$

Soil to water ratio: 1:1
Inoculum types: Peat

$$pH = 10.09 + 0.81375A - 0.3135B - 2.0x10^{-3}C + 0.024AB - 8.16667x10^{-3}AC + 1.36667x10^{-3}BC \quad (2)$$

Soil to water ratio: 1:3
Inoculum types: Alluvium

$$pH = 8.7365 + 0.88375A - 0.3135B + 4.16667x10^{-3}C + 0.024AB - 8.16667x10^{-3}AC + 1.36667x10^{-3}BC \quad (3)$$

Soil to water ratio: 1:1
Inoculum types: Alluvium

$$pH = 10.17 + 0.81375A - 0.3135B - 2.0x10^{-3}C + 0.024AB - 8.16667x10^{-3}AC + 1.36667x10^{-3}BC \quad (4)$$

Where A = reaction time, B = temperature and C = agitation speed. A, B and C were referred as the main effect while AB, AC and BC were referred as the interaction effect.

Table 3 Experimental results

Run	Factors					Response
	Reaction Time, day (A)	Temperature, °C (B)	Agitation speed, rpm (C)	Soil to water ratio (D)	Inoculum types (E)	pH
1	3	25	150	1:3	Alluvium	7.43
2	5	25	150	1:3	Peat	7.95
3	3	30	150	1:3	Peat	7.22
4	5	30	150	1:3	Alluvium	8.05
5	3	25	180	1:3	Peat	7.91
6	5	25	180	1:3	Alluvium	7.89
7	3	30	180	1:3	Alluvium	7.85
8	5	30	180	1:3	Peat	8.09
9	3	25	150	1:1	Peat	7.61
10	5	25	150	1:1	Alluvium	8.06
11	3	30	150	1:1	Alluvium	7.53
12	5	30	150	1:1	Peat	8.00
13	3	25	180	1:1	Alluvium	7.85
14	5	25	180	1:1	Peat	7.71
15	3	30	180	1:1	Peat	7.88
16	5	30	180	1:1	Alluvium	8.11
17	4	27.5	165	1:3	Peat	7.50
18	4	27.5	165	1:1	Peat	7.90
19	4	27.5	165	1:3	Alluvium	7.60
20	4	27.5	165	1:1	Alluvium	8.20

Table 4 ANOVA for Biological pH Treatment

Source	Sum of squares	df	Mean square	F value	p-value Prob>F
Model	1.07	10	0.11	4.4	0.018
A-Reaction time	0.42	1	0.42	17.1	0.0025
B-Temperature	$6.4x10^{-3}$	1	$6.4x10^{-3}$	0.26	0.6204
C-Agitation speed	0.13	1	0.13	5.33	0.0464
D-Soil to water ratio	0.092	1	0.092	3.80	0.0830
E-Inoculum types	0.032	1	0.032	1.32	0.2810
AB	0.058	1	0.058	2.37	0.1583
AC	0.24	1	0.24	9.87	0.0119
AD	0.02	1	0.02	0.81	0.3928
BC	0.042	1	0.042	1.73	0.2213
CD	0.034	1	0.034	1.41	0.2660
Residual	0.22	9	0.024		
Cor total	1.29	19			
Std. Dev	0.16		R-Squared		0.8301
Mean	7.82		Adj R-Squared		0.6414
C.V. %	2		Pred R-Squared		0.4445
Press	0.72		Adeq Precision		8.135

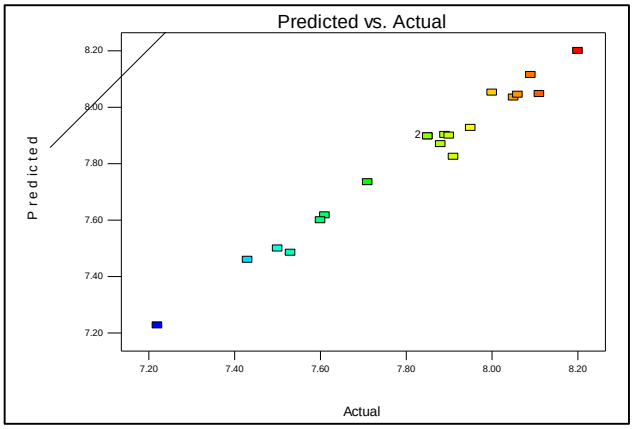


Figure 1 Predicted versus actual data for biological pH treatment

3.3 Main Effect on Biological pH treatment

The contribution for each factor on biological pH treatment was presented in Table 5. Reaction time gives the highest contribution which was at 29.81%. Experiments were carried out by varying the reaction time from 3 days to 5 days. Low pH value was detected at a short reaction time. It had been observed that pH value was changed during the reaction time and this was affected by population growth of microbes in the treatment process [13]. Microbes in inoculum can bind enzymes and organisms thus affecting the movement of cells through the inoculum and the breakdown of organic matter [14]. This situation makes the microbial population growth increase and increasing its performance in rising up the pH value.

Agitation speed gives contribution at about 9.29%. Experiments were carried out by varying the agitation speed from 150rpm and 180rpm. Agitation plays an important role in biological pH treatment. It ensured a proper mixing between substrate and inoculum. With the addition of agitation, the microbial activities were increased [15]. This had been observed that agitation can help in speed up the microorganism activities thus increased the microbial performance [16]. According to Yan *et al.* [13] pH value was directly proportional with the microbial growth. This lead to the increasing of microbial performance thus increased the pH value.

Table 5 Main and interaction factor effect percentage contribution

Term	Effect	Sum Sqr	% Contribution
A-Reaction time	0.3225	0.416025	29.814
B-Temperature	0.04	0.0064	0.45865
C-Agitation speed	0.18	0.1296	9.28766
D-Soil to water ratio	0.152053	0.09248	6.62749
E-Inoculum types	0.0894427	0.032	2.29325
AB	0.12	0.0576	2.4527
AC	-0.245	0.2401	17.2065
AD	-0.07	0.0196	1.40462
BC	0.1025	0.042025	3.01168
CD	-0.0925	0.034225	4.12785

3.4 Interaction Effect between Reaction Time and Agitation Speed

The interaction between reaction time and agitation speed give the highest contribution which was at 17.21%. The interaction graph was presented in Figure 2. From the Figure 2, it shows that the pH value was directly proportional with reaction time and agitation speed. In anaerobic process, pH and reaction time were interacting to each other. Anaerobic process was an effective method to increase the microbial performance [17]. Most of the microbes gave better performance in anaerobic condition compared to aerobic condition [17]. It had been observed by other researcher that microbial performance was decreased as reaction time decreased [18]. The microbial activities were increased in the stirred culture thus it also directly proportional with agitation speed [15]. Thus at higher agitation speed the pH value was increased.

At reaction time of 3 day, the pH value was higher at agitation speed of 180rpm compared to the agitation speed at 150rpm. Agitation helps to speed up the activity of microorganism [16]. Thus higher agitation speed provides a better mixing to the treatment. Figure 2 also shows the interaction of reaction time and agitation speed at day 5. At agitation speed of 180rpm and 150rpm, the almost similar pH value could be observed. This showed the reaction between substrate and inoculum approaching its maximum value at day 5. Based on preliminary study that was done the reaction had been completed during the 5 days of reaction time. Agitation was important to make sure that substrate and inoculum were properly mixed during the treatment [19].

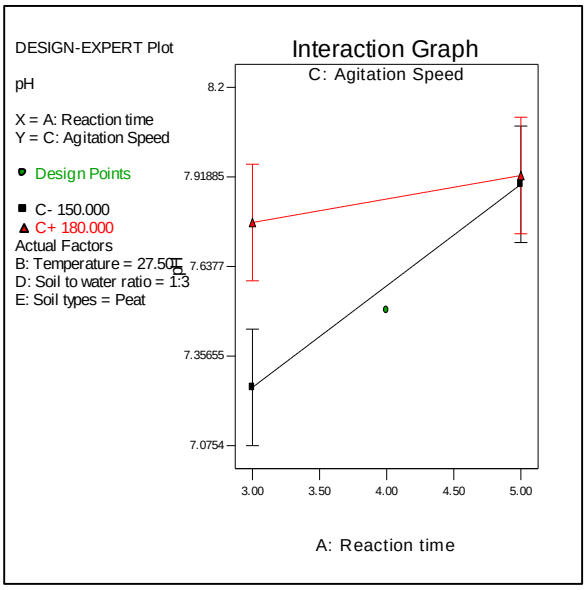


Figure 2 Interaction graph between reaction time and agitation speed for biological pH treatment

3.5 Interaction Effect between Temperature and Agitation Speed

The effect of interaction between temperature and agitation speed for biological pH treatment was presented in Figure 3. From the Figure 3, it shows that pH value was directly proportional with the temperature and agitation speed. The pH value was increased gradually with temperature at agitation speed 180rpm from 7.78 to 7.92. At agitation speed 150rpm, the pH value was decreased gradually from 7.61 to 7.55 with addition of temperature. This situation occurs due to the fermentation process where the final pH value decreased with increasing of temperature from 20-35°C [20].

It was reported from previous study that pH value was decreased with increasing of temperature [20] but with addition of agitation speed it increase the final pH value. This situation can be seen in Figure 3 where pH value increase at agitation speed of 180rpm and decreased at agitation speed of 150rpm. Different agitation speed used gave significant impact on biological pH treatment. This was confirmed by Kaparaju *et al.* [21] that the type of agitation used affects the microbial performance. In order to treat wastewater biologically, temperature plays an important rules where higher temperature (>40°C) was not suitable for microbial growth [20]. Agitation was required in microbial

growth where it speeds up the microbial activities and increased its performance.

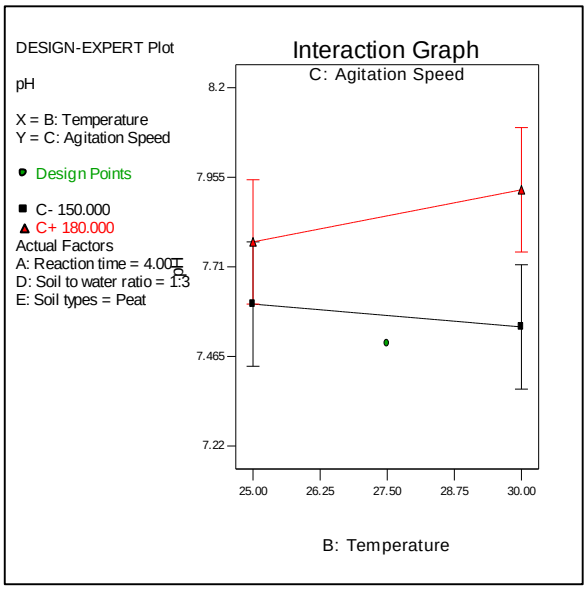


Figure 3 Interaction graph between temperature and agitation speed for biological pH treatment

3.6 Interaction Effect between Agitation Speed and Soil to Water Ratio (s/w)

The effect of interaction between agitation speed and soil to water ratio (s/w) for biological pH treatment was presented in Figure 4. From the Figure 4, it shows that pH value was directly proportional with the agitation speed and s/w. The s/w also can be known as soil concentration. Soil concentration was directly proportional with pH value. This can be shows in Figure 4 where at high s/w the pH value was increased. With the addition of agitation on this research, it increased the microbial performance [15].

At agitation speed 150rpm the pH value was high at s/w 1:1 compared to s/w 1:3. These situations happen due to large amount of microbes that exists in the s/w 1:1 [6]. These microbes were used in biological pH treatment to break down the waste water properties. It was observed that at agitation speed of 180rpm, the pH value was almost same for both s/w. It was expected that the reaction between microbes in inoculum and substrate had been completed.

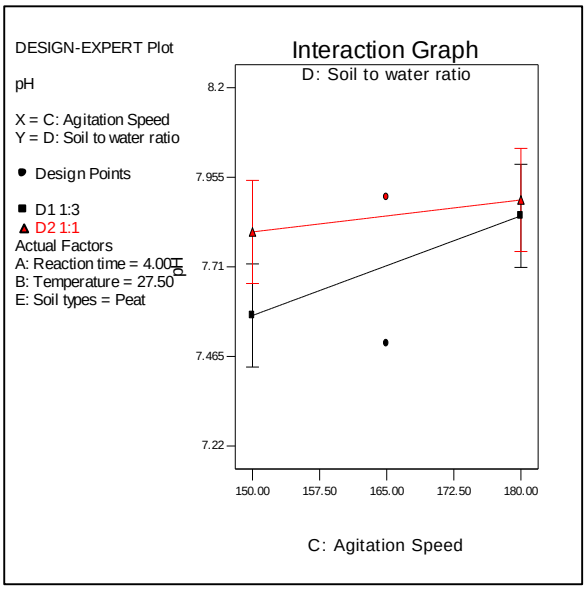


Figure 4 Interaction graph between agitation speed and soil to water ratio for biological pH treatment

4.0 CONCLUSION

This study investigated the interaction effect between the factors in biological pH treatment of acidic palm oil mill effluent (POME) by using response surface method (RSM). RSM was used to construct the experimental design table and analyzed the experimental data. Five factors selected in this study were reaction time, temperature, agitation speed, soil to water ratio and inoculum types. From the experimental data analysis, two most significant factors that affect biological pH treatment of POME were reaction time and agitation speed. Complete reaction was accomplished according to the selected reaction time. With help of agitation, it increased microbial performance thus increasing the pH value. The experimental data was analyzed using analysis of variance (ANOVA) and the coefficient of determination (R^2) of 0.8301 was obtained. The final pH value of POME was increase from 4 to 8 which was from acidic condition to alkaline condition.

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