Research article

The Application of Design of Experiments in the Hatchery Wastewater Treatment through Biological Method

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Abstract

Keywords

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chemical oxygen demand;

nitrate-N;

total phosphorus

This study was conducted to evaluate the most influential factors contributing to the removal of chemical oxygen demand (COD), nitrate-N, and total phosphorus in hatchery wastewater to optimize the removal process with the application of acclimatized mixed culture (AMC). The factors, namely, the ratio of AMC to synthetic wastewater (SW), support media, agitation speed, and retention times, were selected and were analyzed through two-level factorial analysis. The results suggested that the best conditions for maximum removal of each response was the ratio of 1 to 3 AMC: SW, absence of support media, 0 rpm agitation, and 4 days of retention time; where the removal values of COD, nitrate-N, and total phosphorus were 26.5%, 76.5%, and 42.9%, respectively. The two largest contributing factors obtained from the factorial analysis were analyzed by the Design-Expert software to determine the optimum conditions for waste removal through the central composite design of the Response Surface Methodology (RSM). The obtained removal values were up to 62%, 94%, and 46% for COD, nitrate-N, and phosphorus at each optimum condition. The results obtained were higher than those from factorial analysis for all responses. To sum up, the treatment of contaminated wastewater through biological treatment via acclimatized mixed culture can be applied. The optimum conditions determined from this study should bee helpful in scaling up the wastewater treatment process.

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1. Introduction

The biological treatment of wastewater is a technology that manipulates the efficiencies of microorganisms to clean water by the breakdown of organic pollutants. Biological treatment depends on microorganisms such as bacteria and fungi [1] to break down organic compounds with the aid of oxygen into carbon dioxide and water [2]. One important process of biological treatment is to design a system where decomposition outcomes are readily collected for an appropriate disposition. The proper treatment of wastewater is needed to ensure safety and to meet discharge regulations. Breaking down and removing organic contaminants from biological treatment systems can be practical and cost-effective. Biological treatment has been widely used [3] due to its prominent economic benefit in terms of operational costs and capital expenditure compared to other treatment methods. The biological treatment of wastewater is one of the most effective means of defending against the transmission of water-borne infectious diseases and poor quality of water.

Several types of contaminants in wastewater causing the increase in chemical oxygen demand (COD) include nitrogen (N), and phosphorus (P). According to Li and Wu [4], nitrogen and phosphorus are two nutrients that induce eutrophication in receiving waters. COD and nitrogen are the two main indicators indicating poor wastewater quality. Also, the accumulation of nitrogenous wastes in aqua culturing systems has given rise to the toxicity to microorganisms [5]. The continuous production of wastes from domestic and industrial sectors occurs at an alarming rate nowadays, and disposal of wastes regularly takes place without proper treatment and management [3]. With the need to abide by the National Pollution Discharge Elimination System (NPDES) rules, an alternative treatment method that involves the application of the biological method for hatchery treatment was developed. Biological treatment methods that are environmentally safe as well as economically realistic have attracted researcher attention recently [2]. Hence, intending to depreciate the current environmental crisis, this study aimed to assess the most influential factors contributing to the removal of the wastes in polluted hatchery wastewater and to optimize the removal process with the manipulation of acclimatized mixed culture (AMC). The outcome of this study should be useful in scaling up the treatment of wastewaters as the optimum conditions for the removal of the waste have been determined.

2. Materials and Methods

2.1 Collection of wastewater and mixed culture

Both mixed culture and wastewater were sampled from a pond located at Setiu, Terengganu. The cultures were obtained from the sediment, while the wastewater was sampled from the pond [6]. Store-bought stones with a diameter of 1.5 to 2 cm were chosen.

2.2 Preparation of synthetic wastewater

The synthetic wastewater (SW) was formulated in accordance with the hatchery wastewater characterization to achieve 250 mg/L of COD and 10 mg/L of nitrate-N and total phosphorus each. The commercial nutrient solution (CNS) was supplemented whenever the proportion was too low in order to reach the design basis. To prepare 10 liters of synthetic wastewater, 7 g of CNS solution was mixed with 10 L of wastewaters from AB Hatchery and autoclaved at 121°C for 15 min and 250 mL of prepared synthetic wastewater was used to feed the mixed cultures daily for 14 days.

2.3 Acclimatization of mixed cultures

Acclimatization was carried out to ensure the adaptation of the mixed culture to the COD content in the sample and 1250 mL of mixed culture was mixed with 3750 mL of nutrient stock in a bioreactor [7]. The total solid concentrations and COD were determined, which were 29294 mg/L and 285.67 mg/L, respectively. This process was conducted daily for two weeks by providing 250 mL of nutrient stock each day.

2.4 Preliminary experiment

A new mixture of synthetic wastewater was acquired for the preliminary experiment as a substitute for the wastewater from the hatchery. The effect of retention time on COD removal was preliminarily investigated, within a time-frame of 0 and 10 days. The other factors were kept constant at a condition of 1:3 AMC to SW ratio, without support media and agitation and 50 mL of AMC was added to 150 mL of SW to give 200 ml volume for every 11 containers following the retention time.

2.5 Experimental setup for factorial analysis

Four factors that contributed to the removal of contaminants from the wastewater were selected and recognized as AMC to SW ratio (1:3 and 2:3), support media (absence and presence), agitation (0 and 100 rpm), and retention time (4 and 8 days). The experiment was conducted according to the setup displayed in Table 1, and constructed by the Design-Expert (Ver.7) software, with randomized factors. The output was analyzed by the software to determine the most significant factors and the best conditions for the removal of wastes, with COD, nitrate-N, and total phosphorus as responses. Another wastewater mixture was prepared using Artemia to substitute for the hatchery wastewater for factorial analysis. Two cycles of the experiment were performed with 8 and 4 days for the first and second cycle, respectively. Eight conical flasks were prepared and labeled for the first cycle. For a 1:3 ratio, 4 conical flasks were equipped with a mixture of 150 mL of SW and 50 mL AMC, while the other 4 flasks were supplied with 80 mL of AMC and 120 mL of SW. Stones were used as the support media in this experiment. Four beakers were supplemented with 80 mL of stones according to the Design-Expert run table. The experiment was conducted in conditions with and without agitation. Four flasks were agitated at 100 rpm on an orbital shaker for 8 days. The same procedure was applied for the second cycle and was tested after 4 days of retention time.

Table 1. Factors and ranges of low and high levels for factorial analysis

Factors	Range	
	Low level	**High level**
AMC: SW ratio	1:3	2:3
Support media	Absent	Present
Agitation (rpm)	0 rpm	100 rpm
Retention time	4 days	8 days

2.6 Validation experiment

An experiment was carried out to validate the best conditions suggested by the Design-Expert software. The criteria for selecting the best conditions included 1:3 AMC: SW ratio, support media in range (absence or presence), 0 rpm agitation speed, and four days of retention time, with all responses being maximized.

2.7 Analysis of total solids

A dry weight measurement method was used to determine the total solids. An empty aluminium cup was first weighed and recorded. After that, the mixed culture sample (50 mL) was placed in the aluminium cup which was weighed again before being placed in the oven for drying at 120° C for 2 h. The sample was heated up until dry. The calculation was simplified as below in equation (1).

$$Total\ solids = \frac{weight\ of\ sample - weight\ of\ empty\ cup}{volume\ of\ sample} \quad (1)$$

2.8 Analysis of sample

The analysis of the sample with a Hach spectrophotometer was performed after four and eight days. The three responses, namely, COD, nitrate-N, and total phosphorus removal, were analyzed, and the data were recorded in the Table accordingly.

2.8.1 Analysis of COD removal

The UESPA Reactor Digestion Method (Method 8000) was used to determine the removal of COD. The mixture of AMC and SW was first diluted with deionized water with ten dilution factors. The COD value was then determined by using a Hach spectrophotometer.

2.8.2 Analysis of nitrate-N removal

To calculate the nitrate-N, the sample and the blank were prepared by adding 10 mL of each sample into a sample bottle. A NitraVer 5 nitrate reagent pillow was added to the sample bottle. Both sample and blank were tested using a DR900 Hach spectrophotometer.

2.8.3 Analysis of total phosphorus removal

The Molybdovanadate acid persulfate digestion method was used to determine the total phosphorus in the sample. A mixture of AMC and SW was diluted with deionized water by 10 and 5 dilution factors, respectively. A Hach Spectrophotometer was used to determine the removal value of total phosphorus.

2.9 Data analysis

The data acquired from the analysis were documented in the Design-Expert software and were evaluated by the analysis of variance (ANOVA), based on the 95% confidence level of a p-value. The analysis of variance was conducted to figure out the most significant influential factor that contributed to the removal of the wastes.

2.10 Experimental set-up for optimization study

The factors and ranges of the central composite design (CCD) experimental setup are shown in Table 2. The experimental setup was constructed following the CCD by the Response Surface

Methodology (RSM) in two variables, namely acclimatized mixed culture (AMC) content and retention time, in order to determine the optimum process parameters. In this section, the term acclimatized mixed culture content (AMC content) was used instead of AMC to SW ratio, as the ratio was converted to the percentage value. Table 3 displays the experimental setup of CCD constructed by Design-Expert software. The experiment was conducted accordingly with 13 experimental runs, and the software was further used to analyze the experimental outputs using ANOVA based on the p-value with a 95% confidence level.

Table 2. Setup of two-level factorial analysis by Design-Expert software

Run	Factor			
	A: AMC: SW ratio	B: Support media	C: Agitation (rpm)	D: Retention time (days)
1	1:3	Yes	0	4
2	2:3	Yes	0	4
3	1:3	No	0	4
4	2:3	No	0	4
5	1:3	Yes	100	4
6	2:3	Yes	100	4
7	1:3	No	100	4
8	2:3	No	100	4
9	1:3	Yes	0	8
10	2:3	Yes	0	8
11	1:3	No	0	8
12	2:3	No	0	8
13	1:3	Yes	100	8
14	2:3	Yes	100	8
15	13	No	100	8
16	2:3	No	100	8

Table 3. Experimental design table of CCD

Std	Run	Factor	
		AMC content (%)	Retention time (days)
1	6	20.00	3.50
2	9	30.00	3.50
3	3	20.00	4.50
4	5	30.00	4.50
5	1	15.00	4.00
6	2	35.00	4.00
7	11	25.00	3.00
8	7	25.00	5.00
9	10	25.00	4.00
10	13	25.00	4.00
11	12	25.00	4.00
12	8	25.00	4.00
13	4	25.00	4.00

3. Results and Discussion

3.1 Preliminary results

Figure 1 illustrates the results of the preliminary experiment for the effect of retention time on COD removal. The value of COD was measured daily for ten days, and the outcome was used to evaluate the suitable range of retention times to be used in the factorial experiment. The removal value of the COD increased from day 0 until day 6, fluctuated a bit on day 7, kept on rising on day 8, but dropped again until day 10. The maximum removal of COD obtained was on day 8, which was 80%. The increase in the removal value implied that the degradation of organic matters by the mixed culture had occurred in the system. Neoh *et al*. [8] mentioned that the removal of COD was documented at 71% when a single culture of *Ochrobactrum* sp. was applied for the treatment of palm oil mill effluent (POME) under aerobic conditions. Tangahu *et al*. [7] discovered that both biological oxygen demand (BOD) and COD removal, which were up to 89% and 97%, respectively, were far better when applying a mixed culture of *Scirpus grossus* and *Iris pseudacorus* rather than using their single culture for the treatment of Batik wastewater. According to Velvizhi [9], the reduction of hydraulic retention time from 10 to 2.5 days caused a decrease in the removal efficiency of COD from 74 to 57%. Therefore, from the outcome of the preliminary experiment, day 4 and day 8 were chosen as the range for retention times since the highest removal obtained was on day 8, and after day 4, the removal showed a gradual increment. The test was discontinued after ten days as it took a long time for the samples to remove COD.

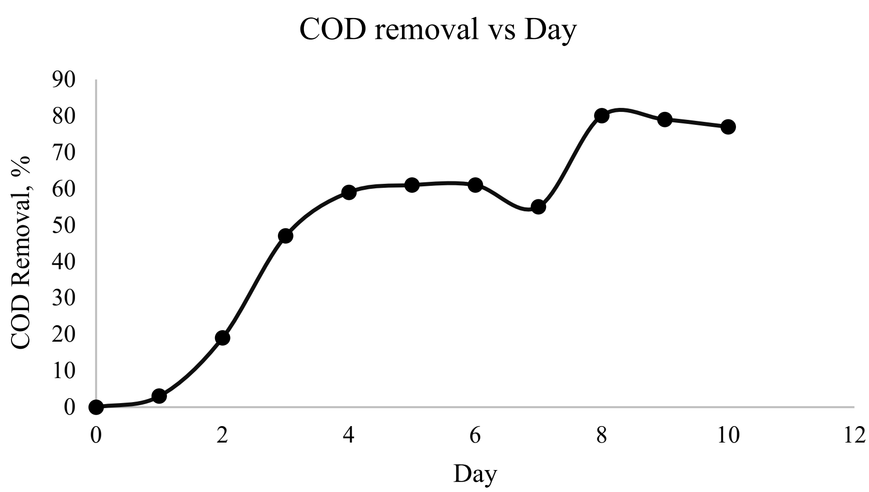

Figure 1. Graph of COD removal versus day of the preliminary study

3.2 Factorial analysis

Table 4 presents the removal outputs of COD, nitrate-N, and total phosphorus from the samples. The results were evaluated using ANOVA to determine the significant factors for all responses. The range of COD removal was from 0 to 75.35%, while for nitrate-N, the value was between 47 and 100%. The removal values of total phosphorus obtained ranged between 0 and 75.68%. It can be concluded from the outputs that the maximum removal value for each response varied, and experimental setups differently contributed to the removal values.

Table 4. Experimental results of COD, nitrate-N, and total phosphorus

Std	Factor				Response		
	A	B	C	D	COD	Nitrate-N	Total Phosphorus
1	1:3	Yes	0	4	23	83	51.27
2	2:3	Yes	0	4	20	99	32.7
3	1:3	No	0	4	40	52	1.3
4	2:3	No	0	4	0	100	75.68
5	1:3	Yes	100	4	48	47	36.27
6	2:3	Yes	100	4	67	80	64.26
7	1:3	No	100	4	64	70	0
8	2:3	No	100	4	47	11	27.35
9	1:3	Yes	0	8	36	96	0
10	2:3	Yes	0	8	47	100	41.34
11	1:3	No	0	8	50	100	0
12	2:3	No	0	8	75.35	69	51.89
13	1:3	Yes	100	8	38	88	0
14	2:3	Yes	100	8	69	80	51
15	1:3	No	100	8	65	100	51.44
16	2:3	No	100	8	68	64	0

3.3 Statistical analysis for each response

3.3.1 Analysis of variance (ANOVA)

ANOVA summary for COD, nitrate-N, and total phosphorus removal are presented in Tables 5, 6, and 7, respectively. The p-values were 0.0177, 0.0025, and 0.0032 for COD, nitrate-N, and total phosphorus, respectively. Each had a value of less than 0.05, indicating that all models were significant. The R^2 values for COD, nitrate-N, and total phosphorus were 0.9051, 0.9996, and 0.9958, respectively. The high values of R^2, which were higher than 0.8, implied that the models were acceptable and that those models could represent the process. The R^2 values within the range of 0.75 to 1 implied that it had a good statistical model [10].

Table 5. ANOVA table for COD removal

Source	Sum of Square	Df	Mean Square	F value	P-value Prob>F	
Model	5896.80	9	655.20	6.36	0.0177	*significant*
A: AMC: SW ratio	53.84	1	53.84	0.52	0.4969	
B: Support media	235.24	1	235.24	2.28	0.1815	
C: Agitation	1906.41	1	1906.41	18.50	0.0051	
D: Retention time	1213.65	1	1213.65	11.78	0.0139	
AB	469.26	1	469.26	4.55	0.0768	
AC	113.69	1	113.69	1.10	0.3339	
AD	774.93	1	774.93	7.52	0.0336	
BD	354.85	1	354.85	3.44	0.1129	
CD	774.93	1	774.93	7.52	0.0336	

Table 6. ANOVA table for nitrate-N removal

Source	Sum of Square	Df	Mean Square	F value	P-value Prob>F	
Model	9292.31	13	714.79	394.37	0.0025	*significant*
A: AMC: SW ratio	68.06	1	68.06	37.55	0.0256	
B: Support media	715.56	1	715.56	394.79	0.0025	
C: Agitation	1580.06	1	1580.06	871.76	0.0011	
D: Retention time	1501.56	1	1501.56	828.45	0.0012	
AB	945.56	1	945.56	521.69	0.0019	
AC	715.56	1	715.56	394.79	0.0025	
AD	742.56	1	742.56	409.69	0.0024	
BD	126.56	1	126.56	69.83	0.0140	
CD	540.56	1	540.56	298.24	0.0033	

Table 7. ANOVA table for total phosphorus removal

Source	Sum of Square	Df	Mean Square	F value	P-value Prob>F	
Model	10552.71	12	879.39	59.15	0.0032	*significant*
A: AMC: SW ratio	2599.47	1	2599.47	174.85	0.0009	
B: Support media	299.12	1	299.12	20.12	0.0207	
C: Agitation	35.58	1	35.58	2.39	0.2196	
D: Retention time	542.42	1	542.42	36.48	0.0091	
AC	553.90	1	553.90	37.26	0.0088	
BC	363.86	1	363.86	24.47	0.0158	
BD	519.38	1	519.38	34.93	0.0097	
CD	111.72	1	111.72	7.51	0.0713	

3.3.2 Main and interaction effect between factors on the removal percentage for each response

Figure 2 displays the Pareto chart of the main and interactive effects of the factors on COD removal. As seen in the Figure, three factors were observed to contribute to the removal of COD, namely AMC to SW ratio (A), agitation (C), and retention time (D). Agitation contributed the most to the removal of COD, as indicated by the highest bar, above the t-value limit line. Nayl *et al.* [11] mentioned that the application of agitation speed of 100 to 700 rpm contributed to 92.8% and 95.4% of BOD and COD removal, respectively, which indicated a significant increment in the removal percentage with increased agitation speed. The effect of retention time was also significant proven by the t-value of effect above the limit line. Li *et al.* [12] mentioned that a removal of COD of between 95 to 97% was recorded within 5 to 40 days of retention time for domestic wastewater treatment. This indicated that a longer retention time contributed to higher percent removal of COD. For interaction effects, the interaction between factors CD and AD was seen to contribute the most compared to other interactions, with negative and positive effects, respectively.

Figure 3 illustrates the Pareto chart of both the main and interactive effects of the parameters for nitrate-N removal. It was observed that agitation (C) contributed the most to the removal of nitrate-N, followed by retention time (D). As also seen in the Figure, all main effects lay above the t-value limit line, with support media (B), agitation (C), and retention time (D) extended above the Bonferroni line. As mentioned by Banala *et al.* [13], a coefficient with a t-value of effect beyond the Bonferroni line indicates the significance of the coefficient. On the other hand, a coefficient with a t-value of effect that lies beneath the t-limit line shows an insignificant response.

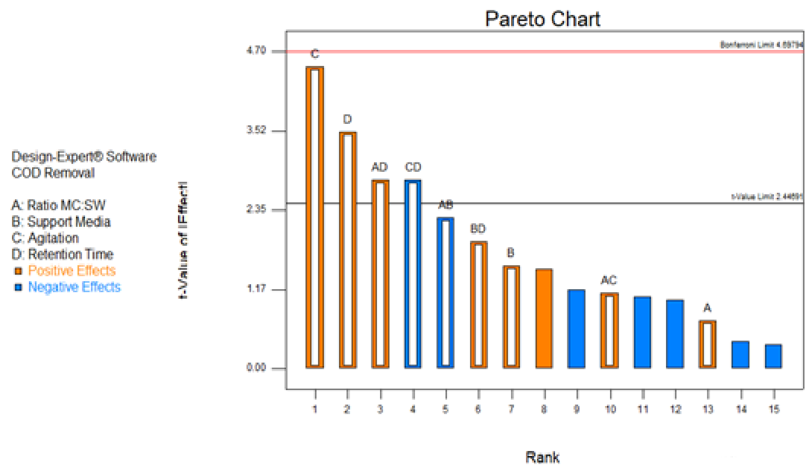

Figure 2. Pareto chart for COD removal

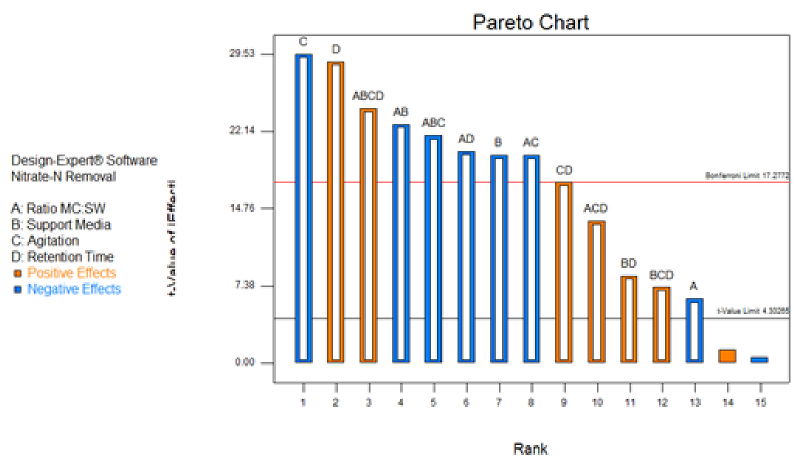

Figure 3. Pareto chart for nitrate-N removal

As illustrated in Figure 2, agitation contributed negatively to the removal of nitrate-N. The disproportion of the factor and the response value resulted in a negative effect. Therefore, an increment in agitation speed caused the nitrate-N removal value to decrease. Omar *et al*. [14] observed that the nitrate percent removal decreased from 68% to 52% with an increase in agitation speed from 100 rpm to 300 rpm. For interaction effects, all the factors presented a positive maximized contribution to nitrate-N removal, except for the factors BD and CD.

Figure 4 demonstrates the Pareto chart of the main and interactive effects of the parameters on total phosphorus. The ratio of AMC to SW (A) was the main contributing factor to the removal of phosphorus, represented by the highest bar, above the t-value limit line. The other main effects were insignificant, as their t-value of effect was beneath the t-value limit line. Besides, the AMC to

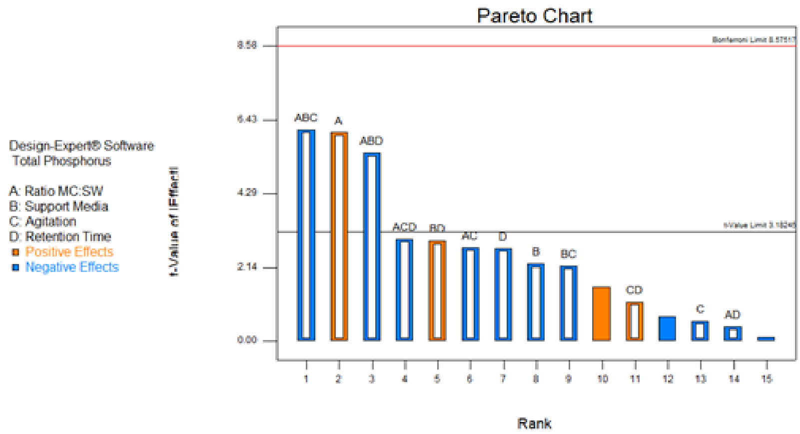

Figure 4. Pareto chart for total phosphorus removal

SW ratio was the only factor contributing positively to the total phosphorus removal, among the other main effects. A positive effect is indicated as the factor proportionally related to the response value. Factor AC presented the largest positive contribution to total phosphorus removal for the interaction effects, followed by factors BD and BC. Delgadillo-Mirquez *et al*. [15] highlighted that the maximum removal of total phosphorus in the wastewater was attributed to the impact of stirring of flask stirring and the nature of the mixed culture ratio being used.

To sum up, it was observed that each factor contributed differently to the removal process for each response. The ratio of AMC to SW was observed to contribute to the removal of total phosphorus with 24.35%. Agitation contributed to 30% of the removal of COD and 17 % to the removal of nitrate-N. The percentage contribution of retention time was seen as the highest in the two responses, indicating the significance of the factor. Meanwhile, the low percent contribution displayed by factor agitation suggested that the factor was the less significant in the removal process of wastes, except for COD and nitrate-N removal. Therefore, it can generally be concluded that factor A (AMC: SW ratio) and factor D (retention time) were the two most significant factors that influenced the waste removal process in the hatchery wastewater.

3.4 Best condition determination

The best condition suggested by the Design-Expert software for the maximal removal of each response was at 1:3 of AMC: SW ratio, absence of support media, 0 rpm agitation, and 4 days of retention time. A validation experiment was carried out accordingly in triplicate. The error was calculated between predicted and actual data on the removal of each response, where all the error values were in an acceptable rule of thumb range, as exhibited in Table 8.

Table 8. Percentage errors for COD, nitrate-N, and total phosphorus

	Predicted	Actual	Error %
COD	24.0	26.5	6.18
Nitrate-N	82.4	76.5	7.71
Total phosphorus	53.6	46.5	15.28

3.5 Optimization of waste removal

3.5.1 Fitting the model

Table 9 displays the experimental data and the responses. Among the center point of 25% AMC content and 4 days retention time, run 11 exhibited the highest percent removal for COD and nitrate-N. Meanwhile, for total phosphorus removal, run 10 was the highest. It was also observed that different parameters contributed to the removal of the pollutants differently. The highest removal of COD was observed at 62%, 98% for nitrate-N, and 46% for total phosphorus. Fitting the data to various models (linear, two-factorial, quadratic, and cubic) and their subsequent analysis of variance showed that COD and total phosphorus were described most properly with a quadratic polynomial model. Meanwhile, the cubic polynomial model was the most suitable for nitrate-N. The adjusted R^2 of the quadratic model for COD (0.9577) and total phosphorus (0.8907) was the highest of the linear, two factorial, and cubic models. Meanwhile, the adjusted R^2 of the cubic model was higher than that of linear and two factorial models for nitrate-N (0.7366). The quadratic polynomial model was found to be insignificant for the nitrate-N model.

Table 9. Experimental data of CCD

Run	Factors		Responses		
	A: AMC content	B: Retention time	COD	Nitrate-N	Total P
1	20	3.50	26	98	0
2	30	3.50	14	69	36
3	20	4.50	30	56	17
4	30	4.50	50	41	6
5	15	4.00	24	62	0
6	35	4.00	38.8	94	5
7	25	3.00	19	98	0
8	25	5.00	62	62	1.6
9	25	4.00	28	74	40
10	25	4.00	27	75	46
11	25	4.00	31.5	78	42
12	25	4.00	30	72	35
13	25	4.00	31.5	77	38

3.5.2 Analysis of variance (ANOVA)

ANOVA summaries for COD, nitrate-N, and total phosphorus are presented in Tables 10, 11, and 12, respectively. The p-value for all models exhibited a value below 0.05, which indicated that the models were all significant. The R^2 for COD, nitrate-N, and total phosphorus were 0.9753, 0.8903, and 0.9362, respectively. An R^2 value of more than 0.8 demonstrates that the estimated model fits the experimental data satisfactorily.

3.5.3 Effect of independent and interactive parameters

Figure 5 shows the influence of the two independent variables on COD removal. COD removal increased with the increase of AMC content from 20% to 30%, as displayed in Figure 5(a). The increase in retention time also caused an increase in COD removal, as presented in Figure 4(b). Oljira *et al.* [16] proved that the amount of COD removal in the brewery effluents was increased

Table 10. ANOVA table for COD removal

Source	Sum of squares	Df	Mean square	F value	P-value Prob > F
Model	1864.23	5	372.85	55.35	< 0.0001
A-AMC Content	117.81	1	117.81	17.49	0.0041
B-Retention time	1323.00	1	1323.00	196.42	< 0.0001
AB	256.00	1	256.00	38.01	0.0005
A^2	3.71	1	3.71	0.55	0.4824
B^2	164.23	1	164.23	24.38	0.0017
Residual	47.15	7			
Cor Total	1911.38	12			
R^2	0.9753				
R_{adj}	0.9577				

Table 11. ANOVA table for nitrate-N removal

Source	Sum of squares	Df	Mean square	F value	P-value Prob > F
Model	2942.54	7	420.36	5.80	0.0354
A-AMC Content	512.00	1	512.00	7.06	0.0451
B-Retention time	648.00	1	648.00	8.93	0.0305
AB	49.00	1	49.00	0.68	0.4485
A^2	5.93	1	5.93	0.082	0.7864
B^2	23.31	1	23.31	0.32	0.5953
Residual	362.69	5			
Cor Total	3305.23	12			
R^2	0.8903				
R_{adj}	0.7366				

Table 12. ANOVA table for total phosphorus

Source	Sum of squares	Df	Mean square	F value	P-value Prob > F
Model	4054.03	7	810.81	20.55	0.0005
A-AMC Content	102.08	1	102.08	2.59	0.1518
B-Retention time	8.00	1	8.00	0.20	0.6660
AB	552.25	1	552.25	14.00	0.0073
A^2	2081.67	1	2081.67	52.76	0.0002
B^2	2271.45	1	2271.45	57.57	0.0001
Residual	276.18	5	39.45		
Cor Total	4330.21	12			
R^2	0.9362				
R_{adj}	0.8907				

with increased incubation time. Also, rather than a single culture of bacteria, the combination of three different bacteria produced a remarkable increment in COD removal. The removal efficiency increased with increase in incubation time. The result from Oljira *et al*. [16] was in line with this current study, where the removal rate of COD increased with time, and the bacteria culture content plays a crucial role in scaling up the removal process.

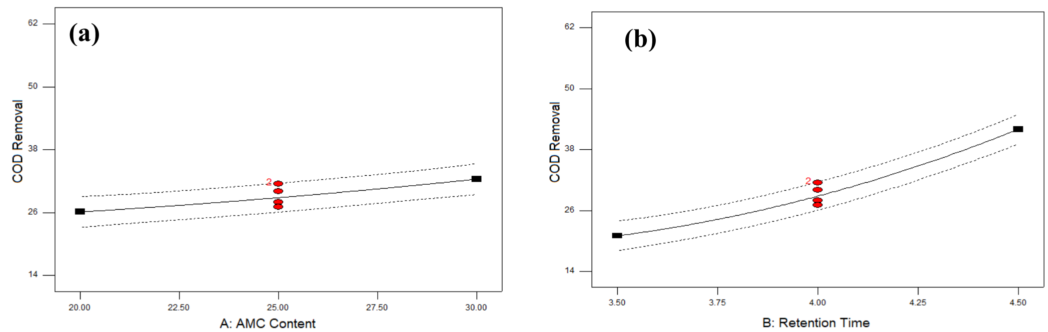

Figure 5. Effect of (a) AMC content and (b) retention time on COD removal.

The interaction effect between parameters of AMC content and retention time on COD removal was represented graphically by a contour plot and 3D response surface, as depicted in Figure 6. From the plot, it was observed that the COD removal at any AMC content was affected by the retention time, and an increase in retention time caused an increase in COD removal.

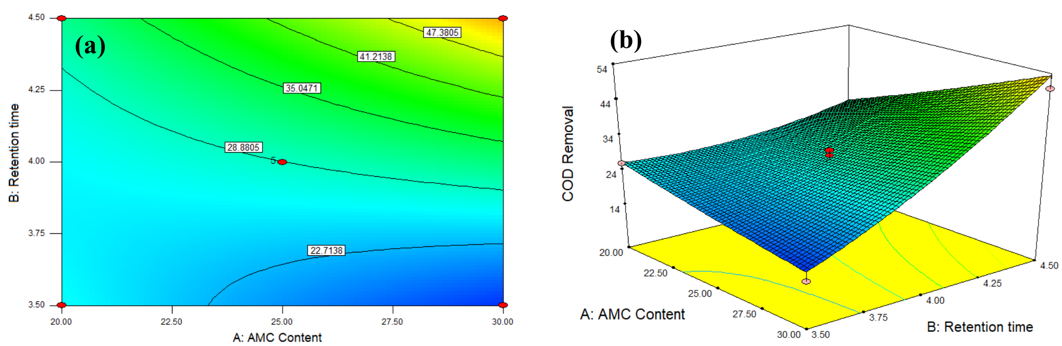

Figure 6. Contour plot (a) and 3D response surface (b) of COD removal as a function of AMC content and retention time

Figure 7 displays the effect of two independent parameters on nitrate-N removal. From Figure 7(a), the removal of nitrate-N increased with an increase of AMC concentration; meanwhile, an increase in retention time caused a reduction of nitrate-N removal, as illustrated in Figure 7(b). These may imply that three days was long enough for the microbes to remove nitrate-N, as the maximum removal was achieved on day 3. Also, the removal of nitrate through the denitrification process requires a large amount of oxygen [5, 17]; therefore, a longer retention time causes the reduction of oxygen, consequently reducing the amount of nitrate removed. According to Lu *et al.* [18], 0.7h was the optimum hydraulic retention time for nitrogen removal instead of 12 h. Similarly, the decrease of hydraulic retention time from 30 to 5 h had caused a remarkable increment in nitrite oxidation activity [12].

Figure 8 displayed the interaction effect between parameters of AMC content and retention time on nitrate-N removal, presented graphically by contour plot and 3D response in Figures 8(a) and 8(b), respectively. From the plot, the optimum removal of nitrate-N was up to 94% at the conditions of 25% AMC content and 3.5 days of retention time. An increase in retention time did not necessarily increase the removal of nitrate-N. It was also noted that the removal of nitrate-N at any AMC content was affected by the retention time.

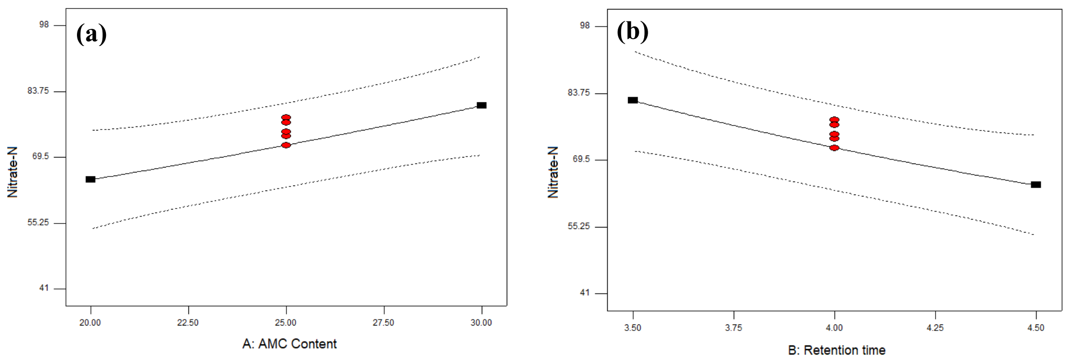

Figure 7. Effect of (a) AMC content and (b) retention time on nitrate-N removal

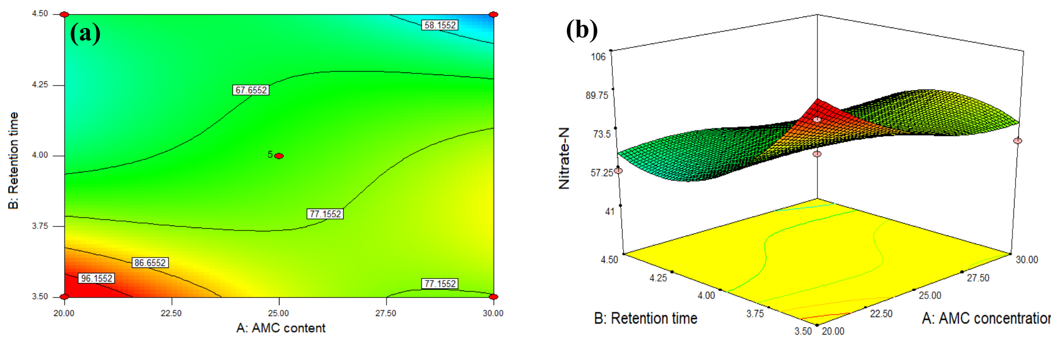

Figure 8. Contour plot (a) and 3D response surface plot (b) of nitrate-N removal as a function of AMC content and retention time

The effect of two independent parameters on the removal of total phosphorus was presented in Figure 9. As can be seen in Figure 9(a), the removal of total phosphorus increased with increase of AMC content, and reached a maximum value of 46% at the center point of 25% AMC content and 4 days of retention time. The removal of total phosphorus then decreased when it reached beyond a maximum value. A similar pattern was also observed for the retention time parameter, as depicted in Figure 9(b). The removal of total phosphorus increased with increased retention time and achieved its maximum value at the center point. The interaction effect between both parameters was portrayed in Figures 10(a) and 10(b) for contour plot and 3D response, respectively. An elliptical contour plot indicated that the interaction between the parameters was significant. It was well noted from the Figure that the optimum removal of total phosphorus was obtained at 25% AMC content and at 4 days of retention time.

3.6 Optimum condition for COD, nitrate-N, and total phosphorus removal

The optimum condition suggested were AMC content of 29% and 4 days of retention time, with the removal value of COD, nitrate-N, and total phosphorus being 28%, 80%, and 36%, respectively. However, by separating the optimum condition to achieve the optimal removal value for each

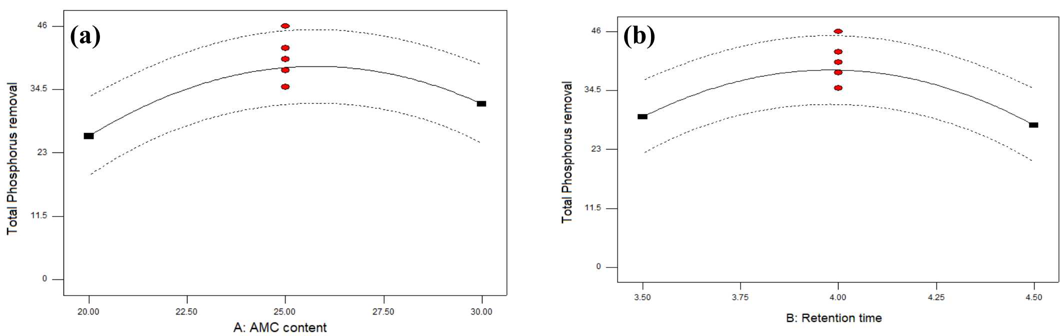

Figure 9. Effect of (a) AMC content and (b) retention time on total phosphorus removal

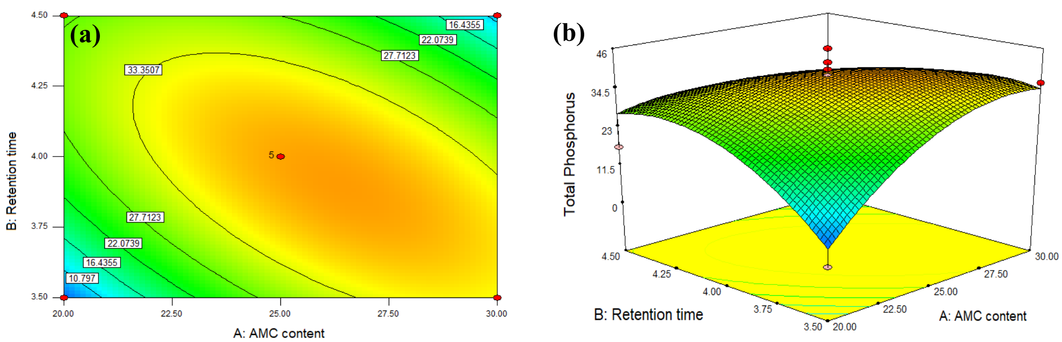

Figure 10. Contour plot(a) and 3D response surface plot (b) of total phosphorus removal as a function of AMC content and retention time

response, the conditions varied among responses. To reach the maximum removal of 62% for COD, the optimum condition was at 25% AMC content and 5 days of retention time. Meanwhile, for nitrate-N, a condition of 25% AMC content and 3 days of retention time gave rise to a removal value of up to 94%. The maximum removal of 46% for phosphorus was achieved at 25% AMC content and 4 days of retention time. All responses exhibited the same AMC content of 25% for the maximum removal with varied retention times.

Arbianti *et al*. [19] found that adding 5 mL of selective mixed culture resulted in better removal of COD and BOD than 1 mL application. Their finding suggested that a higher content of mixed culture led to a significant increment of COD being removed, which was in line with the current study. For nitrate-N, as the maximum removal value was at 25% AMC content and 3 days on retention time, it seemed that at 3 days of retention time, 25% AMC content was sufficient for the maximum removal of nitrate-N. The case was similar to that of phosphorus, where 25% of AMC was adequate for the removal process by the mixed culture. Durai *et al*. [20] evaluated the influence of inoculum concentration of mixed culture on the removal of COD with 1% to 4% concentrations. The result showed that 2% was the optimum concentration for COD removal. It meant that a high concentration of mixed culture content might not necessarily spur the removal of pollutants in the wastewater. The possible reason might be due to the pollutant concentrations and the content of mixed culture, as the removal may reach a certain limit for both pollutant concentration and mixed culture content. A clear observation that could be made from the results was that different pollutants required different optimal conditions for their maximal removal in the wastewater. However, further investigations are recommended in order to further understand the optimum condition for the removal for each response.

4. Conclusions

Results from the factorial analysis indicated that retention time and agitation speed were the two significant factors contributory to the removal of COD and nitrate-N. Meanwhile, for total phosphorus, the AMC to SW ratio was influential in its removal. Design-Expert software suggested that the best conditions for the removal of pollutants were a 1:3 ratio of AMC: SW, no agitation and support media, and 4 days retention time, which produced removals of 26.5% of COD, 76.5% of nitrate-N, and 42.9% of total phosphorus. The optimum conditions suggested by Design-Expert were 29% of AMC content and 4 days of retention time with 28%, 80%, and 36% removal of COD, nitrate-N, and phosphorus, respectively. The obtained removal values for COD and nitrate-N were higher than those from factorial analysis, and for total phosphorus the value was about the same. These results suggest that optimization led to an increment in the removal values of each pollutant. Furthermore, it can be concluded that AMC can be potentially applied in the biological treatment of wastewater as a natural waste removal agent. The biological treatment with AMC can be deemed as a good substitution for the physical and chemical treatment of wastewater, and with further research the treatment process can be improved.

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