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Polishing of Treated Palm Oil Mill Effluent Using *Azolla pinnata*

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ABSTRACT

Phytoremediation was used in this study to polish treated palm oil mill effluent using *Azolla pinnata*. The effectiveness of phytoremediation using *A. pinnata* can be proved through the reduction of the contamination concentration of wastewater before and after treatment. Preliminary study was made to determine the range of variables that are required for Response Surface Methodology (RSM) software. The variables for this study are the quantity of *A. pinnata* (20-30 grams), the concentration of palm oil mill effluent (60% -10%), and retention time (1-5 days). Characterization of palm oil mill effluent showed the BOD concentration (285 mg/L), and oil and grease (68.1 mg/L) above the emission standards, the Environment Quality Act 1974, (2005). The highest percentage of effectiveness to eliminate contamination after treatment is ammonia nitrate with an average efficiency of 93%. Meanwhile, the process of BOD removal efficiency percentage is the lowest at 46%. The optimization process has been done using the software Design Expert®. Through Variance analysis, the percentage of effectiveness to eliminate the BOD is not significant for the quadratic equation. Optimum conditions for phytoremediation of the palm oil mill effluent using *A. pinnata* are quantity of *A. pinnata* (30 g), the concentration of palm oil mill effluent (10%) and retention time (4.7 days) with the reliability or desirability at 0.9. Maximum percentage of effectiveness removal for BOD, COD, TS, TSS, oil and grease, NH₃-H and phosphate are 63.67%, 80.02%, 85.89%, 80.58%, 81.50%, 98.44%, 92.92% and 0.90%, respectively.

INTRODUCTION

Palm oil is a food product extracted from palm fruit fiber called mesocarp. Palm oil is oil and fat against 17 other crops such as soybean, rapeseed, sunflower, and animal fat in the world's productions [1,2]. Palm oil is a good source of oil and fats, it can produce 4,080 kg of crude palm oil and 456 kg of crude palm kernel oil [1]. The Palm oil industry in Malaysia focuses on the upstream process to obtain crude palm oil and palm kernel oil rather than downstream processes to produce palm oil-based products such as biodiesel and cooking oil [2]. The palm oil production process requires a large volume of water. Approximately, 5 to 7.5 tons of water are needed to produce 1 ton of palm oil and 50% of that water will contribute to the generation of wastewater [2,3]. Basically, palm oil processing will produce wastewater during sterilization, separation of sludge, and the cyclone process [4]. Characteristics of wastewater produced from palm oil industries are influenced by the quality of their raw material

and processing environment. On average, palm oil wastewater contains 25000 mg/L of BOD, 51000 mg/L of COD, 40250 mg/L of TS, 18500 mg/L of TSS, 6505 mg/L of oil, and grease, 35mg/L of ammonia nitrate and 745 mg/L of total nitrogen [1,4,]. Typically, oil palm wastewater is treated in several stages that involve few processes. Firstly, wastewater undergoes a screening process to remove large particles and then will be transferred to a retention tank for 3 days to ensure the temperature of wastewater at ambient temperature and to separate the oil layer. After that, the separated wastewater will be transferred into the anaerobic digester tank for 20 days and later to the sedimentation process where the sludge will be removed. The treated wastewater with no sludge will be treated in the facultative pond next to an algae pond before being released to rivers or open water flow [4,5,6]. However, the final treated effluent from the palm oil mill is still above the effluent discharge standards for crude palm oil mills as shown in **Table 1**.

Table 1. Effluent discharge standards for crude palm oil mills (Environmental Quality Act 1974, (2005).

Parameter	Unit	Value
Temperature	°C	45
pH	-	5-9
Biochemical oxygen demand 3days at 30°C	mg/L	100
Chemical oxygen demand	mg/L	-
Total solid	mg/L	-
Total suspended solid	mg/L	400
Oil and gris	mg/L	50
Ammonia nitrate	mg/L	150
Total nitrogen	mg/L	200

Phytoremediation is proposed as a treatment method for polishing palm oil mills effluent further to support existing treatment. Phytoremediation is one of the green technology processes, easy to handle, low cost and environmentally friendly that use plant or microorganism to remove or decrease contaminations in wastewater [7,8]. Phytoremediation involves several processes in this study which are phytoaccumulation, phytostabilization, rhizofiltration and phytodegradation [8,9].

There are several important factors in choosing plants for phytoremediation. First, the plant has been proven to be effective or to show positive results in phytoremediation [9,10]. Besides, the plant should be able to live in a universal condition for optimum growth of roots. Root factors can divide into the type of root and the root zone depth. Fibrous root and the deepest root zone are the best type for phytoremediation [10,11].

Transpiration and growth rate also become one the important factors in choosing a plant for phytoremediation [10]. It is relevant to state that the plant has rapid growth in terms of root depth, density, volume, and surface area as a plant with great potential as a phytoremediation plant. More than 500 species of plant are listed as potential plants for phytoremediation [9,10]. In this study, *Azolla pinnata* was chosen as plant for the phytoremediation of treated palm oil mill effluent. *A. pinnata* is often found in areas of calm water and it can also prevent mosquito larvae from breeding because it multiplies to the entire surface of the water [4,11, 12]. *A. pinnata* is a unique plant that can utilize atmospheric N₂ due to symbiosis with the blue-green alga, *Anabaena azollae* which grows in the cavities of their leaflets. The presence of this algae also can increase their growth rate then, as great potential in treating wastewater [4]. This study aims to characterize the treated palm oil mill effluent. Then, utilize *A. pinnata* as a medium in phytoremediation for palm oil mill effluent treatment to reduce pollution load and the optimization of the phytoremediation process conditions system.

MATERIALS AND METHODS

Cultivation of *Azolla pinnata*

Azolla pinnata was taken from a greenhouse on Universiti Kebangsaan Malaysia. These plants are grown in a

container with dimensions of 23cm long, 14cm wide and with a height of 10cm. Cultivation of *A. pinnata* was done in a closed room with a temperature of 25°C and sunlight rates of 25-50%. Temperature and pH of medium for growing were controlled at 18 to 26°C and pH 5.5 respectively for maximum growth.

Sources of Palm Oil Mill Effluent and The Characterization

The sample was taken from a final discharge treatment pool at Sri Langat Palm Oil Mill Sdn. Bhd. located at Banting, Selangor. Characterization of the palm oil mill effluent as an initial and control study. The wastewater will be characterized by pH, biochemical oxygen demand for three days, chemical oxygen demand, total solids, total suspended solids, oil and grease, ammonia nitrate and phosphate. The experiment was conducted entirely in Environmental Laboratory, Universiti Kebangsaan Malaysia. The palm oil mill effluent was characterized by the BOD concentration (285 mg/L), and oil and grease (68.1 mg/L) above the emission standards, the Environment Quality Act 1974, (2005) as shown in **Table 2**. Therefore, this study aimed to investigate whether the method of treatment using the phytoremediation process can reduce the concentration of contaminants.

Phytoremediation Process of Palm Oil Mill Effluent

A Preliminary study conducted to determine the range of parameters for RSM involving retention time, the concentration of palm oil mill effluent and the quantity of *Azolla pinnata* been used in phytoremediation process. The preliminary study starts with the physical observation of *A. pinnata* exposed in a 1000 mL beaker then proceeds to the second stage which is using (30×30×30) cm aquarium without dilution of palm oil mill effluent. The last stage is a combination of the preliminary study for the first and second stages. This process was carried out in the Green House, Universiti Kebangsaan Malaysia. *A. pinnata* will be exposed on the surface of palm oil mill effluent in (30×30×30) cm aquarium. The constant variable in this experiment is the volume of palm oil mill effluent. The quantity of *A. pinnata* was calculated using the wet weight method. *A. pinnata* was washed and dried on a tissues paper and allowed to dry at room temperature for few minutes before being weighed for its dried weight.

Table 2. Characterization of treated palm oil mill effluent

Parameter	Unit	Environment Quality Act 1957, (2005)	Characterization
Temperature	°C	45	-
pH	-	5-9	8.34
BOD	mg/L	100	285
COD	mg/L	-	1430
TS	mg/L	-	6432
TSS	mg/L	400	253.3
Oil and Grease	mg/L	50	68.1
Ammonia nitrate	mg/L	150	70.3
Total Nitrogen	mg/L	200	-
Phosphate	mg/L	-	45.7

Analysis for Water Quality Parameter

Analysis of water quality involves two main methods which include field measurement and laboratory experiments. Field measurement includes test for pH,

BOD, COD, NH₃-N and Phosphate whilst the laboratory experiments involve TS, TSS and oil and grease on POME. The following test shown in **Table 3** were conducted on the POME samples before and after the phytoremediation process occur.

Table 3. Measurement method and instrumentation

Parameter	Instrument (Model)	Descriptions
pH	pH meter (827 pH LAB)	-
BOD	DO meter (YSI 5100)	Calculated using BOD ₃ =DO ₀ -DO ₃ × dilution factor
COD	spectrometer (HACH DR/2100)	Wavelength 420 nm
NH ₃ -N	spectrometer (HACH DR/2100)	Wavelength 425 nm
Phosphate	spectrometer (HACH DR/2100)	Wavelength 890 nm
Equation		
TS	$= \frac{\text{Weight of beaker after dried} - \text{Initial weight of beaker (mg)}}{\text{Sample volume (L)}}$	
TSS	$= \frac{\text{Weight of filter paper after dried} - \text{Initial weight of filter paper (mg)}}{\text{Sample volume (L)}}$	
Oil and Grease	$= \frac{\text{Weight of volumetric flask after evaporation process} - \text{Initial weight of volumetric flask (mg)}}{\text{Sample volume (L)}}$	

Optimization of the Treatment

This study uses response surface methods (RSM) to optimize variables based on software Expert Design® 6.0.10. Each of the chosen variables is expected to be operated at an optimum range to maximize the benefit of the process, therefore, optimization becomes very important [13,14,15]. This optimization process involves seven responses which are biochemical oxygen demand, chemical oxygen demand, total solids, total suspended solids, oil and grease, ammonia nitrate and phosphate. Optimization for wastewater treatment uses quadratic equation for the relationship between responses and independent variable. The quadratic equation can be represented by **Eqn. 1**.

$$X = \alpha + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} A B + \beta_{13} A C + \beta_{23} B C \quad (\text{Eqn. 1})$$

with, X = Response, α = constant, β = coefficient and A, B, C = independent variables (A = time retaining, B = concentration of sample, C = quantity of *A. pinnata*)

Various studies of diagnostic and statistics are available from the software RSM. Analysis of Variance (ANOVA) including F test sequence, the test 'Lack-of-fit' and others are being analyzed by RSM in this research. ANOVA was also able to confirm the efficiency of the model.

RESULTS AND DISCUSSION

Phytoremediation of Treated Palm Oil Mill Effluent

Based on preliminary study, retention time was increased from 3 days to 6 days for *A. pinnata* absorb contaminants mean it still can survive after 5 days exposed to the dilute palm oil mill effluent. *A. pinnata* cannot survive in long period on the surface of oil palm wastewater without dilution because of its toxic material that is too high. Quantity of the *A. pinnata* also affect the lifespan of the plant to carry out the process of absorption of contaminants or phytoremediation [12]. The results of preliminary experiments, the range for process optimization have been identified as shown in **Table 4**.

At a concentration of 60% palm oil mill effluent, changes in the physical appearance of the *A. pinnata* began to be seen as early as the first day. There are some populations of plants that changed color from green to blackish color. On the third day, plants seem to die more than those that survived. Next, all plants died after 5 days of exposure. The phytoremediation process has occurred, and dead plant biomass can be used as fertilizer to improve soil fertility [4,15]. At a concentration of 35% palm oil mill effluent, no significant changes can be seen on the first day. Some plants died, changed colour from green to black on the third day of exposure.

Table 4. Variables for the optimization process.

Variables	Unit	Range
Retention Time	Days	1 - 5
Concentration of Palm Oil Mill Effluent	%	10 - 60
Quantity of <i>A. pinnata</i>	grams	20 - 30

Physical plants that die can be seen clearly because there is a difference in color with plants that are still alive. Next, on the fifth day showed a small number of plants still survived. For the 10% concentration of palm oil mill effluent, no changes could be observed on the first day. Physical plant on the third day also still no significant changes can be seen. This can be explained that the concentration of contaminants also can become as source of nutrients for the plant [16]. The physical appearance of the small number of plants begins to wilt and turn to black on the fifth day. From observations made, it is clearly found, concentrations that are suitable for the phytoremediation process is at a concentration of 10%.

Water Quality Analysis in Phytoremediation

The percentage of efficiency was calculated to obtain the required response by RSM software for process optimization. **Table 5** shows the overall percentage of the effectiveness of each run conducted in accordance with the parameters tested. The highest percentage of effectiveness is ammonia nitrate, while the lowest percentage of effectiveness is the BOD. Based on **Table 5**, the average percentage of removal efficiency of BOD, COD, TSS, oil and grease, and $\text{NH}_3\text{-H}$ are 46.07%, 68.73%, 63.83%, 54.78%, and 93.00%, respectively.

Optimization of BOD, COD, TSS, Oil and Grease and $\text{NH}_3\text{-N}$ Removal

Analysis of variance (ANOVA) was applied to optimize the chosen variables to achieve the correlation between process variables and the responses. [17,18]. Meanwhile, experimental data were used to verify the adequacy of the used model [17]. The optimization results according to RSM and the verified result based on laboratory experimental are presented in Table 5. There were 20 solutions given in the RSM optimization process. To confirm the validity of the statistical experimental strategies, a verification experiment was run at optimum conditions determined based on RSM solutions (Daud et al. 2018)

The highest percentage of BOD removal efficiency (92.98%) at run number 9 with a 35% concentration of sample, retention time at 3 days and quantity *A. pinnata* of 25 grams. The highest percentage of COD removal efficiency was (86.01%) at run number 1 with 60% concentration of sample, retention time at 1 day and quantity *A. pinnata* of 30 grams. The highest percentage of total suspended solid (TSS) removal efficiency was (94.75%) at run number 5 with 10% concentration of sample, retention time at 1 day and quantity *A. pinnata* of 20 grams. The highest percentage removal efficiency of oil and grease was 79.74% at run number 11 with a 35%

concentration of the sample, the retention time of 3 days and quantity *A. pinnata* of 30 grams. The highest of $\text{NH}_3\text{-N}$ removal efficiency was (98.86%) at the run number 2 with 60% concentration of sample, retention time at 1 day and quantity *A. pinnata* of 20 grams.

According to [12], the aquaculture method using *Azolla filiculoides* could be an appropriate option for removing total phosphorus (TP), total nitrogen (TN) and chemical oxygen demand (COD) from wastewater. Results from response surface methodology (RSM) study showed that the removal efficiency of COD, TP and TN were 77.5%, 66.8% and 78.1% respectively. Results indicate that removal efficiency increase as *Azolla* growth increase as also shown in this study. Meanwhile study by, [19], an extraordinary decrease in organic matter as measured by BOD and COD (96% and 94% respectively) was recorded during the experimental duration of 4 weeks using a density of 30 Vetiver plants. The next best result of BOD at 32 mg/L was obtained when using 30 Vetiver plants after 24 days for medium concentration of palm mill oil effluent (initial BOD 175 mg/L).

To graphically analyze the interactive relationships among the factors and the response, the Design Expert generated the 3D surface as shown in **Fig. 1**. **Fig. 1** illustrates 3D response plots and 2D contour plots of the quadratic model in terms of retention time and concentration respectively.

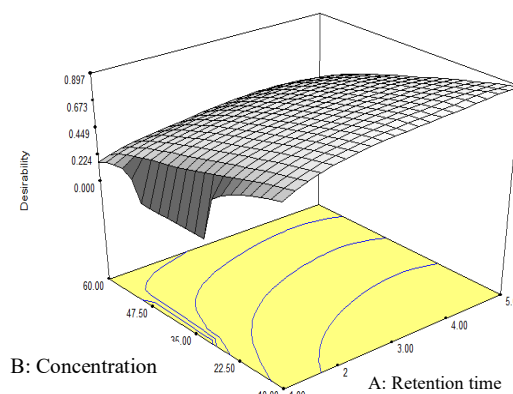


Fig. 1. Optimum condition for responses removal.

The purpose of this graph was to estimate the correlation relationship between independent variables and the responses of the models [20,21]. According to **Fig. 1**, the optimum condition for the treatment process is at 4.7 days retention time, 10% sample concentration and 30 gram of *A. pinnata*. This optimum condition achieved when all variables are set in the range and the responses were set in maximum condition. The maximum removal of BOD, COD, TSS, oil and grease and $\text{NH}_3\text{-H}$ are 63.67%, 80.02%, 80.58%, 81.50%, 98.44% and 92.92% respectively. According to [14,22], the plots depict the sensitivity of the responses due to the change of factor levels with the degree of their interactions. The optimization of the process variables was aimed at finding the levels of the factors to obtain a maximum desirability for all the responses within the design space.

Table 5. Optimization results according to RSM and the verified result based on laboratory experimental.

Standard order	Efficiency of BOD			Efficiency of COD			Efficiency of TSS		
	Actual value	Predicted value	Error (%)	Actual value	Predicted value	Error (%)	Actual value	Predicted value	Error (%)
1	5.26	31.34	-26.08	86.01	81.62	4.39	73.67	69.47	4.20
2	17.89	21.06	-3.17	79.37	83.76	-4.39	82.08	79.30	2.78
3	78.95	82.50	-3.55	49.65	54.31	-4.66	5.25	3.57	1.68
4	36.84	47.48	-10.64	48.43	38.51	9.91	71.06	73.71	-2.64
5	73.68	82.50	-8.81	36.89	47.19	-10.30	94.75	88.87	5.88
6	52.63	68.53	-15.90	86.57	82.30	4.27	81.56	80.00	1.56
7	57.89	74.18	-16.29	50.00	45.99	4.01	10.50	10.04	0.46
8	42.11	35.48	6.62	58.39	63.17	-4.78	60.52	61.48	-0.96
9	92.98	38.25	54.73	70.45	63.89	6.57	42.08	54.31	-12.22
10	36.84	13.76	23.08	68.53	73.55	-5.01	84.21	84.94	-0.73
11	57.89	3.94	53.96	81.12	75.08	6.03	68.42	82.83	-14.42
12	36.84	12.99	23.85	47.38	51.86	-4.48	42.08	40.62	1.46
13	63.16	19.73	43.43	66.61	71.86	-5.25	57.88	63.88	-6.01
14	73.68	39.30	34.38	73.78	66.97	6.80	60.52	67.47	-6.95
15	-68.42	4.30	-72.72	71.33	69.75	1.58	68.42	69.36	-0.95
16	-1.75	4.30	-6.05	66.78	69.75	-2.97	71.06	69.36	1.70
17	-1.75	4.30	-6.05	66.78	69.75	-2.97	71.06	69.36	1.70
18	-68.42	4.30	-72.72	71.33	69.75	1.58	68.42	69.36	-0.95
19	5.26	4.30	0.96	69.58	69.75	-0.17	81.56	69.36	12.20
20	5.26	4.30	0.96	69.58	69.75	-0.17	81.56	69.36	12.20

Standard order	Efficiency of Oil and Grease			Efficiency of Ammonia Nitrate		
	Actual value	Predicted value	Error (%)	Actual value	Predicted value	Error (%)
1	38.47	36.25	2.22	97.72	97.45	0.28
2	81.50	73.02	8.48	98.86	98.13	0.73
3	26.14	25.06	1.08	74.09	74.86	-0.76
4	38.62	34.67	3.95	87.62	88.92	-1.30
5	37.44	39.28	-1.83	97.86	96.55	1.32
6	77.24	76.19	1.05	98.29	97.52	0.77
7	23.94	30.29	-6.35	77.65	78.37	-0.72
8	39.94	40.04	-0.10	92.46	92.72	-0.26
9	28.93	24.04	4.89	87.90	88.01	-0.11
10	33.92	47.30	-13.38	95.59	95.53	0.06
11	79.74	89.65	-9.91	98.72	101.82	-3.10
12	67.40	65.98	1.43	91.17	88.12	3.05
13	43.47	59.19	-15.73	95.02	93.96	1.06
14	70.63	63.39	7.24	94.31	95.41	-1.11
15	70.19	65.21	4.98	95.73	95.43	0.30
16	64.90	65.21	-0.30	95.73	95.43	0.30
17	64.90	65.21	-0.30	95.73	95.43	0.30
18	70.19	65.21	4.98	95.73	95.43	0.30
19	69.02	65.21	3.81	94.88	95.43	-0.55
20	69.02	65.21	3.81	94.88	95.43	-0.55

CONCLUSIONS

The characterization of the treated palm oil mill effluent consists of the BOD (285 mg/L), and oil and grease (68.1 mg/L) above the emission standards provided by Environment Quality Act 1974, (2005). The highest effectiveness percentage of the phytoremediation treatment using *A. pinnata* is 93%. Meanwhile, the BOD removal is about 46%. The optimization process shows that the optimum conditions for the phytoremediation process using *A. pinnata* is at 10% concentration with retention time of 4.71 days and 30 grams of *A. pinnata* with the value of desirability is 0.9.

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