Jurnal
Teknologi

Full Paper

PHOTO-FENTON TREATMENT OF SAGO WASTEWATER: RSM OPTIMIZATION AND TOXICITY EVALUATION

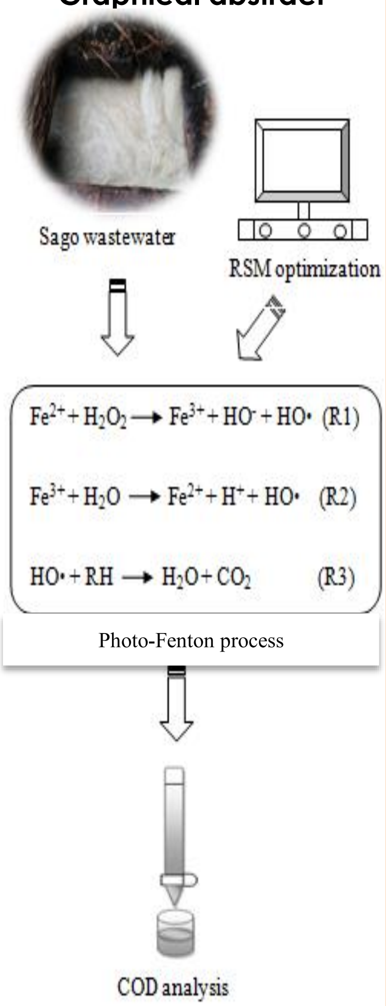
Devagi Kanakaraju[a*], Wong Soon Pang[a], Wan Azelee Wan Abu Bakar[b]

[a]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[b]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

Article history
Received
3 April 2017
Received in revised form
23 August 2017
Accepted
1 November 2017

*Corresponding author
kdevagi@unimas.my

Graphical abstract



Abstract

Due to the fact that organic matter in sago wastewater is not effectively removed by current traditional methods, this study was designed to systematically investigate the performance of photo-Fenton treatment. Despite being ratified for its high efficiency in improving wastewater quality, there remains a paucity of evidence on its performance on sago wastewater. Thus, the objective of this study was to optimize the conditions of the photo-Fenton process by employing the response surface methodology (RSM) using the chemical oxygen demand (COD) removal as the target parameter. Fenton's reagent (Fe^{2+} and H_2O_2 concentration) and pH were used as the independent variables to be optimized. Under optimum conditions, 90.0% of COD removal efficiency was obtained when the wastewater sample was treated at pH 2.66 in the presence of 4.01 g/L of $H_2O_2$and 5.07 g/L Fe^{2+}ion. Despite the high COD removal, the total organic carbon (TOC) removal under the same optimized condition was lower, only 48.0% indicating incomplete mineralization of stable intermediates present in the solution. Toxicity evaluation revealed that the mortality of *Artemia salina* was less than 50%, which means that the treated sago wastewater can be considered as non-toxic. The regression value (R^2> 0.99) of the models indicates a high degree of correlation between the parameters evaluated. The results obtained indicate the feasibility of photo-Fenton treatment to the sago wastewater as an appealing alternative approach.

Keywords: Chemical oxygen demand, degradation, Box-Behnken design, wastewater, Fenton

Abstrak

Disebabkan oleh kandungan bahan organik dalam sisa air sagu yang tidak dapat dirawat secara berkesan oleh kaedah rawatan tradisional, kajian ini telah dijalankan untuk menyiasat prestasi rawatan foto-Fenton secara sistematik. Walaupun kaedah foto-Fenton telah diperakui tahap keefisienannya untuk memperbaiki tahap kualiti air sisa, masih terdapat kekurangan bukti atau data yang kukuh bagi sisa air sagu. Oleh itu, objektif kajian ini adalah untuk mengoptimumkan kondisi proses foto-Fenton dengan menggunakan kaedah gerak balas permukaan (GBP) dan penyingkiran permintaan oksigen kimia (POK) sebagai parameter sasaran. Reagen Fenton (kepekatan Fe^{2+} dan H_2O_2) dan pH telah digunakan sebagai pembolehubah tak bersandar untuk dioptimumkan. Dalam keadaan yang optimum, sebanyak 90.0% kecekapan penyingkiran POK telah diperolehi apabila sampel air sisa sagu yang pada pH 2.66 dan 4.01 g/L H_2O_2 and 5.07 g/L Fe^{2+} ion. Walaupun penyingkiran POK yang diperolehi adalah tinggi, jumlah karbon organik yang diperolehi pada keadaan yang optimum didapati rendah iaitu hanya 48% menunjukkan mineralisasi yang tidak sempurna bagi bahan perantaraan stabil yang hadir dalam larutan. Ujian ketoksikan menunjukkan tahap kematian *Artemia salina* *adalah kurang daripada 50% dimana ia menunjukkan* air sisa sagu yang dirawat adalah tidak toksik. Nilai regrasi (R^2> 0.99) model menunjukkan tahap korelasi yang tinggi di antara parameter-parameter yang dinilai. Keputusan kajian menunjukkan rawatan foto-Fenton dapat digunakan sebagai kaedah alternatif yang menarik dalam rawatan sisa air sagu.

Kata kunci: Permintaan oksigen kimia, degradasi, reka bentuk kotak-Behnken, air sisa, Fenton

1.0 INTRODUCTION

Solid or liquid waste derived from series of post-processing steps in agricultural industry could impose significant impacts on environmental degradation if not properly treated or managed. Agro-based industries contribute significantly to economic growth of countries around the globe. Nevertheless, from the environmental point of view this industry also inevitably tend to increase the accumulation of wastes which demand considerable attention. One of the most important crops for starch production is sago palm (Metroxylon sagu). Sago palm, known as the highest starch-producing crop at 25/t/ha/year are mainly concentratedin the State of Sarawak, Malaysia with more than 90 % of the total planting areas [1, 2]. The sago residues from sago starch processing mills are abundant and readily available. Wastewater effluent generated during sago debarking and sago processing is generally discharged into nearby rivers [3]. A study reported that approximately 400 tons of effluent are estimated to be generated from a sago mill, which typically consumes about 1,000 logs/day [4]. About 94-97% of the bulk of the sago wastewater is liquid, while the remaining portion is solid waste referred as roughage or 'hampas'. Sago wastewater effluent is complex and acidic in nature and typically characterized by elevated chemical oxygen demand (COD), 780-5130mg/L, biochemical oxygen demand (BOD), 910-1300 mg/L and total suspended solids (TSS) 19-20,000 mg/L [5,6] and emits obnoxious odour. As the direct discharge of sago wastewater effluent may cause issues related to environmental deterioration, its treatment for either re-using or improving its discharged water quality has been the main interest of the sago mill operators.

Thus far, biological methods have been widely used to treat sago wastewater in other countries particularly in India include high-rate anaerobic treatment such as anaerobic fluidized beds and filters [7], anaerobic tapered fluidized bed reactor [8], three-phase fluidized bed bioreactor [9], and hybrid upflow anaerobic sludge blanket reactor [10]. Also, bio-management methods using bacteria and fungi have been utilized to treat sago wastewater [11, 12]. These biological methods although known for their efficiency, are unable to achieve the complete degradation of organic matter because of bio-refractory nature of substrates and require additional treatment [13]. In this viewpoint, advanced oxidation processes (AOPs), a non-biological method could be a desirable alternative to degrade organics present in the sago effluent as this treatment has emerged as an effective method for the degradation of various biorecalcitrant compounds in water and wastewater.

AOPs which are chemical oxidation processes have been proven as highly effective for the oxidation of industrial wastewater containing toxic and organic materials [14]. AOPs involve the generation of reactive oxygen species such as hydroxyl (HO•) radical, a strong oxidant, which reduces organic materials into biodegradable and simpler end products, CO_2 and H_2O [15, 16]. Among various AOPs, Fenton and photo-Fenton processes appear as an attractive option for their efficiency in industrial effluent abatement. Due to the short reaction time and high efficiency, photo-Fenton could be considered as an alternative method in industrial applications [17, 18]. Photo-Fenton process involves an artificial light source or irradiation with sunlight, which improves the rate of contaminant degradation [19]. When H_2O_2 reacts with Fe^{2+} ions in the presence of ultra violet (UV) light, HO• radicals are generated by the decomposition of H_2O_2 [20]. The generated HO• radicals will attack the organic materials and mineralize them into CO_2 and H_2O *via* the formation and decomposition of intermediates [21]. Although photo-Fenton treatment has been extensively studied, the application of this treatment on sago wastewater effluent has not been investigated.

The factors that affect the effectiveness of the photo-Fenton process are the amount of Fe^{2+} and H_2O_2 and also the initial pH [22]. Optimization of such parameters plays a crucial role towards the enhancement of the photo-Fenton reaction. In the present study, the experimental design using a statistical-based technique, commonly known as response surface methodology (RSM), was used to study the optimization of the photo-Fenton treatment process. Various published studies have applied photo-Fenton combined with RSM in their wastewater degradation studies [23, 12, 24]. RSM is generally applied to optimize the parameters of photo-Fenton's reagent in order to maximize the degradation efficiency and also reduce the number of experiments needed to be conducted [25]. Process optimization by RSM is more economical and a faster method for obtaining experimental research results than the rather full-factorial or time-consuming one-factor-at-a-time approach [26]. To the best of our knowledge, no studies have been performed on the photo-Fenton treatment on sago wastewater effluent. Also, sago wastewater effluent failed to be recognized as nuisance compared to the great attention given to converting solid residues into value added products. As RSM is known for its superiority in optimizing chemical reactions, this study was designed to use this statistical tool in obtaining the optimal conditions for the degradation of sago wastewater effluent primarily focusing on COD as the target parameter.

The aim of this work was to investigate the effectiveness of photo-Fenton process on the degradation of sago wastewater effluent by optimizing the process variables, the concentration of H_2O_2 and Fe^{2+} ion and initial pH *via* RSM. The toxicity of the treated effluent was also tested using *Artemia salina* bioassay.

2.0 METHODOLOGY

2.1 Materials

Iron (II) sulfate-7-hydrate and hydrogen peroxide (H_2O_2, 35%) were purchased from Bendosen. Sulfuric acid (H_2SO_4, 95-97%) and sodium hydroxide (NaOH) were obtained from Merck (Germany).

2.2 Sago Wastewater

Sago wastewater sample was collected from the cooling pond of Herdsen Sago Mill located in Pusa, Sarawak, Malaysia. The wastewater samples were filled into polyethylene bottles and transported to the laboratory and stored at 4^{0}C prior to further analysis. The collected wastewater samples were characterized for water quality parameters namely pH, BOD, COD, TSS, dissolved oxygen (DO) and turbidity.

2.3 Photo-Fenton Experiment

In a typical photo-Fenton experiment, different concentrations of Fe^{2+} ion (2 to 6 g/L) and hydrogen peroxide (3 to 5 mL/L) were added to a beaker containing 200 mL of filtered sago wastewater sample. Sago wastewater sample was filtered prior to treatment to ensure that the roughage or known as 'hampas', being the major portion of the wastewater does not interfere with the final COD reading and block the penetration of UV light during the photo-Fenton experiment. The concentration of both Fe^{2+} ion and H_2O_2 were optimized by RSM. The pH value of the solution was adjusted to the desired values with dilute H_2SO_4 or NaOH solution. pH was measured using a pH meter (PHS-3BW model, Bante instrument)before being subjected to photo-Fenton oxidation. The solution mixture was subsequently stirred at 750 rpm in the dark condition for 30 min to establish the absorption and equilibrium. The mixture was irradiated using a UVC lamp (15 W) (λ = 254 nm, Phillips TUV 8 W) for 2 h. Temperature and light intensity ranging between 30 and 32°C and light intensity of 120-173 lux, respectively were recorded during the photo Fenton experiments. Samples were collected prior to irradiation and at fixed time intervals throughout the 2 h irradiation period. To ensure the complete decomposition of H_2O_2 and Fe^{2+} ion in the collected sample prior to COD determination, NaOH pellet was added to the sample to increase the pH>8. After the samples have stood overnight, they were centrifuged and filtered to remove Fe^{2+}. The supernatant was subjected to COD analysis. Figure 1 summarizes the typical steps involved in the lab-scale photo-Fenton experiment performed.

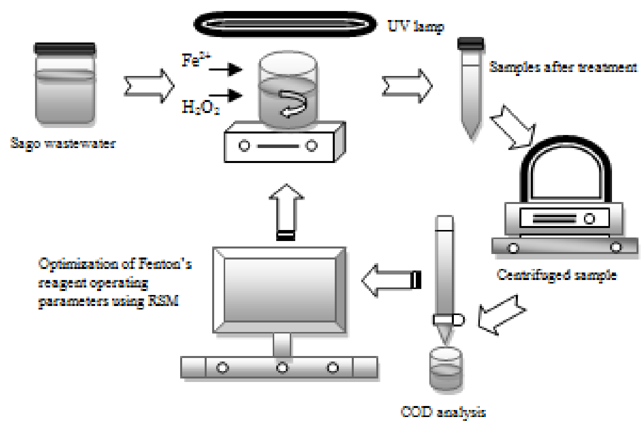


Figure 1 Schematic diagram of a lab-scale photo-Fenton experiment

2.4 Analytical Determinations

DO was recorded using a dissolved oxygen meter (Milwaukee brand, MW 600 model). The turbidity of the water sample was determined by means of a turbidity meter (Mi 415 model, Martini Instrument). COD, BOD, TSS, and total organic carbon (TOC) analyses were performed based on the Standard Methods for Water and Wastewater Examination [27]. UV-Vis spectrophotometer (Jasco V-630) was used to record the changes of UV absorbance of sago wastewater samples. TOC was measured using OI Analytical (Aurora 1030 model) TOC analyzer.

2.5 Experimental Design

Box-Behnken Design (BBD) which is a widely used form of RSM was utilized for the optimization of photo-Fenton-like treatment of sago wastewater. The benefits of BBD consist of fact that they require variables to be run at only three levels and are all spherical designs [28]. Besides, BBD also provides an accurate result and minimum effort instead of complete factorial design. In the present study, the concentration of Fe^{2+} ion and H_2O_2, and initial pH were considered as the independent variables, while the COD removal efficiency was considered as the dependent variable (response). These independent variables were coded as -1 (low), 0 (centre point) and +1 (high) in the ranges identified by a preliminary work. Table 1 summarizes the experimental range and levels of independent variables considered in this study. Based on a three-level BBD, a total number of 17 experiments were carried out in a randomized order with five centre points. The initial design included 17 tests, based on a three-level BBD shown in Equation (1) [22]:

$$\eta\ (\%) = \frac{COD_0 - COD_f}{COD_0} \times 100, \quad (1)$$

where η represents the percentage of COD removal; COD_0 and COD_f designate the measured COD values before and after the photo-Fenton process, respectively. Table 2 shows the 17 experiments with the ranges and levels in coded units.

Table 1 Range and levels of independent variables for RSM

Variables	Coded	Range and levels		
		-1	0	1
H_2O_2 (mL/L)	X_1	3.00	4.00	5.00
Fe^{2+} (g/L)	X_2	2.00	4.00	6.00
pH	X_3	2.00	2.50	3.00

Table 2 RSM for the three independent variables in corresponding natural values and coded units

Run	Independent variables			Coded variables		
	H_2O_2 (mL/L)	Fe^{2+} (g/L)	pH	X_1	X_2	X_3
1	4.00	6.00	2.00	0	+1	-1
2	4.00	4.00	2.50	0	0	0
3	3.00	4.00	2.00	-1	0	-1
4	4.00	6.00	3.00	0	+1	+1
5	4.00	2.00	2.00	0	-1	-1
6	3.00	2.00	2.50	-1	-1	0
7	5.00	2.00	2.50	+1	-1	0
8	3.00	6.00	2.50	-1	+1	0
9	4.00	2.00	3.00	0	-1	+1
10	5.00	6.00	2.50	+1	+1	0
11	4.00	4.00	2.50	0	0	0
12	5.00	4.00	2.00	+1	0	-1
13	4.00	4.00	2.50	0	0	0
14	3.00	4.00	3.00	-1	0	+1
15	5.00	4.00	3.00	+1	0	+1
16	4.00	4.00	2.50	0	0	0
17	4.00	4.00	2.50	0	0	0

A trial version of Design-Expert 7.1.6 software was utilized to analyze the experimental data and fitted to a second-order polynomial equation. The interaction between the dependent and independent variables was attained through Equation (2) [29]:

$$\eta = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (2)$$

where η represents the predicted response (dependent variable), k is the number of patterns, i and j are the index numbers for pattern, β_0 is the offset or free term called intercept term, x1, x2,…, xk are the coded independent variables, β_i is the first-order (linear) main effect, β_{ii} is the quadratic (square) effect, β_{ij} is the interaction effect, and ε designates the random error between experimental and predicted values. Due to the regression and mean square residual error, ANOVA was used to test the statistical significance of the ratio of mean square variation [28]. The value of correlation coefficient (R^2) was used to express the quality of the fit of the polynomial model. The main indicators including the probability value (Prob>F), model F-value (Fisher variation ratio), and adequate precision showed the adequacy and significance of the employed model. To find an optimum condition, the response plots such as contour and 3D plot were used. Fischer's F-Test was utilized to determine the statistical significance and optimize the desired target fixed for maximum removal of COD. For process optimization, the desired target for the response (COD removal) was selected as to "maximize", while independent variables (concentration of Fe^{2+} and H_2O_2, initial pH) were chosen to be "within the range". For maximization to find the best and optimize local maximum, these individual targets were combined into an overall desirability function by the software [30].

2.6 *Artemia salina* Bioassay

Bioassay study using *Artemia salina* was performed to determine the toxicity of sago wastewater after being treated by photo-Fenton treatment. *A. salina* eggs were added into a clean beaker filled with natural seawater for hatching purpose. The hatching beaker was illuminated by a tungsten filament light and aerated with air at room temperature (25^{0}C) [31]. After 24 h, the hatched *A. salina* was transferred to fresh natural seawater. The hatched *A. salina* was incubated under artificial light with air sparging for further 24 h. For toxicity test, an aliquot of 0.5 mL of treated sago wastewater was poured into micro-wells and added with 5 mL of fresh seawater. Each micro-well was then placed with 10 *A. salina* nauplii and incubated at room temperature with low ambient light [32]. The number of death *A. salina* nauplii was recorded after 24 h.

3.0 RESULTS AND DISCUSSION

3.1 Sago Wastewater Characterisation

The characteristics of sago wastewater effluent are summarized in Table 3. The supernatant of sago effluent is pale brownish in colour with a pungent smell. The sago wastewater demonstrated acidic value. A large amount of TSS (4000-8200 mg/L) would have caused the high BOD and COD of the wastewater, ranging from 800-3300 mg/L and 6192-34048 mg/L, respectively.

The BOD, COD and TSS values obtained in this study were compared to the wastewater characteristics from another sago mill, Nitsei Sago Mill Sdn Bhd in Mukah published by the Department of Environmental, Sarawak (2001). The BOD, COD and TSS values of sago wastewater from Nitsei Sago Mill were 2528-4647 mg/L, 6048-12594 mg/L, and 3368-15890 mg/L, respectively. The BOD and TSS values of sago wastewater from Nitsei Sago Mill were slightly higher than that of recorded in the Herdsen Sago Industry. The TSS, which are mainly roughage or 'hampas', suspended in the sago wastewater would have caused the high turbidity. Relatively, the characteristics of sago wastewater from Herdsen Sago Industry were comparable to the Nitsei Sago Mill although the differences in their water qualities are notable for several parameters as mentioned previously. It appears that the traditionally practiced cooling pond system failed to improve the discharge quality of sago wastewater effluent. As the effluent quality did not comply with the regulatory requirement of the DOE (2010) [33] (Table 3), this thus necessitates the application of a more powerful treatment tool prior to discharge.

Table 3 Characteristics of sago wastewater effluent

Parameters	Range	Average value ± SD	Parameters limits of effluent (Standard B)*
pH	5.12-6.06	5.58± 0.50	5.50-9.00
TSS (mg/L)	4000-8200	6730.00 ±1777.80	100.00
BOD (mg/L)	800-3300	1916.67±124 6.46	50.00
COD (mg/L)	6192-34048	19304.00± 95 65.11	200.00
Turbidity (NTU)	109-566	328.00± 239. 77	Not available
DO (mg/L)	0.16-1.00	0.53 ±0.41	Not available

*DOE (2010) [33]

3.2 Model Fitting

According to the experimental design, 17 sets of experiments with operating conditions were conducted in order to investigate the variables (concentration of Fe^{2+} ion and H_2O_2, and initial pH) involved in the photo-Fenton reaction (Table 2). The data from the experimental results were fitted to the polynomial equation to quantify the curvature effects. The second-order fitting polynomial equation of coded factors was expressed as shown in Equation (3):

$$\eta\ (\%) = 85.18 - 4.70X_1 + 21.17X_2 + 5.15X_3 + 11.10X_1X_2 - 2.26X_1X_3 + 7.70X_2X_3 - 20.51X_1^2 - 22.17X_2^2 - 14.46X_3^2 \quad (3)$$

The complete 17 sets of the experimental design matrix and response based on experimental results and predicted values on COD removal proposed by BBD are tabulated in Table 4. The obtained percentage of COD removal efficiencies varied between 5.66 to 87.02% (Table 4) and the model predicted values matched these experimental results satisfactorily (Figure 2). A graph of predicted values and experimental results for COD removal is plotted in Figure 2. From Figure 2, which depicts a regression coefficient, R^2 value of 0.99 confirmed the results between the experimental and predicted data. If the R^2 value is less than 0.80, the model will be rejected [34]. Accordingly, it is reasonable to declare that the polynomial model (Equation (2)) is a reliable tool to delineate the Fenton reaction behaviour in sago wastewater treatment.

Table 4 Experimental results and predicted values of η for percentage of COD removal using RSM

Run	COD removal, η (%)	
	Experimental results	Predicted values
1	57.14	56.87
2	83.33	85.18
3	47.37	47.51
4	82.50	82.56
5	30.00	29.94
6	37.21	37.14
7	5.66	5.53
8	57.14	57.27
9	24.56	24.83
10	70.00	70.07
11	85.00	85.18
12	42.42	42.61
13	84.85	85.18
14	62.50	62.31
15	48.53	48.39
16	87.02	85.18
17	85.71	85.18

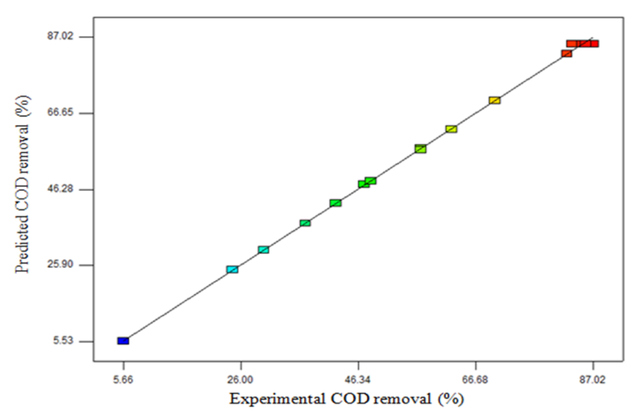


Figure 2 Predicted versus experimental values for COD removal (%) (R^2 = 0.99)

3.3 Statistical Analysis

Statistical analysis of variance (ANOVA) using Design-Expert 7.1.6 software was performed to further assess the polynomial model (Equation (3)) taking into account the interaction of factors. Fisher's F-test, at which the F-value is the ratio of the mean square of regression to the mean square of the error, was used to determine the statistical significance of the factors towards the response. Table 5 shows the ANOVA results for the percentage of COD removal. As shown in Table 5, the F values are higher for all regressions. The large F value shows that most of the variation in the response can be interpreted by the regression equation. The probability values (p-values) act as a tool to investigate the significance of the model. Generally, if the significance probability value (p>F) is less than 0.05, the model is considered to be statistically significant and acceptable [35]. For all the regressions, the p-values are lower than 0.01, which means that minimum one of the terms in the regression equation has a significant correlation with the response (dependent) variable.

Based on the ANOVA analysis, the model F-value was 1029.70 which revealed that the model was significant. This indicates that a "model F-value" has < 0.01% probability to occur due to noise. For the response, the square of correlation coefficients was computed as the coefficient of determination (R^2). It depicted high significant regression at 95% confidence level. The adequacy of the model was tested *via* the lack-of-fit F-tests [36]. The lack of fit F-value of 0.06 indicates that the lack of fit was not significantly relative to the pure error. This implies that there was 98.00% probability that a "lack of fit F-value" as large as this would occur due to noise. For further analysis, the quadratic model was chosen as the adjusted R^2 value (0.9983) and predicted R^2 value (0.9984) was found to be the maximum, respectively. Adequate precision measures the signal to noise ratio or, in other words, is a measure of the range in predicted response relative to its associated error. The desired ratio of adequate precision is 4 or greater [37]. The ratio of 100.087 shows an adequate signal (Table 5). Thus, this model can be utilized to navigate the designed space. Low values of coefficient of variation (CV) (1.78%) indicate good precision and reliability of the experiments. The greater the value of CV indicates the lower or poorer reliability of the experiment [38].

Table 5 ANOVA for the percentage of COD removal

Source	Sum of Squares	DF	Mean Square	F value	p-value Prob> F
Model	9977.89	9	1108.65	1029.70	< 0.0001
X_1	176.81	1	176.81	164.22	< 0.0001
X_2	3584.93	1	3584.93	3329.64	< 0.0001
X_3	211.77	1	211.77	196.69	< 0.0001
$X_1 X_2$	493.06	1	493.06	457.95	< 0.0001
$X_1 X_3$	20.34	1	20.34	18.89	0.0034
$X_2 X_3$	237.16	1	237.16	220.27	< 0.0001
X_1^2	1771.59	1	1771.59	1645.43	< 0.0001
X_2^2	2069.00	1	2069.00	1921.66	< 0.0001
X_3^2	880.96	1	880.96	818.23	< 0.0001
Residual	7.54	7	1.08		
Lack of Fit	0.31	3	0.10	0.057	0.9800
Pure Error	7.23	4	1.81		
Cor Total	9985.43	16			

R^2 = 0.9992, Adj R^2 = 0.9983, Pred R^2 =0.9984, Adeq Precision = 100.087

3.3.1 *Effect of Fe^{2+} and H_2O_2 Dosage on the COD Removal*

The three-dimensional (3D) response surface and two-dimensional (2D) contour plots of the model-predicted responses were obtained by the Design-Expert software. From the plots, two variables varying within the experimental ranges and one factor kept at constant were plotted and utilized to assess the interactive relationships between the variables and response for photo-Fenton treatment of sago wastewater. Figures 3a and b show the 3D surface and 2D plot of the model, respectively for COD removal with respect to the dosage of Fe^{2+} ion and H_2O_2 when the pH was kept constant at 2.50. The percentage of COD removal increased when the Fe^{2+} ion concentration was increased to about 5 g/L (Figure 3a). However, at higher concentration of Fe^{2+} ion, the COD removal rate was observed to decrease. This simply means that an increase in the concentration of Fe^{2+} ion does not improve the COD removal efficiency of the treated wastewater. Higher H_2O_2 concentration in the presence of Fe^{2+} produced more hydroxyl (•OH) radicals and thus improved the process efficiency. Nevertheless, excessive amounts of H_2O_2 and Fe^{2+} ion particles may inhibit the COD removal efficiency. The undesirable reactions between excessive amounts of H_2O_2 and Fe^{2+} ions and hydroxyl/hydroperoxyl radicals can be explained through Equations 4-7 [23]:

$$H_2O_2 + \bullet OH \rightarrow HO_2^{\bullet} + H_2O \quad (4)$$

$$Fe^{2+} + \bullet OH \rightarrow OH^- + Fe^{3+} \quad (5)$$

$$Fe^{2+} + HO_2^{\bullet} \rightarrow HO_2^- + Fe^{3+} \quad (6)$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow O_2 + Fe^{2+} + H^+ \quad (7)$$

Although higher Fe^{2+} ions concentration may benefit COD removal in the photo-Fenton process, the scavenging of the hydroxyl/hydroperoxyl radicals may occur due to the high Fe^{2+} ion concentration [23]. Likewise, the percentage of COD removal increased with the increasing concentration of H_2O_2 up to 4 mL/L after which the COD removal decreased (Figure 3a). Lower removal of obtained COD upon reaching the optimum amount of H_2O_2 could be due to the excess amount of H_2O_2 remaining in the solution, which becomes a hindrance in the result of COD of the final effluent [15]. Thus, it can be concluded that optimal dosage of both H_2O_2 and Fe^{2+} ion concentrations are crucial in the photo-Fenton treatment.

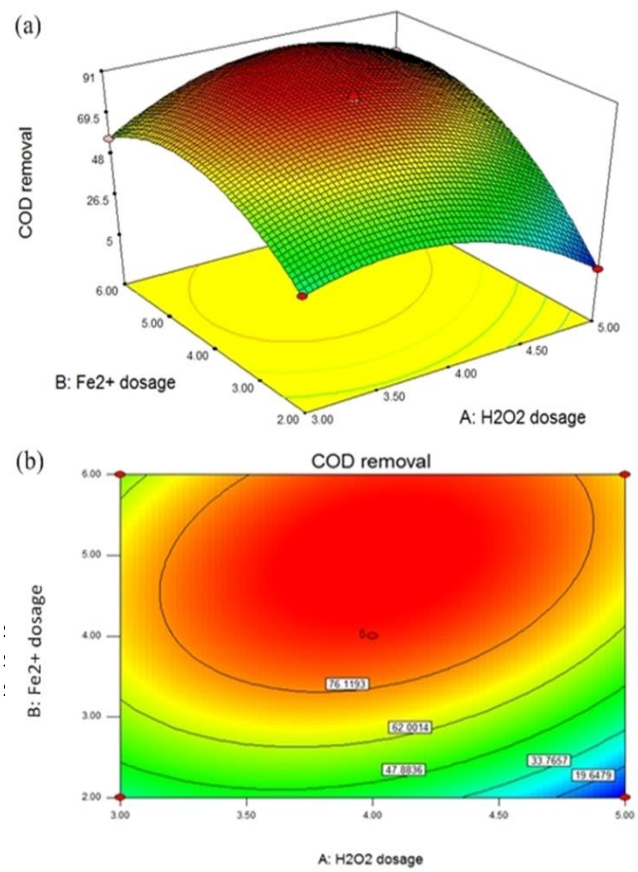


Figure 3 (a) 3D surface and (b) contour plot of response surface curve for COD removal showing interaction between Fe^{2+} and H_2O_2

3.3.2 *Effect of H_2O_2 Dosage and Initial pH on the COD Removal*

In the photo-Fenton process, H_2O_2 which acts as a dominant source of •OH radicals plays an important role in the organic wastewater treatment [39]. In the present experiment, the concentration of H_2O_2 is directly associated with the number of •OH radicals generated, and therefore, to the performance achieved. 3D surface and 2D contour plot in Figures 4a and b revealed that the combination of H_2O_2 concentration and initial pH has a significant effect on the COD removal. From Figure 4, the percentage of COD removal increased with the increasing concentration of H_2O_2 from 3.00 to 4.00 mL/L at an initial pH of 2.00 to 2.50 when Fe^{2+} ion concentration was kept constant at 4.00 g/L. The highest COD removal (>80.00%) was achieved at pH 2.50 and H_2O_2 concentration of 4.00 mL/L. However, the COD removal percentage was reduced when the H_2O_2 was higher than 4.00 mL/L and when the initial pH was beyond 2.50. The detrimental effect of higher H_2O_2 concentration may be due to the recombination of both •OH radicals and the auto-decomposition of H_2O_2 into oxygen and water [40]. Moreover, the oxidation potential of •OH radicals is known to decrease when the pH increases [41].

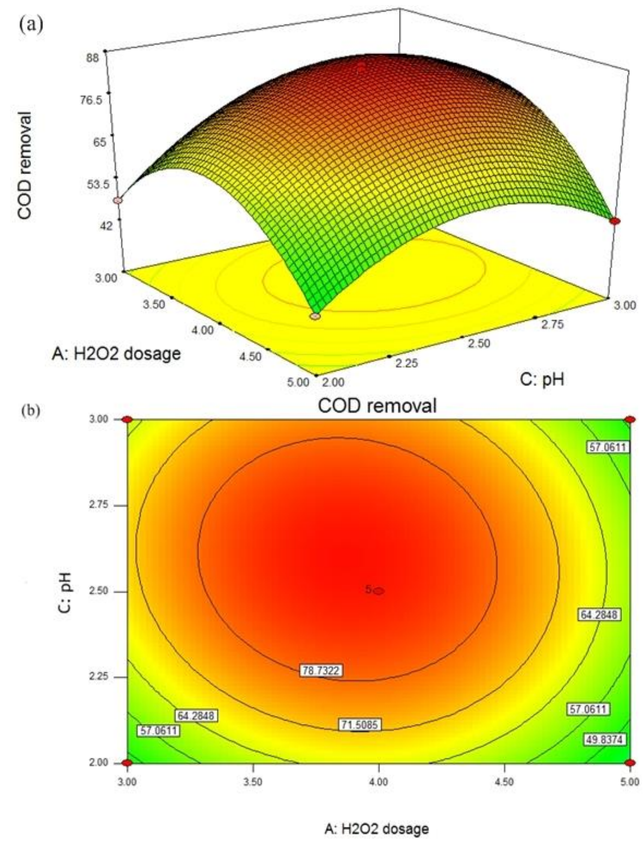


Figure 4 (a) 3D surface and (b) contour plot of response surface curve for COD removal showing interaction between H_2O_2 and pH

3.3.3 *Effect of Fe^{2+} Dosage and Initial pH on the COD Removal*

One of the main parameters that influence the photo-Fenton process is the amount of Fe^{2+} ion [41]. Besides that, the photo-Fenton process is strongly pH dependent as the pH value affects the generation of •OH radicals and therefore, the oxidation efficiency. Figures 5a and b demonstrate the 3D surface and 2D plot for the interaction of Fe^{2+}ion dosage and initial pH in terms of COD removal percentage (%), respectively. Based on Figure 5, the efficiency of COD removal can be seen to increase with the increasing concentration of Fe^{2+}ion from 2.00 to 5.00 g/L at all the pHs studied when the H_2O_2 dosage was kept constant at 4.00 mL/L. However, the COD removal percentage slightly decreased at higher concentration of Fe^{2+} (6 g/L). As reported by Samet *et al.* [41], the amount of Fe^{2+} ions in the Fenton process must be as low as possible for economic and environmental reasons. High amounts of Fe^{2+} ions may lead to the formation of Fe^{3+} sludge in the treated effluent. As a result of this, an additional step would be required for the removal of the iron sludge which would incur in cost and manpower [41]. As can be seen in Figure 5, the initial pH is also a determinant factor. On the pH axis shown in Figure 5a, at two sides of the middle pH (2.50), the efficiency of COD removal decreased as pH changed. At pH <2.50, the reaction between H_2O_2 and Fe^{2+} ion was significantly affected and thus causing a reduction in the production of •OH radicals due to the •OH radical scavenging by H^+ ions [41].

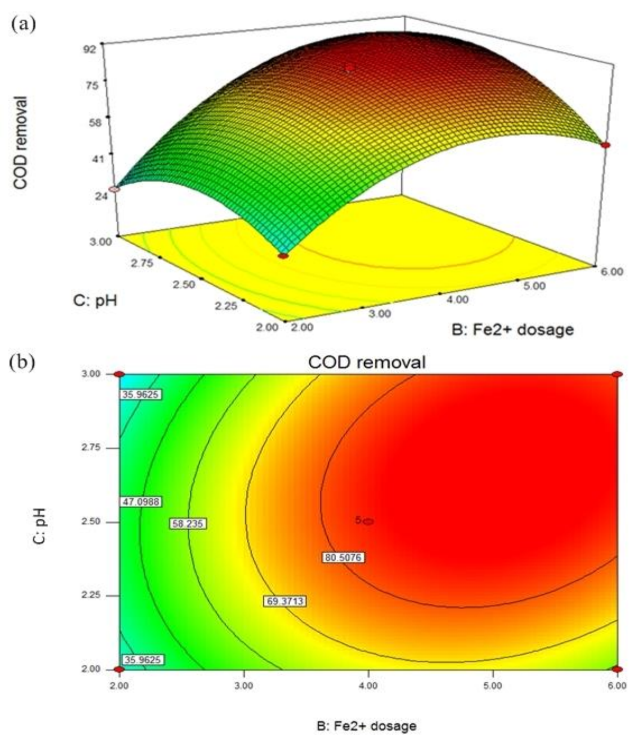


Figure 5 (a) 3D surface and (b) contour plot of response surface curve for COD removal showing interaction between Fe^{2+} and pH

3.4 Optimization Analysis and Verification of Results

In the present study, the desired target in terms of COD removal (%) is defined as "target" to achieve the highest performance of treatment. The process variables such as the concentration of H_2O_2 and Fe^{2+} and initial pH are chosen to be "in range". The optimal conditions derived from the statistical software are shown in Table 6.

Table 6 Optimum values of the photo-Fenton parameters for maximum efficiency

Parameter	Optimum value
COD removal, Y (%)	91.65
H_2O_2 (mL/L)	4.01
Fe^{2+} (g/L)	5.07
pH	2.66

Three duplicate experiments using the optimum operating conditions were conducted to validate the efficiency of the model. An average percentage of the COD removal of 90.00% was yielded from the duplicate experiments. Table 7 shows the efficiency of the predicted COD removal (%) *via* the polynomial model (Equation (2)). After the photo-Fenton treatment under the optimum operating conditions, the TSS and BOD of treated sago wastewater were reduced to 4330 mg/L and 427 mg/L, respectively which corresponded to 35.7% and 77.7% removal, respectively. Plotting shown in Figure 2 indicates that a good agreement of the data between the experimental and predicted can be obtained with an R^2 value of 0.99. Thus, it is reasonable to consider that the polynomial model (Equation (2)) is a reliable and acceptable model to describe the Fenton reaction behaviour in wastewater treatment.

Table 7 Experimental and predicted values for the COD removal at optimum conditions

Type of value	COD removal, (%)
Experimental	90.00
Predicted	91.65

3.5 Total Organic Carbon Analysis

TOC removal for sago wastewater under optimized photon-Fenton treatment is depicted in Figure 6. TOC reduction of about 48% was obtained after 2 h of photo-Fenton treatment under the optimized conditions (Figure 6) which mean only 48% of organic conversion or decomposition took place. TOC removal was rather low compared to the COD removal (90%) under similarly optimized conditions. A recent study on the solar TiO_2 photocatalytic treatment of petroleum wastewater using RSM reported similar trend to this study whereby the experimental results of COD (45%) removal was relatively higher than the TOC (16.5%) removal [42]. The COD analysis is used to measure the total quantities of oxygen required to oxidize organic compound present in wastewater into simpler forms such as water and carbon dioxide [43] while TOC measures the amount of the organic carbon in wastewater. COD parameter, however, has been reported to be more accurate when investigating the efficiency of a treatment in wastewater [42].

The lower TOC conversion obtained could be attributed to the stable intermediates or partially oxidized by-products or organic matter which remained in the solution [44]. UV absorption of sago wastewater samples, 0 min (initial)and 120 min (final) treated under optimized photo-Fenton conditions which were recorded using UV-Vis spectrophotometer (Figure 7) supported the above reasoning. The UV profile at 0 min and 120 min indicates that the strong presence of compounds even at the end of the photo-Fenton treatment and bathochromic shift is notable. Judging from the UV profile, the formation or presence of stable intermediates at 120 min of photo-

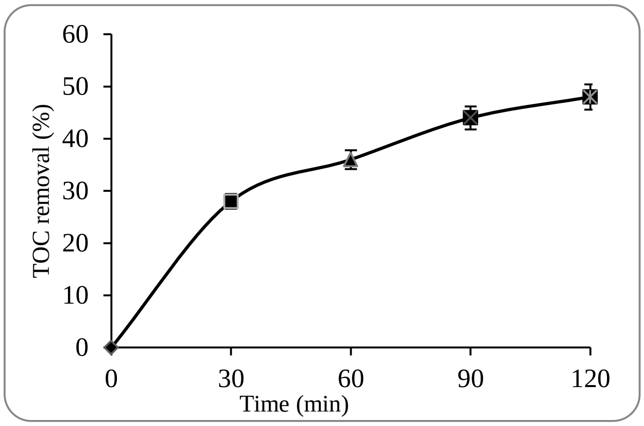


Figure 6 TOC removal against time for sago wastewater degradation under optimized photo-Fenton condition ([Fe^{2+}] = 5.07 g/L; [H_2O_2] = 4.01 mL/L; pH = 2.66)

Fenton treatment could have inhibited from the complete mineralization of organics [45]. These stable intermediates could be present at high concentration in the solution, as indicated by the higher absorbance at absorption band at 276 nm (120 min) (Figure 7). Therefore, one possible way for achieving conversion of organic compounds present in the sago wastewater effluent is by extending the reaction time. The identification of degradation products will be undertaken in future studies as it is beyond the scope presented in this work.

The mechanism of sago wastewater degradation was proposed. In the photo-Fenton process, the •OH radicals formed will attack the organics present in the sago wastewater effluent to form the intermediates before decomposing and mineralizing them into simpler molecules, CO_2 and H_2O, as shown in Equation (8)[21].

$$\text{Pollutant} + \bullet OH \rightarrow \text{intermediates} + \bullet OH \rightarrow \text{products } (CO_2 \,\&\, H_2O) \quad (8)$$

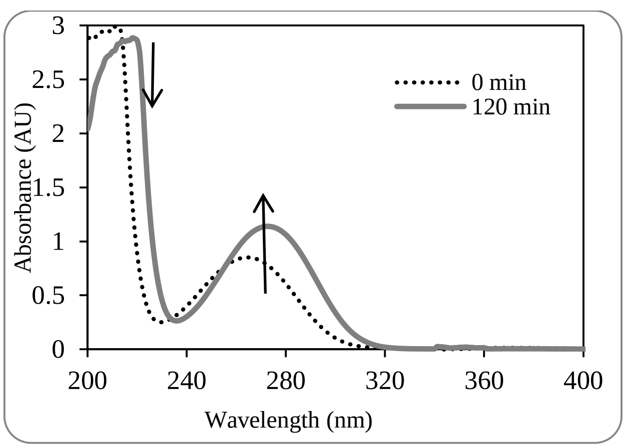


Figure 7 UV spectra of untreated sago wastewater and samples (initial and final) of optimized photo-Fenton treatment ([Fe^{2+}] = 5.07 g/L; [H_2O_2] = 4.01 mL/L and pH = 2.66)

3.6 Toxicity Evaluation

LC_{50} value is the lethal concentration of toxin that can kill 50% of the organisms in the test population [46]. Under the optimized concentration of Fe^{2+} = 5.07 g/L and H_2O_2 = 4.01 mL/L, the mortality of *A.salina* in the treated sago wastewater was less than 50%, as shown in Figure 8. This simply means that the effluent produced under the treated under optimized concentration of Fe^{2+} ion and H_2O_2 can be regarded as non-toxic. The mortality of *A. salina* for the control test was 10% in three replicates. Dojcinovic *et al.* [47] stated that if the mortality in the control test did not exceed 10%, then the tests can be considered as valid. The mortality of *A. salina* for all the reaction times was found to be less than 50% except for the 60 minutes, as which the mortality was found to be 66.7% (Figure 8). This could be explained by the partial oxidation reaction that occurs in the photo-Fenton process. Organic pollutants are converted into stable intermediates such as formic acids, aldehydes and carboxylic acids [44]. Depending on the intermediate products formed, the partial oxidation of organic pollutants is able to lead to less or more toxic components in the wastewater [31]. Hence, the highest mortality at 60 min might be due to the presence of stable intermediates in wastewater that could be toxic. Also, it is known that the presence of iron increases toxicity, depending on the iron concentration used in the treatment [31]. Although the addition of iron could increase the toxicity of H_2O_2 towards *A. salina*, the initial form of iron (either ferric or ferrous) likely not to significantly influence the toxicity [48].

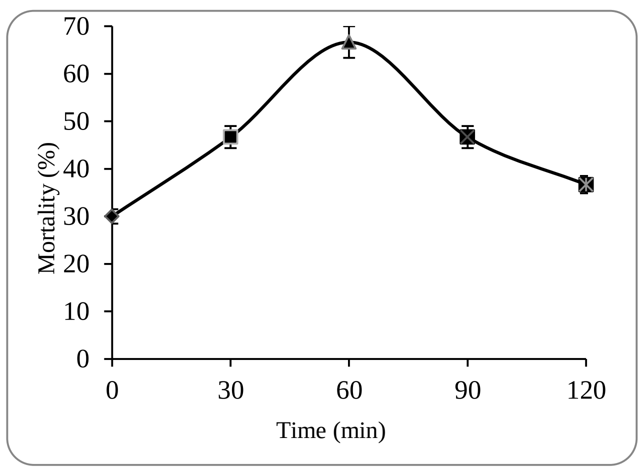


Figure 8 Mortality of *A. salina* in treated sago wastewater for different reaction times under optimized photo-Fenton condition ([Fe^{2+}] = 5.07 g/L; [H_2O_2] = 4.01 mL/L and pH = 2.66)

4.0 CONCLUSION

The optimal values of three independent variables (Fe^{2+} and H_2O_2 concentration, and initial pH) yielded a maximum of 90.00% COD removal from the sago wastewater under the applied experimental conditions. The optimum conditions of the photo-Fenton process were found to be H_2O_2 of 4.01 mL/L, Fe^{2+} of 5.07 g/L and pH of 2.66. Despite the high COD removal, the TOC removal was found to be low (48.0%) under optimized condition. Under the optimum concentration of H_2O_2 and Fe^{2+}, the effluent mortality of *A. salina* was less than 50%, in which the toxicity of treated sago wastewater can be considered as non-toxic. The R^2 value of the models (R^2>0.99) depicts a very high degree of correlation between the parameters. Photo-Fenton treatment can be regarded as a suitable treatment for sago wastewater degradation. Although the treated solution demonstrated non-toxic characteristics, the presence of stable intermediates or partially mineralized organics during the course of the treatment confirmed from TOC and UV-Vis analyses, suggests that there are rooms to improve this non-biological method in the future as this study is the first of its kind. Further photo-Fenton studies can be performed in the presence of aeration such as oxygen purging to increase the degradation rate and oxidation of organics. Greener approach using solar irradiation as photon source could be investigated to initiate the photo-Fenton process for sago wastewater degradation.

Acknowledgement

This study was supported by a research grant from the Research Acculturation Collaboration Effort (RACE) with grant number of (RACE/b/1245/201(01)). The author wishes to thank Unimas for sponsoring the Zamalah postgraduate scholarship and the financial support, Mybrain 15 scholarship by the Malaysian government.

References

[1] Singhal, R. S., J. F. Kennedy, S. M. Gopalakrishnan, A. Kaczmarek, C. J. Knill, and P. F. Akmar. 2008. Industrial Production, Processing, and Utilization of Sago Palm-Derived Products. *Carbohydrate Polymers.* 72(1): 1-20.

[2] Bujang K. 2015. *Rejuvenation of an Old Crop: Roles of Sago in the Food and Energy Industries*. Sarawak, Malaysia: Universiti Malaysia Sarawak (UNIMAS).

[3] Awg-Adeni, D. S., S. Abd-Aziz, K. Bujang, and M. A. Hassan. 2010. Bioconversion of Sago Residue into Value Added Products. *African Journal of Biotechnology.* 9: 2016-2021.

[4] Bujang, K. 2008, April. Sago Starch Factory Effluent: Salvaging a Potential Pollutant. *Asia Research News.*

[5] Ibrahim, S., S. Vikinswary, S. Al-Azad, and L. L. Chong. 2006. The Effects of Light Intensity, Inoculums Size, and Cell Immobilisation on the Treatment of Sago Effluent with *Rhodopseudomonas palustris* Strain B1. *Biotechnology and Bioprocess Engineering.* 11: 377-381.

[6] Rashid, W. A., H. Musa, S. K. Wong, and K. Bujang. 2010. The Potential of Extended Aeration System for Sago Effluent Treatment. *American Journal of Applied Sciences*. 7(5): 616-619.

[7] Saravanane, R., D. V. S. Murthy, and K. Krishnaiah. 2001. Anaerobic Treatment and Biogas Recovery for Sago Wastewater Management Using a Fluidized Bed Reactor. *Water Science & Technology*. 44(6): 141-146.

[8] Parthiban, R., P. V. R. Iyer, and G. Sekaran. 2008. Anaerobic Tapered Fluidised Bed Reactor for Treatment of Sago Industry Effluent. *Indian Chemical Engineer*. 50(4): 323-333.

[9] Rajasimman, C., and M. Karthikeyan. 2007. Starch Wastewater Treatment in a Three Phase Fludized Bed Bioreactor with Low Density Biomass Support. *Journal of Applied Sciences and Environmental Management*. 11(3): 97-102.

[10] Banu, J. R., S. Kaliappan, and D. Beck. 2006. High Rate Anaerobic Treatment of Sago Wastewater using HUASB with PUF as Carrier. *International Journal Environmental Science and Technology*. 3(1): 69-77.

[11] Ayyasamy, P. M., R. Banuregha, G. Vivekanandhan, S. Rajakumar, R. Yasodha, S. Lee, and P. Lakshmanaperumalsamy. 2008. Bioremediation of Sago Industry Effluent and Its Impact on Seed Germination (Green Gram and Maize). *World Journal of Microbiology and Biotechnology.* 24(11): 2677-2684.

[12] Savitha, S., S. Sadhasivam, K. Swaminathan, and F. H. Lin. 2009. A Prototype of Proposed Treatment Plant for Sago Factory Effluent. *Journal of Cleaner Production.* 17(15): 1363-1372.

[13] Sangeetha, V., V. Sivakumar, A. Sudha, and K. Kannan. 2015. Electrochemical Degradation of Sago Wastewater Using Ti/PbO_2 Electrode: Optimisation Using Response Surface Methodology. *International Journal of Electro-chemical Science*. 10(2): 1506-1516.

[14] Yazdanbakhsh, A. R., A. S. Mohammadi, M. Sardar, H. Godini, and M. Almasian. 2014. COD Removal from Synthetic Wastewater Containing Azithromycin Using Combined Coagulation and a Fenton-Like Process. *Environmental Engineering and Management Journal*. 13(12): 2929-2936.

[15] Abdulah, S. H. Y. S., M. A. A. Hassan, Z. Z. Noor, and A. Aris. 2011. Optimization of Photo-Fenton Oxidation of Sulfidic Spent Caustic by Using Response Surface Methodology. *IEEE*. 7: 1-7.

[16] Kanakaraju, D., C. A. Motti, B. D. Glass, and M. Oelgemöller. 2014. Titanium Dioxide Photocatalysis for Pharmaceutical Wastewater Treatment: A Review. *Environmental Chemistry Letters.* 12: 27-47.

[17] Expósito, A. J., J. M. Monteagudo, I. Díaz, and A. Durán. 2016. Photo-Fenton Degradation of a Beverage Industrial Effluent: Intensification with Persulfate and the Study of Radicals. *Chemical Engineering Journal.* 306: 1203-1211.

[18] Novoa-Luna, K. A., A. Mendosa-Zepeda, R. Natividad, R. Romero, M. Galar-Martinez, and L. M. Gomez-Olivan. 2016. Biological Hazard Evaluation of a Pharmaceutical Effluent Before and After a Photo-Fenton Treatment. *Science of the Total Environment.* 569-570: 830-840.

[19] Romero, V., S. Acevedo, P. Marco, J. Gimenez, and S. Esplugas. 2016. Enhancement of Fenton and Photo-Fenton Processes at Initial Circumneutral pH for the Degradation of the β Blocker Metoprolol. *Water Research.* 88: 449-457.

[20] Elmolla, E., and M. Chaudhuri. 2009. Improvement of Biodegradability of Synthetic Amoxicillin Wastewater by Photo Fenton Process. *World Applied Sciences Journal.* 5: 53-58.

[21] Tokumura, M., M. Shibusawa, and Y. Kawase. 2013. Dynamic Simulation of Degradation of Toluene in Waste Gas by the Photo-Fenton Reaction in a Bubble Column. *Chemical Engineering Science*. 100: 212-224.

[22] Tony, M. A., and Z. Bedri. 2014. Experimental Design of Photo-Fenton Reactions for the Treatment of Car Wash Wastewater Effluents by Response Surface Methodological Analysis. *Advanced Environmental Chemistry*. 2014: 1-8.

[23] Saber, A., H. Hasheminejad, and A. Taebi. 2014. Optimization of Fenton-Based Treatment of Petroleum Refinery Wastewater with Scrap Iron Using Response Surface Methodology. *Applied Water Science*. 4: 283-290.

[24] Aljuboury, D. A., P. Palaniandy, H. B. A. Aziz, and F. Shaik. 2016. Evaluation of the Solar Photo-Fenton Process to Treat

the Petroleum Wastewater by Response Surface Methodology (RSM). *Environmental Earth Sciences*. 75: 333-345.

[25] Muthukumar, A., and M. Muthuchamy. 2014. Contribution of Ozone, pH and Temperature in the Inactivation of a Foodborne Pathogen *Listeria monocytogenes*-A Study Using Response Surface Methodology. *International Journal on Applied Bioengineering*. 8(1): 5-12.

[26] Alim, M. A., J. -H. Lee, C. C. Akoh, M. -S. Choi, M. -S. Jeon, J. -A. Shin, and K. -T. Lee. 2008. Enzymatic Transesterification of Fractionated Rice Bran Oil with Conjugated Linoleic Acid: Optimization by Response Surface Methodology. *Swiss Society of Food Science and Technology*. 41: 764-770.

[27] APHA. 1999. *Standard Methods for the Examination of Water and Wastewater*. 18th ed. Washington, DC: America Public Health Association.

[28] Sangeetha, V., V. Sivakumar, A. Sudha, and K. S. P. Devi. 2014. Optimization of Process Parameters for COD Removal by Coagulation Treatment Using Box Behnken Design. *International Journal of Engineering & Technology*. 6(2): 1053-1058.

[29] Yetilmezsoy, K., S. Demirel, and R. J. Vanderbei. 2009. Response Surface Modelling of Pb(II) Removal From Aqueous Solution by *Pistaciavera*L.: Box-Behnken Experimental Design. *Journal of Hazardous Materials*. 171(1-3): 551-562.

[30] Arslan-Alaton, I., G. Tureli, and T. Olmez-Hanci. 2009. Treatment of Azo Dye Production Wastewaters Using Photo-Fenton-Like Advanced Oxidation Processes: Optimization by Response Surface Methodology. *Journal of Photochemistry and Photobiology A: Chemistry*. 202: 142-153.

[31] Dantas, T. L. P., H. J. Jose, and R. F. P. M. Moreira. 2003. Fenton and Photo-Fenton Oxidation of Tannery Wastewater. *Acta Scientiarum Technology*. 25(1): 91-95.

[32] Metcalf, J. S., J. Lindsay, K. A. Beattie, S. Birmingham, M. L. Saker, A. K. Torokne, and G. A. Codd. 2002. Toxicity of Cylindrospermopsin to the Brine Shrimp *Artemia salina*: Comparisons with Protein Synthesis Inhibitors and Microcystins. *Toxicon*. 40: 1115-1120.

[33] Department of Environment (DOE). 2010. *Environmental Requirements: A Guide for Investors.* Edisi Ke-11. Putrajaya, Malaysia: Department of Environment.

[34] Montgomery, D. 1991. *Design and Analysis of Experiments.* Edisi Ke-5. New York: John Wiley & Sons.

[35] Kumar, A., B. Prasad, and I. M. Mishra. 2007. Process Parametric Study for Ethane Carboxylic Acid Removal onto Powder Activated Carbon Using Box-Behnken Design. *Chemical Engineering & Technology*. 30(7): 932-937.

[36] Hadavifar, M., A. A. Zinatizadeh, H. Younesi, and M. Galehdar. 2010. Fenton and Photo-Fenton Treatment of Distillery Effluent and Optimization of Treatment Conditions with Response Surface Methodology. *Asia-Pacific Journal of Chemical Engineering*. 5: 454-464.

[37] Mason, R. L., R. F. Gunst, and J. L. Hess. 2003. *Statistical Design and Analysis of Experiments.* Edisi Ke-2. Hoboken, New Jersey: John Wiley & Sons, Inc.

[38] Sadler, B. 2013. *Light Metals 2013*. Hoboken, New Jersey: John Wiley & Sons.

[39] Wang, N. N., T. Zheng, G. S. Zhang, and P. Wang. 2016. A Review on Fenton-Like Processes for Organic Wastewater Treatment. *Journal of Environmental Chemical Engineering.* 4(1): 762-787.

[40] Sarria, V., S. Kenfack, O. Guillod, and C. Pulgarin. 2003. An Innovative Coupled Solar-Biological System at Field Pilot Scale for the Treatment of Biorecalcitrant Pollutants. *Journal of Photochemistry and Photobiology A: Chemistry*. 159(1): 89-99.

[41] Samet, Y., E. Hmani, and R. Abdelhedi. 2012. Fenton and Solar Photo-Fenton Processes for the Removal of Chlorpyrifos Insecticide in Wastewater. *Water SA*. 3(4): 537-542.

[42] Aljuboury, D. A., P. Palaniandy, H. B. A. Aziz, and S. Feroz. 2015. Evaluating the TiO_2 as a Solar Photocatalyst Process by Response Surface Methodology to Treat the Petroleum Waste Water. *Karbala International Journal of Modern Science*. 1(2): 78-85.

[43] Kanakaraju, D., C. A. Motti, B. D. Glass, and M. Oelgemöller. 2016. Solar Photolysis Versus TiO_2-Mediated Solar Photocatalysis: A Kinetic Study of the Degradation of Naproxen and Diclofenac in Various Water Matrices. *Environmental Science and Pollutant Research*. 23: 17437-17448.

[44] Rabelo, M. D., C. R. Bellato, C. M. Silva, R.B. Ruy, C. A. B. da Silva, and W. G. Nunes. 2014. Application of Photo-Fenton Process for the Treatment of Kraft Pulp Mill Effluent. *Advances in Chemical Engineering and Science*. 4: 483-490.

[45] Amilcar Machulek Jr., Frank H. Quina, Fabio Gozzi, Volnir O. Silva, Leidi C. Friedrich and Jose E. F. Moraes. 2012. Fundamental Mechanistic Studies of the Photo-Fenton Reaction for the Degradation of Organic Pollutants. In Tomasz Puzyn and Aleksandra Mostrag-Szlichtyng (eds.). *Organic Pollutants Ten Years After the Stockholm Convention-Environmental and Analytical Update*. Croatia: InTech.

[46] Letourneau, D. K., and B. E. Burrows. 2001. *Genetically Engineered Organisms: Assessing Environmental and Human Health Effects*. Boca Raton, Florida: CRC Press.

[47] Dojcinovic, B., B. M. Obradovic, M. M. Kuraica, M. V. Pergal, S. D. Dolic, D. R. Indic, T. B. Tosti, and D. D. Manojlovic. 2016. Application of Non-Thermal Plasma Reactor for Degradation and Detoxification of High Concentrations of Dye Reactive Black 5 in Water. *Journal of Serbian Chemical Society*. 81(7): 829-845.

[48] Twiner, M. J., S. J. Dixon, and C. G. Trick. 2001. Toxic Effects of *Heterosigma akashiwo* do not Appear to be Mediated by Hydrogen Peroxide. *Limnology and Oceanography*. 46(6): 1400-1405.