Optimization and performance evaluation for nutrient removal from palm oil mill effluent wastewater using microalgae

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Abstract. Palm oil mill effluent (POME) wastewater was produced in huge amounts in Malaysia, and if it discharged into the environment, it causes a serious problem regarding its high content of nutrients. This study was devoted to POME wastewater treatment with microalgae. The main objective was to find the optimum conditions (retention time, and pH) in the microalgae treatment of POME wastewater considering retention time as a most important parameter in algae treatment, since after the optimum conditions there is a diverse effect of time and pH and so, the process becomes costly. According to our knowledge, there is no existing study optimized the retention time and pH with % removal of nutrients (ammonia nitrogen NH\textsubscript{3}-N, and orthophosphorous PO\textsubscript{4}\textsuperscript{3-}) for microalgae treatment of POME wastewater. In order to achieve with optimization, a central composite rotatable design with a second order polynomial model was used, regression coefficients and goodness of fit results in removal percentages of nutrients (NH\textsubscript{3}-N, and PO\textsubscript{4}\textsuperscript{3-}) were estimated. WinQSB technique was used to optimize the surface response objective function for the developed model. Also experiments were done to validate the model results. The optimum conditions were found to be 18 day retention time for ammonia nitrogen, and pH of 9.22, while for orthophosphorous, 15 days were indicated as the optimum retention time with a pH value of 9.2.

1. Introduction

Raw palm oil mill effluent (POME) wastewater is a colloidal suspension containing 95-96% water, 0.6-0.7% oil, and 4-5% total solids, including 2-4% suspended solids, also POME contains high concentrations of proteins, carbohydrate, nitrogenous compounds, lipids and minerals that may be converted into useful materials using microbial processes. If this untreated POME was thrown out to the environment, it has a negative effect on it, therefore it must be a critical needful solution savings the equilibrium between environmental safety and sustainable energy that can be produced from nutrient materials existing in the POME [1]. Bio-treatment of POME with microalgae is the solution because the algae grows in photosynthesis method that has the ability to use solar energy and transforming it to a profitable biomass take advantages from nitrogen and phosphorous compounds presented already in POME. Microalgae culture becomes an economizing solution to the problem treats the wastewater by consuming nutrients by microalgae growth and transform it to a biomass...
which is in turn a major source of biofuel [2]. Colak et al (1988) [3], used the microalgae for industrial wastewater treatment. Mohammed (1994) [4], used algae to treat wastewater with high rates of nitrogenous compounds. The usual methods of experimentation in any process have been neglecting the interaction effects between the operating parameters rather than it's considered as time consuming methods. Thus, the efficiency of the optimization process is negatively affected by these restrictions, and as a result, the response surface methodology (RSM) statistical experimental design is detected. Some researchers optimized the biological treatment using different microalgae strains to treat the wastewater of various sources [5-10]. This study is devoted to the treatment of POME wastewater using biological method, consisting of microalgae treatment of the wastewater, then optimization of the parameters affecting the biological treatment process using RSM statistical experimental design. Because there is no specific literature existed on how the retention time and pH of algal solution would affect along with the treatment of POME in range of applicable experimental variables. Thus the main goal of the present study was to investigate the effect of retention time (0-23 days) and pH (7.48-9.26) on the efficiency of algae treatment of POME, and optimize these operating parameters to find the optimum conditions to reach the highly efficient algae treatment with highest removal percentages of ammonia nitrogen (AN), and orthophosphorus (OP). Also, this process is considered as a completely green process without using any chemicals that produces environmental injured byproducts.

2. Materials and methods

2.1 Palm oil mill effluent (POME)
Palm oil mill effluent (POME) samples were taken directly after the last anaerobic digester from East Mill Sime Darby Plantation, Carey Island, Selangor, Malaysia, the samples were kept in a cool room at a temperature around 4°C before using, they were being centrifuged at 8000 rpm for 5 minutes to obtain the supernatant of the samples. The characteristics after centrifugation are shown in Table 1.

Table 1. Characteristic of POME after centrifugation.

| Parameter                        | Value       |
|----------------------------------|-------------|
| pH (-)                           | 7.48        |
| Chemical Oxygen Demand (COD), (mg/L) | 4045.0     |
| Ammonia Nitrogen (NH₃-N), (mg/L) | 334.0       |
| Orthophosphorus (PO₄³⁻), (mg/L)  | 465.0       |
| Total Suspended Solids (TSS), (mg/L) | 607.0       |
| Turbidity (NTU)                  | 395.5       |

2.2 Microalgae
In this research, the microalgae strain selected was *Botryococcus Braunii* which was obtained from the Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment in the National University of Malaysia. *Botryococcus braunii* in the concentration of 0.3 g/l was cultivated in Bold Basal Medium (BBM) for 10 days before transferring it into a conical flask containing two litters of AnPOME. The medium was aerated using a peristaltic pump with aeration of 1 l/hr placed on the bottom. The flask was placed under two continuous fluorescent lamps from both sides with an average light intensity of 1000 lux and a constant temperature of 21 ℃.

2.3 Analytical method
Ammonia nitrogen (NH₃-N) was measured using Nessler method while orthophosphorus (PO₄³⁻) was measured using Molybdovanadate method. NH₃-N, and PO₄³⁻ were analysed with the help of HACH DR/3900 spectrophotometer. For pH, a pH meter was used.

2.4 Experimental Procedure
After the microalgae strain of *B. braunii* was cultivated for 10 days, 200 ml was inoculated into 2 liters of POME that was pretreated with centrifugation for removal of solids. The reactor consists of a 2 liter conical flask with providing aeration from the bottom using a peristaltic pump. The top of the conical flask was covered with double layer aluminum foils for prevention of contamination with other microalgae strains. Two continuous fluorescent lamps were placed from both sides above the reactor with a constant temperature of 21 °C. The samples were taken with an interval of 3 days, from day 0 to day 15 retention time (RT) and the sample taken was on day 23 retention time. Throughout the entire treatment, the pH was not adjusted by any chemicals. As the samples were collected, another round of centrifugation was done to obtain the supernatant of all the samples. Then analysis of NH$_3$-H, PO$_4$-3, and pH were done to observe the performance of microalgae treatment with different retention time.

### 3. Optimization technique

In order to achieve with the RSM of experimental design, we must firstly calculate the number of experiments required as $2^K + 2K + 1$, where, $K$ is the number of variables. It means that for two operating variables, we need at least 9 experiments, plus two experiments represent the probability of experimental errors. According to RSM of experimental design, the operating variables that need to be optimized such as retention time ($X_1$), and pH ($X_2$), were correlated in a non-linear second order polynomial model equation, which is more sufficient proposed model to show the interaction effects between variables. The coefficients of model equation were estimated using a STATISTICA program (version 8.0). The proposed model was:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_1X_2 + a_4X_1^2 + a_5X_2^2$$

Where: $x_1$ and $x_2$ are operating variables representing retention time (day), and pH, while, $Y$ represents the objective function as percentage removal of nutrients (%). The optimization process was done using a winQSB technique (winQSB software version 1.0) in order to find the optimum values of operating conditions for the purpose of enhancing the efficiency of the POME treatment process using the microalgae.

### 4. Results and discussion

#### 4.1 Multiple regression analysis results

In order to achieve with the objective, a polynomial model regression coefficients and goodness of fit results in removal percentages of nutrients were estimated as:

**4.1.1 Ammonia nitrogen (AN)**

For ammonia nitrogen (AN), the second order polynomial is:

$$Y = 3389.118 + 124.819X_1 - 856.201X_2 - 13.4514X_1X_2 + 0.007139X_1^2 + 53.8917X_2^2$$

With goodness of fit analysis as: Correlation coefficient ($R$)=0.99955, the variance explained=99.91%, mean square error=0.84943, and mean absolute error=0.7115.

**4.1.2 Orthophosphorous (OP)**

For orthophosphorous (OP), the second order polynomial is:

$$Y = -6494.96 - 105.026X_1 + 1522.328X_2 + 11.9975X_1X_2 - 0.19423X_1^2 - 88.5063X_2^2$$

With goodness of fit analysis as: Correlation coefficient ($R$)=0.9992, the variance explained=99.84%, mean square error=0.51285, and mean absolute error=0.5535. Table 2, shows the results of experimental design.
4.2 Optimum condition results and effect of operating parameters

The optimum conditions that give optimum objective functions are explained as follows: The optimum conditions for % removal of AN are: Time = 18 days, and pH = 9.22 gives maximum removal of 92.857%. Figure 1, shows the optimum retention time, and figure 4, shows the effect of time and pH on % removal of AN as a response surface. It was clearly obvious that 18 days is the best time for maximum removal of AN, since before day 18, the microalgae still grown by feeding with nitrogen and phosphorous compounds in the POME wastewater, and after a day (18), the values of removal percentages of AN were lowered because that microalgae capacity to eliminate AN was reduced and the microalgae became older. Also, when the microalgae removed AN, it was simultaneously transferred it to the biomass as a result of concentration equilibrium and mass conservation, that's clarified why the removal percentage was decreased after optimum retention time. But at the optimum it has a maximum removal and maximum conversion of AN into biomass, which is more useful in biofuel production. The optimum conditions for % removal of OP are: Time = 15 days, and pH = 9.2 gives maximum removal of 56.5%. Figure 2, shows the optimum retention time. The retention time (15) day represents the optimum retention time for OP removal. It is the same reason for AN removal, dealing with equilibrium and mass conservation of OP in the algal solution, figure 5, shows the effect of time and pH on % removal of OP as a response surface. Figure 3, shows the effect of pH values on AN and OP concentrations, it can be clearly distinguished that at optimum value of pH, found a minimum concentration of nutrients. In addition to that the mirage consumption of AN is more significant and has a sharper decreasing than OP within the experimental range of operating variables, this can be attributed to chemical composition and initial concentration of each compound in the raw POME wastewater.

| Coded values | Real values | OP removal (%) | AN removal (%) |
|--------------|-------------|----------------|----------------|
| Retention time (day) | pH (-) | Retention time (day) | pH (-) | predicate | observed | predicate | observed |
| -1 | -1 | 0 | 7.48 | -0.00223 | 0.000 | -0.00289 | 0.000 |
| -1 | 0 | 0 | 9.14 | 25.362 | 25.255 | 65.473 | 65.320 |
| -1 | +0 | 0 | 9.26 | 42.127 | 43.101 | 81.720 | 80.980 |
| 0 | -1 | 12 | 7.50 | 44.532 | 44.962 | 83.209 | 83.090 |
| 0 | 0 | 12 | 9.26 | 48.833 | 47.995 | 85.857 | 85.160 |
| 0 | +0 | 12 | 9.14 | 52.948 | 52.600 | 89.050 | 88.600 |
| +1 | -1 | 23 | 7.50 | 47.899 | 47.560 | 90.642 | 89.940 |
| +1 | 0 | 23 | 9.14 | 48.552 | 48.521 | 91.012 | 90.970 |
| +1 | +0 | 23 | 9.26 | 49.490 | 49.400 | 91.517 | 91.400 |
| 0 | 0 | 12 | 9.14 | 52.948 | 52.600 | 89.050 | 88.600 |

Table 2. Results of RSM experimental design.
Figure 1. Effect of retention time on % removal of ammonia nitrogen.

Figure 2. Effect of retention time on % removal of orthophosphorous.

Figure 3. Effect of pH on ammonia nitrogen and orthophosphorous concentrations.
4.3 Evaluation of the process efficiency
From the point of view of researcher, the process is succeed and cost effective, since it used only biological source in the treatment and no chemicals were present, therefore it considered as a totally green treatment process and environmentally friendly. In addition, its useful biomass could be use in a recently highly developed biofuel production.

5. Conclusions
From this experimental work and optimization, we can conclude that: biotreatment using microalgae is considered as a promising method for POME wastewater treatment in addition to biofuel production, distinguished as totally green process without any chemicals used. From optimization done on operating variables of microalgae treatment, it was shown that retention time is a very important parameter needs to be estimated carefully in order to keep the treatment process useful and cost effective. According to optimization process using experimental design technique, the optimum conditions for % removal of ammonia nitrogen was 18 days retention time, and pH value of 9.22, while for % removal of orthophosphorous, it was 15 days retention time, and 9.2 for pH value.
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Acknowledgments

The authors would like to thank the staff and support of Faculty of Engineering and Build Environment in Universiti Kebangsaan Malaysia/ UKM for their financial support in doing the research.