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# Factorial Analysis of Biological Treatment for Contaminated Wastewater from Hatchery Industry

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# Factorial Analysis of Biological Treatment for Contaminated Wastewater from Hatchery Industry

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**Abstract**. The wastewater from a hatchery was dissimilar from a production farm in terms of quality and quantity of waste. The sources of wastewater were basically from uneaten food and fish feces. Biological treatment was a very promising process for removing dissolved organics from refinery wastewater. However, the low availability of clean water has become a critical issue to the shrimp grower. Further, carbon and phosphorus are the main constituents of most wastes, and removal of such elements from waste effluents can reduce the environmental stress and minimize ecosystem deterioration. The purpose of this study is to evaluate the factors affecting the removal of chemical oxygen demand (COD) and total phosphorus (TP) in the contaminated wastewater from hatchery industry. The factors were: ratio acclimatized mixed culture (AMC) to synthetic wastewater (SW) (1:3 and 2:3), the presence and absence of support media (Yes and No), agitation (0 rpm and 100 rpm) and retention time (4 days and 8 days). The responses were COD and TP removal. Design Expert software was used to construct experimental design where all the factor was randomized. As a result, ratio AMC to the SW, agitation and retention time were the most contributing factors to all the responses. The best conditions for the contaminated wastewater from hatchery industry were at ratio (1:3) AMC:SW, present of support media, 0 rpm agitation and 4 days retention time. At this condition the removal for COD and TP were 26.5% and 42.9% respectively. However, by separating the best condition for COD and TP, the removal can achieve 69% and 75.7% respectively. It can be concluded that the biological treatment using AMC can treat contaminated wastewater from hatchery industry.

## 1. Introduction

The wastewater from a hatchery was different from a production farm in terms of quality and quantity of waste [30]. There were two major elements in aquaculture wastewater; nitrogen and phosphorus [1]. Biological treatment was a very important process for removing dissolved organics from refinery wastewater. Most wastewater treatment techniques demonstrated to date had been limited to relatively small systems and had not been shown to be transferable to large-scale systems [16]. In a lab study by Boopathy et al., (2005) a sequencing batch reactor (SBR) was used to reduce COD in shrimp wastewater [16]. A number of physical, chemical and biological methods used in conventional wastewater treatment had been applied in aquaculture systems. These methods do help with total phosphorus removal but were costly in terms of capital investment, energy consumption and maintenance requirements [29]. Some of the benefits of using mixed culture included preventing nitrate, nitrite, ammonia and phosphate levels, increased dissolved oxygen concentrations and promotion of organic matter decomposition [4]. The objectives of this study are to characterize the wastewater and to determine the factors affecting the removal of COD and total phosphorus in the contaminated wastewater from hatchery industry using acclimatized mixed culture. The most contributing factors and interaction between the factors were analyzed via two level factorial analysis (TLFA). This research aimed to determine the best condition for COD and total phosphorus removal. The obtained best conditions from this study could improve the process removal of COD and total phosphorus from hatchery wastewater.

## 2. Materials and Methods

### *2.1. Collection of materials.*

The sample of wastewater and mixed culture were collected from a pond located at Setiu, Terengganu. Wastewater was collected from the pond and mixed culture was collected from the sediment of the pond [19]. The stones were bought from a shop and their diameter were ranged from 1.5 cm to 2.0 cm.

### *2.2. Synthetic wastewater preparation*

The synthetic wastewater prepared according to the total phosphorus and COD contents in the analyzed sample. Commercial nutrient solution (CNS) was added into the wastewater if the COD and phosphorus content was too low. The purpose of adding the CNS was to achieve the design basis. The design basis of total phosphorus and COD is 10 mg/L and 250 mg/L, respectively [13].

### *2.3. Acclimatization of mixed cultures*

The process performed to make the mixed culture adapts to the COD content in the sample. 3750 mL of nutrient stock mixed with 1250 mL mixed culture in a bioreactor [26]. Then the COD and total solid concentrations were determined. The COD and TSS concentration were 285.67 mg/L and 29294 mg/L respectively. This process performed daily for two weeks by giving 250 mL of nutrient stock per day.

### *2.4. Preliminary experiment*

For preliminary experiment, a new mixture of synthetic wastewater prepared to replace wastewater from AB Hatchery.

In this preliminary study, the effect of retention time on COD removal was studied. The range for the time studied was between 0 days until 10 days. The run was based on the fixed condition which is the ratio of AMC to SW was (1:3), no agitation and no support media. Based on the fixed ratio of mixed culture to synthetic wastewater, 50 mL of AMC and 150 mL of SW were mixed to produce 200 mL for every 11 plastic containers according to the retention time. From the preliminary experiment, two retention time were chosen for factorial analysis experiment based on the highest COD removal which was 4 days and 8 days.

### *2.5. Experimental set-up for factorial analysis*

Table 1 shows four selected factors that gave contribution to wastewater treatment. The factors involved were ratio AMC to SW (1:3 and 2:3), presence of support media, agitation (0 rpm and 100 rpm) and retention time (4 days and 8 days). For factorial analysis experiment, a new mixture of SW was needed by using artemia to replace wastewater from AB Hatchery. The experimental setup for factorial analysis was conducted in two cycles which for the first cycle was 8 days and 4 days for the second cycle. For the first cycle, 8 conical flasks were prepared and labeled. 4 conical flask for the ratio (1:3) was 50mL of AMC and 150 mL of SW. Another 4 conical flasks filled with 80mL of AMC and 120mL of SW. The support media for the factorial analysis was stone. 80 mL of stones were added into 4 beakers based on the Design Expert run table. The experiment also is done in two conditions which were with agitation and without agitation. 4 conical flask with agitation were left on an orbital shaker for 8 days with 100 rpm. For the second cycle, the same procedure has been done and the sample was tested after retention time 4 days.

The experiment was conducted according to set-up in Table 2. The experiment set-up in Table 2 was performed by Design Expert software where all the factors were randomized. Then, the experimental data were analyzed by using Design Expert software in order to determine the most contributing factors. There were two responses which were COD removal and total phosphorus removal.

**Table 1.** Selected factor and their range

| Factors | Low level | High level |
|---|---|---|
| Ratio AMC:SW | 1:3 | 2:3 |
| SM | No | Yes |
| Agitation | 0 rpm | 100 rpm |
| Retention time | 4 days | 8 days |

**Table 2.** Experimental set-up that has been constructed by using two level factorial analysis (TLFA) Design Expert software (Version 7)

| | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|---|---|---|---|---|
| Std Run | A: Ratio AMC:SW | 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 |

### *2.6. Validation experiment*

A validation experiment was conducted based on the suggested of the best conditions from Design Expert software. The criteria set-up to select the best processing conditions were given in Table 3.

**Table 3.** Criteria for validation experiment

| Name | Goal | Value |
|---|---|---|
| Ratio MC:SW | is equal to 1:3 | 1:3 |
| Support Media | is in range | Yes – No |
| Agitation | is equal to 0.00 | 0 rpm |
| Retention Time | is equal to 4.00 | 4 days |
| COD Removal | maximize | - |
| Total Phosphorus Removal | maximize | - |

### *2.7. Total Solids Analysis*
The total solids analysis was measured by using dry weight measurement method. First, the empty aluminum cup was weight and the weight was recorded. Then, the 50 ml of sample (mixed culture) was filled in the aluminum cup and weighted. Then it was placed in the oven to dry it using 120° C for 2 hours and heat up until it dry.
Total solids = (weight of sample – weight of empty cup)/ Volume of the sample

### *2.8. Sample analysis*
Sample analysis for factorial analysis was conducted after 4 and 8 days according to the retention time. There were two responses which were chemical oxygen demand, COD and total phosphorus removal.

*2.8.1. COD removal analysis by using HACH Spectrophotomete*r. COD removal was determined by using USEPA Reactor Digestion Method (Method 8000). The synthetic wastewater (SW) mixed acclimatized mixed culture (AMC) was diluted by using deionized water using 10 dilution factor. Then, COD removal was analyzed by using HACH Spectrophotometer.

*2.8.2. Total Phosphorus removal analysis by using HACH Spectrophotometer*. Total phosphorus was determined by using Molybdovanadate with Acid Persulfate Digestion Method (Method 10127). The synthetic wastewater (SW) mixed acclimatized mixed culture (AMC) was diluted by using deionized water using 5 and 10 dilution factor. Then, total phosphorus removal was analyzed by using HACH Spectrophotometer.

### *2.9. Data analysis*
All data obtained were recorded in Design Expert software. The responses were analyzed using Analysis of Variance (ANOVA) based on p-value with 95% confidence level to identify the most contributing factors and interaction between the factors that have an effect on both COD and total phosphorus removal.

## 3. Results and Discussions

### *3.1. Preliminary experiments*
From the preliminary result for retention time, chemical oxygen demand, COD removal was taken daily in Table 4. The result from the COD removal in Figure 1 was used to determine the retention time for the factorial analysis experiment. The COD removal starting increased from day 4 until day 8. COD removal increased from day 4 to day 8 because of the acclimatized mixed culture consume the COD content in the wastewater. Reduction of HRT from 10 to 2.5 days reduced the COD removal efficiency from 74% to 57% [30]. For this preliminary experiment, from Figure 1 shows on day 4, the removal starting increased until day 7 and day 8 the data started fluctuated until day 10. From this

experiment, COD removal was 80% when using mixed culture. According to Neoh et al., (2016), under aerobic conditions, the COD removal only 71% when using pure culture, *Ochrobactrum* sp. for palm oil mill effluent (POME) [21]. Tangahu et al., (2019) studied, by using mixed culture, *Scirpus grossus* and *Iris pseudacorus* showed a better removal than its single culture when COD removal up to 89% and BOD removal up to 97% for Batik wastewater treatment using the intermittent method [26].

In summary, from the preliminary experiment, day 8 has the highest COD removal. The experiment was stopped after 10 days since all the samples took a long time to remove all the COD in wastewater. COD removal recorded 59% for day 4 and 80% for day 8. It showed a positive COD removal from hatchery wastewater. Therefore, 4 days and 8 days were further used for factorial analysis.

**Table 4:** COD test results for preliminary study that contain SW and AMC

| Time (days) | COD Test Reading (mg/L) | | | | COD Removal (%) |
|---|---|---|---|---|---|
| | R1 | R2 | R3 | Average | |
| 0 days | 641 | 610 | 640 | 630.33 | 0 |
| 1 days | 604 | 610 | 615 | 609.67 | 3 |
| 2 days | 513 | 512 | 515 | 513.33 | 19 |
| 3 days | 333 | 336 | 338 | 335.67 | 47 |
| 4 days | 256 | 261 | 260 | 259 | 59 |
| 5 days | 263 | 259 | 218 | 246.67 | 61 |
| 6 days | 238 | 251 | 250 | 246.33 | 61 |
| 7 days | 291 | 281 | 284 | 285.33 | 55 |
| 8 days | 115 | 130 | 133 | 126 | 80 |
| 9 days | 129 | 127 | 135 | 130.33 | 79 |
| 10 days | 148 | 141 | 144 | 144.33 | 77 |

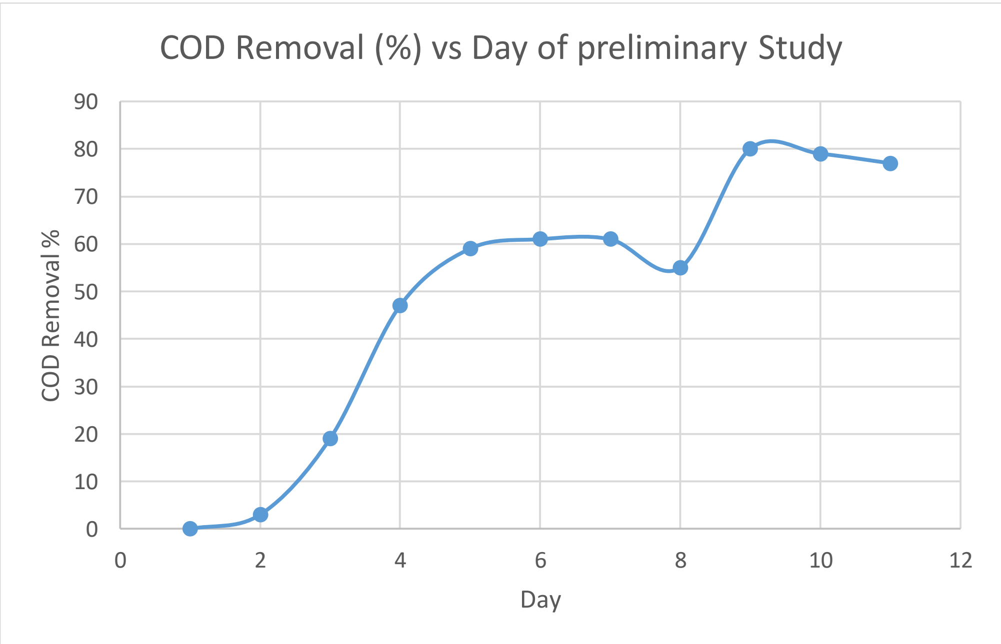



**Figure 1:** Graph of COD removal versus day of preliminary study.

### *3.2. Factorial analysis*

Table 5 shows 16 runs of experiments that were conducted for this study and the results of COD and total phosphorus removal. The responses were analyzed using ANOVA by using Design Expert software V7, based on the value with 95% confidence level to identify the most contributing factors and interaction between the factors on all responses. COD removal was ranged from 0 to 75.35% and total phosphorus removal was ranged from 0 to 75.68%. The lowest value of 0% of COD removal was obtained using ratio AMC to the SW was 2:3, no support media, 0 rpm, and retention time was 4 days. The highest value of 74 % of COD removal was obtained using ratio AMC to the SW was 2:3, no support media, 0 rpm, and retention time was 8 days. Hence, the lowest value of 0% of total phosphorus removal was obtained using ratio AMC to the SW was 1:3, no support media, 100 rpm, and retention time was 4 days; ratio AMC to the SW was 1:3, support media, 0 rpm, and retention time was 8 days; ratio AMC to the SW was 1:3, no support media, 0 rpm, and retention time was 8 days; ratio AMC to the SW was 1:3, support media, 100 rpm, and retention time was 8 days and ratio AMC to the SW was 2:3, no support media, 100 rpm, and retention time was 8 days.

**Table 5.** Experimental results of removal COD and total phosphorus

| Std run | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Response 1 | Response 2 |
|---|---|---|---|---|---|---|
| | A: Ratio AMC:SW | B: Support Media | C: Agitation (rpm) | D: Retention time (days) | COD Removal % | Total Phosphorus Removal % |
| 1 | 1:3 | Yes | 0 | 4 | 23 | 51.27 |
| 2 | 2:3 | Yes | 0 | 4 | 20 | 32.7 |
| 3 | 1:3 | No | 0 | 4 | 40 | 1.3 |
| 4 | 2:3 | No | 0 | 4 | 0 | 75.68 |
| 5 | 1:3 | Yes | 100 | 4 | 48 | 36.27 |
| 6 | 2:3 | Yes | 100 | 4 | 67 | 64.26 |
| 7 | 1:3 | No | 100 | 4 | 64 | 0 |
| 8 | 2:3 | No | 100 | 4 | 47 | 27.35 |
| 9 | 1:3 | Yes | 0 | 8 | 36 | 0 |
| 10 | 2:3 | Yes | 0 | 8 | 47 | 41.34 |
| 11 | 1:3 | No | 0 | 8 | 50 | 0 |
| 12 | 2:3 | No | 0 | 8 | 75.35 | 51.89 |
| 13 | 1:3 | Yes | 100 | 8 | 38 | 0 |
| 14 | 2:3 | Yes | 100 | 8 | 69 | 51 |
| 15 | 13 | No | 100 | 8 | 65 | 51.44 |
| 16 | 2:3 | No | 100 | 8 | 68 | 0 |

### *3.3. Analysis of variance (ANOVA) for COD removal percentage*

Table 6 shows the percentage contribution for each factor towards COD removal percentage. The agitation (C) has the highest percentage with value of 29.26%, followed by retention time (D) with value 18.63%. Continued with support media (B) with value of 3.61% and lastly ratio AMC: SW (A) with value 0.83%. ANOVA summary was shown in Table 7 for COD removal percentage to estimate the coefficient of the model, to check the significance of each parameter and to indicate the interaction strength of each parameter. This model showed the coefficient of determination ($R^2$) was 0.9051. Olmez et al., (2009) suggested that a good fit of a bioprocess model, $R^2$ should be at least 0.80. Since the $R^2$ is higher than 0.8, this model was accepted. It can be concluded that this model could represent the process [24].

**Table 6.** Contribution factor of COD removal percentage

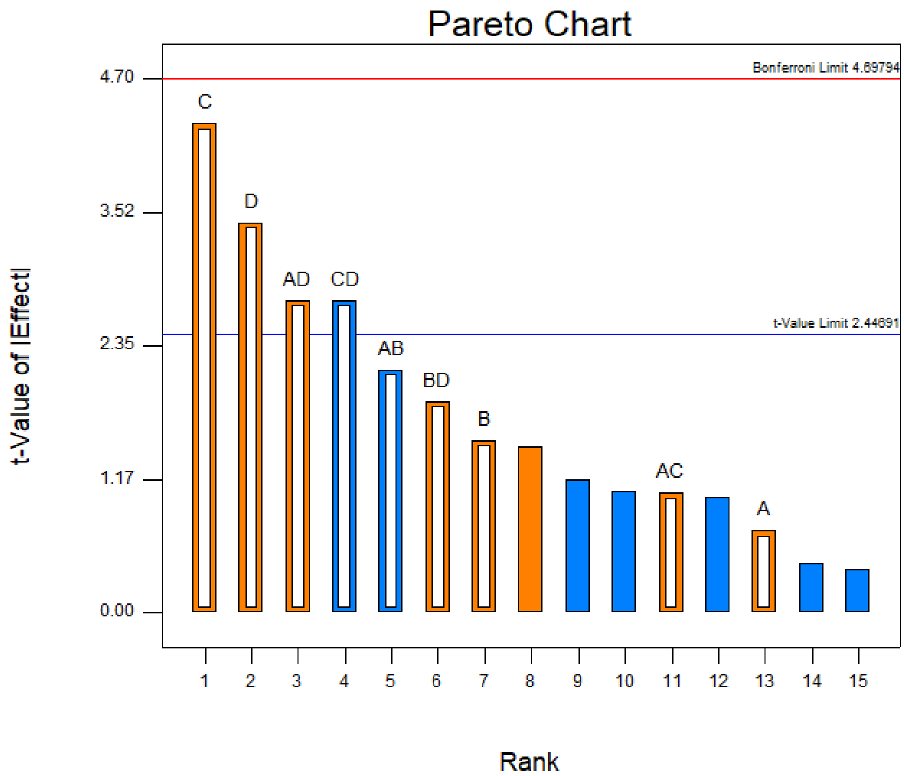
| Factor | % Contribution |
|---|---|
| A – Ratio AMC : SW | 0.83 |
| B – Support Media | 3.61 |
| C – Agitation | 29.26 |
| D – Retention Time | 18.63 |

**Table 7.** *ANOVA* table for COD removal percentage

| *ANOVA* for selected factorial model | | | | | | |
|---|---|---|---|---|---|---|
| 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-Ratio MC:SW | 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 | |

### *3.4. Main effect and interaction effect between factors on COD removal percentage*

The Pareto Chart in Figure 2 shows the main effects and interaction effects of the factors for COD removal. For the main effect, it showed that there are three main factors contribute to COD removal. Agitation (C) has shown the highest effects followed by retention time (D) and ratio AMC: SW (A). In Table 6, Factor C gave the highest contribution among other factors. Based on Table 1, for Factor C, 0 rpm was set as low level and 100 rpm was set as high level. Meanwhile Factor D, 4 days was set as low level and 8 days was set as high level. Positive effect is when the factor is proportional to the response value. From Figure 2, Factor C and Factor D gave positive effect on COD removal. Therefore, when Factor C and Factor D are at high level, the value of COD removal is increasing. For interaction effects, it showed there were two interaction effects that contributed to COD removal which were ratio AMC: SW & retention time (AD) and agitation & retention time (CD) with positive and negative effects, respectively.



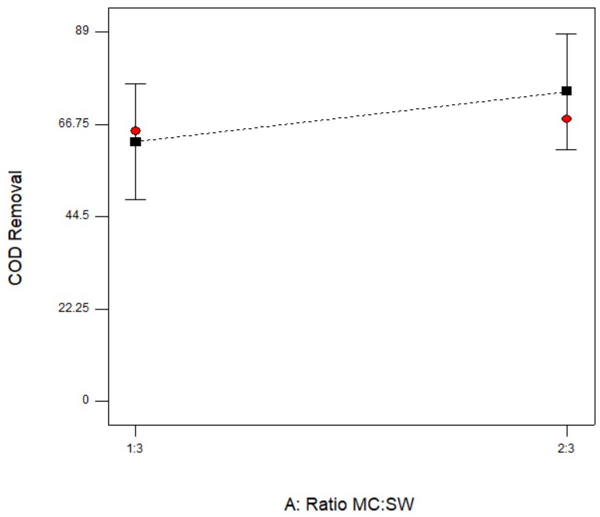

*Keywords:* A: ratio AMC: SW, B: Support media, C: Agitation and D: Retention time

Positive Effects, Negative Effects

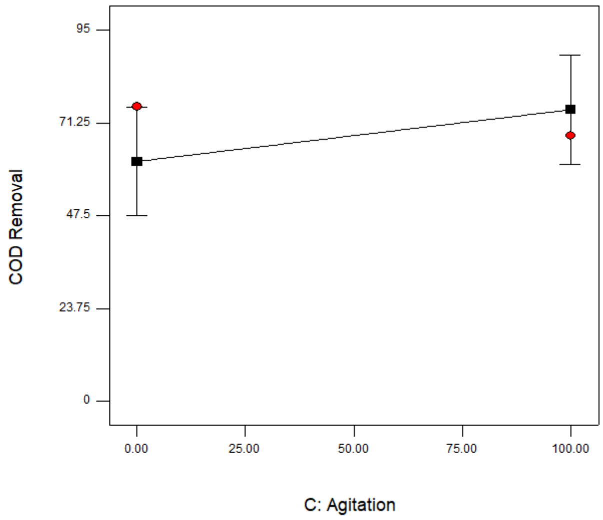
**Figure 2.** Pareto Chart of COD removal percentage

*3.5. Effect of independent processing parameters on COD removal percentage*

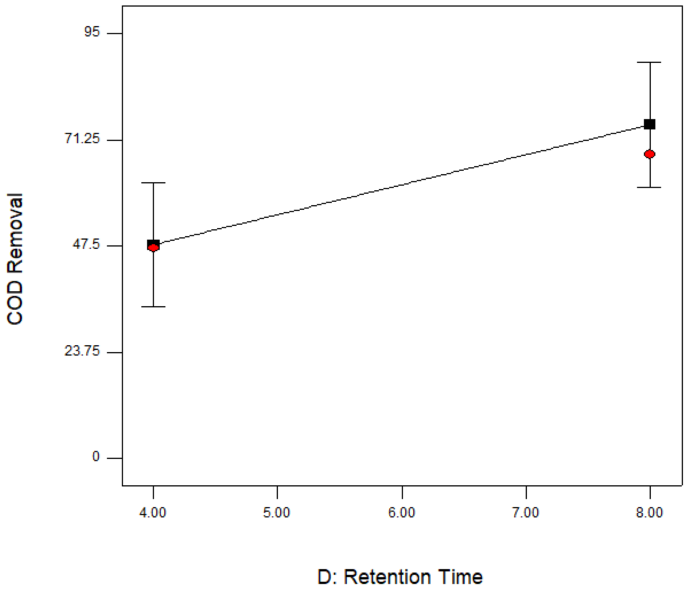
The effect of three independent variables on COD removal which were ratio AMC: SW, agitation, and retention time is shown in Figure 3a, Figure 3b and Figure 3c respectively. From Figure 3a, there was a significant difference in ratio AMC: SW on COD removal because at ratio 1:3, COD removal achieved 62.5% while at ratio 2:3, COD removal was 74.59%. This was supported by the information from the Pareto Chart (Figure 2) where the agitation factor was above the t-value limit. Banala et al., (2013) reported that the coefficients with t-value of effect above Bonferroni line are designated as certainly significant coefficients, and coefficients with t-value of the effect below the t-limit line is statistically insignificant to the response [2]. From Figure 3b, at 0 rpm agitation, COD removal achieved 61.34% while at 100 rpm, COD removal was 74.59% which was higher than 0 rpm. It showed that there was a significant difference between both agitation. Nayl et al., (2017) studied, wastewater from treatment facility was treated using the agitation speed range between 100 to 700 rpm at pH 6 for 150 min and 25 °C contributed to the 95.4% COD and 92.8% BOD removal [20]. From Figure 3c, COD removal was 47.75% when 4 days applied and the value of COD removal become higher which was 74.59% when 8 days was applied. It is supported by Li et al., (2014), by using retention time 5 days up to 40 days to treat the domestic wastewater, COD removal percentages were 95% - 97% illustrating the longer the retention time, the higher the percentage removal COD [15].




(a) Ratio AMC:SW




(b) Agitation

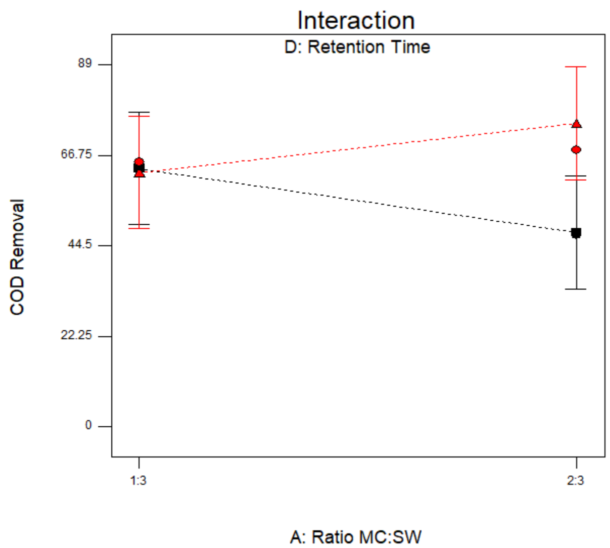



(c) Retention time

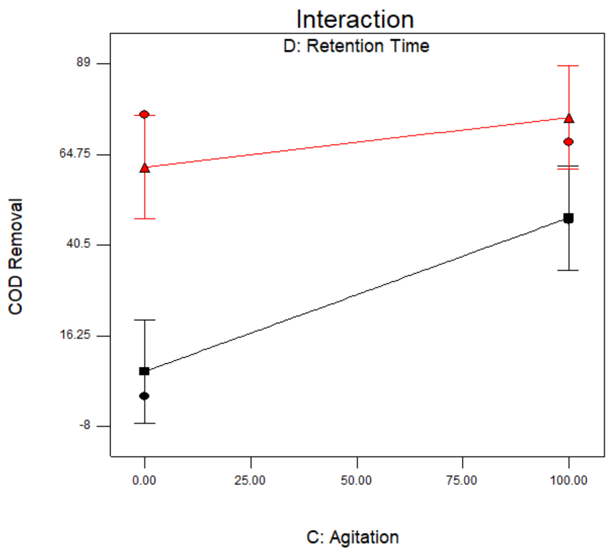
**Figure 3.** Effect of most effective independent factors in COD removal percentage

### *3.6. Interaction effects between factors on COD removal percentage*

Figure 4a shows the interaction effect between ratio AMC: SW and retention time (AD) on COD removal. When 8 days (red line) was applied, it gave better performance on COD removal. There was no significant effect of ratio AMC: SW on COD removal because at ratio 1:3, COD removal achieved 62.5%. Meanwhile at ratio 2:3, COD removal achieved was 74.59%. When 4 days (black line) was applied, ratio AMC: SW showed a significant effect on COD removal. At ratio 1:3, COD removal achieved 63.5% and the value becomes lower which was 47.75% at ratio 2:3. In this study, the amount of mixed culture at ratio 2:3 was more than at ratio 1:3, therefore COD removal achieved better performance at ratio 2:3 when 8 days applied. Figure 4b shows the interaction effect between agitation and retention time (CD) on COD removal. It was the same as interaction effect of AD where COD removal recorded higher value when 8 days (red line) was applied. When 8 days was applied, there was no significant difference of agitation on COD removal because COD removal achieved 61.34% at 0 rpm and 74.59% at 100 rpm. Meanwhile, when 4 days (black line) was applied, there was a significant difference of agitation compare to 8 days on COD removal because at agitation 0 rpm, COD removal achieved 6.67% while at 100 rpm, COD removal was 47.75%. As indicated by the Pareto chart (Figure 2), the most significant factors were agitation and retention time because they were above t-value Limit. The effect of agitation and retention is the most significant when 100 rpm and 8 days were applied.




(a)Factor ratio AMC: SW and retention time (AD)




(b) Factor agitation and retention time (CD)

**Figure 4.** Analysis of interaction effects on COD removal percentage

*3.7. Analysis of variance (ANOVA) for total phosphorus removal*

**Table 8** shows the percentage contribution for each factor towards total phosphorus removal percentage. The ratio AMC: SW (A) has the highest percentage with value of 24.53%, followed by retention time (D) with value of 5.12%. Continued with support media (B) with value of 2.82% and lastly agitation (C) with value 0.34%. The complete removal of total phosphorus approximately 4 to 6 days only which in this experiment, we found the highest total phosphorus removal was on day 4 [6]. ANOVA summary was shown in **Table 9** for COD removal percentage to estimate the coefficient of the model, to check the significance of each parameter and to indicate the interaction strength of each parameter. This model showed the coefficient of determination ($R^2$) was 0.9958. It can be concluded that this model could represent the process.

**Table 8.** Contribution factors of total phosphorus removal

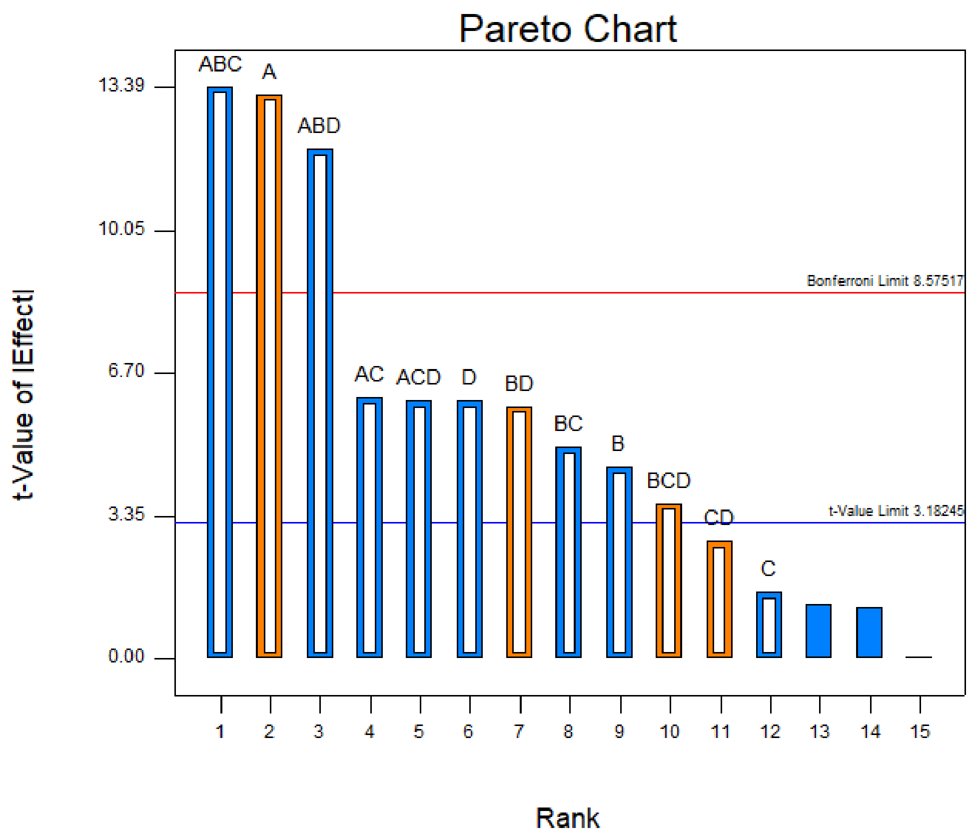
| Factor | % Contribution |
|---|---|
| A – Ratio AMC : SW | 24.53 |
| B – Support Media | 2.82 |
| C – Agitation | 0.34 |
| D – Retention Time | 5.12 |

**Table 9.** *ANOVA* table for total phosphorus removal percentage

| *ANOVA* for selected factorial model | | | | | | |
|---|---|---|---|---|---|---|
| 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-Ratio MC:SW | 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 | |

### *3.8. Main effect and interaction effect between factors on total phosphorus removal*

The Pareto Chart in Figure 5 shows the main effects and interaction effects of the factors to total phosphorus. For the main effect, it shows that ratio AMC: SW (A) is the highest main contributing factor to total phosphorus removal. In Table 8, Factor A gave the highest contribution among other factors. Based on Table 1, for Factor A, 1:3 was set as low level and 2:3 was set as high level. Positive effect is when the factor is proportional to the response value. From Figure 5, Factor A gave positive effect on the total phosphorus removal. Therefore, when Factor A is at high level, the value of total phosphorus removal is increasing. For interaction effects, support media and retention time (AC) gave the highest positive contribution to total phosphorus removal followed by support media and retention time (BD) and support media and agitation (BC). The effect of using stirred flask together with the ratio of the mixed culture used in the wastewater contributed to the highest removal of total phosphorus [6].



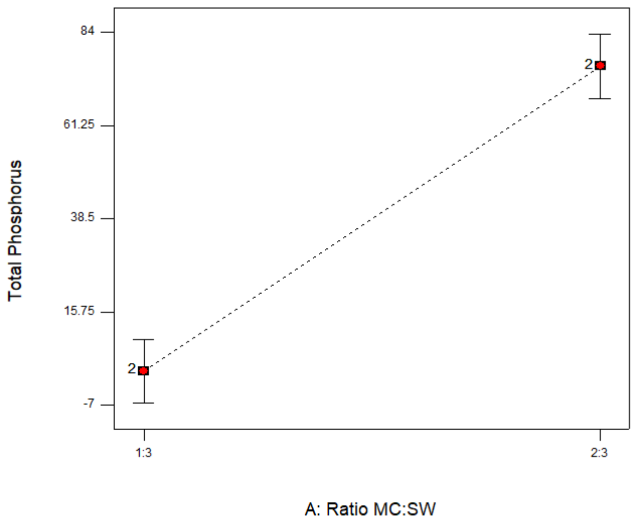

*Keywords:* A: ratio AMC: SW, B: support media, C: agitation and D: retention time

Positive Effects, Negative Effects

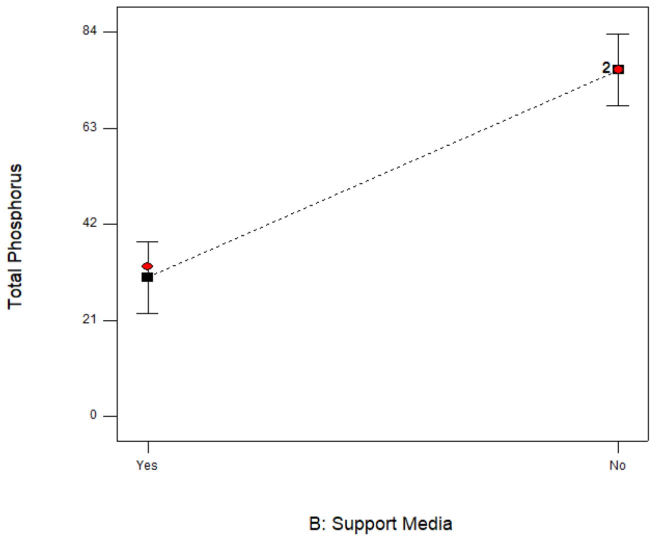
**Figure 5:** Pareto Chart of total phosphorus removal

*3.9. Effect of independent processing parameters on total phosphorus removal*

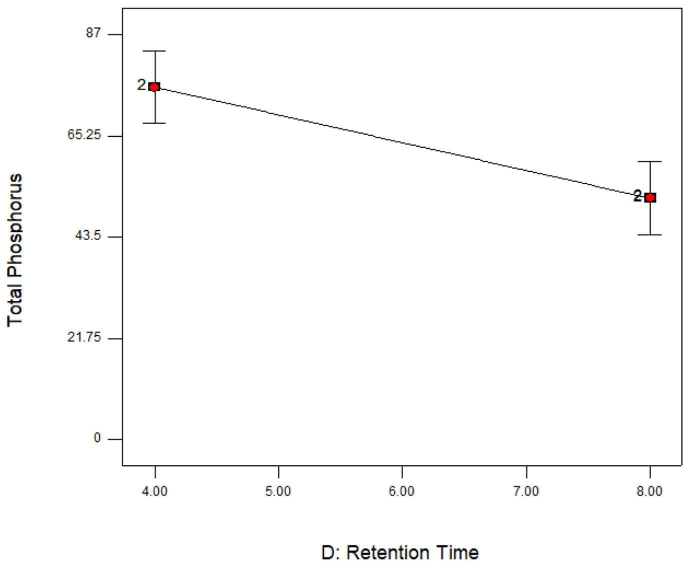
Figure 6a shows the effect of three independent variables (ratio AMC: SW, support media and retention time) on total phosphorus removal. As supported by Pareto Chart Figure 5, the ratio AMC: SW was the most significant factor in total phosphorus removal because it was above Bonferroni Limit in positive effect. Total phosphorus removal achieved 1.26% when ratio 1:3 used, however the value of total phosphorus removal become higher 75.72% when ratio 2:3 was used. When the four types of bacteria were mixed together become the mixed culture, the removal of total phosphorus from rubber wastewater was 69% which was higher compared to the used of each bacteria which was pure culture [25]. Figure 6b shows that studied support media (between yes and no) give a significant effect on total phosphorus removal. Total phosphorus removal was 30.37% at yes which was present of support media while it became higher (75.72%) at no which was absent of support media. The maximum COD and $BOD_5$ reduction achieved when using support media such as PVC was 80.1% and 72.1%, respectively [27]. Figure 6c shows that studied retention time (between 4 days and 8 days) total phosphorus removal was 75.72% when 4 days applied and the value of total phosphorus removal become lower which was 51.80% when 8 days was applied. The best retention time for total phosphorus removal approximately 4 to 6 days only which was contributed to 100% total phosphorus removal [6].




(a) Factor ratio AMC: SW




(b) Factor support media

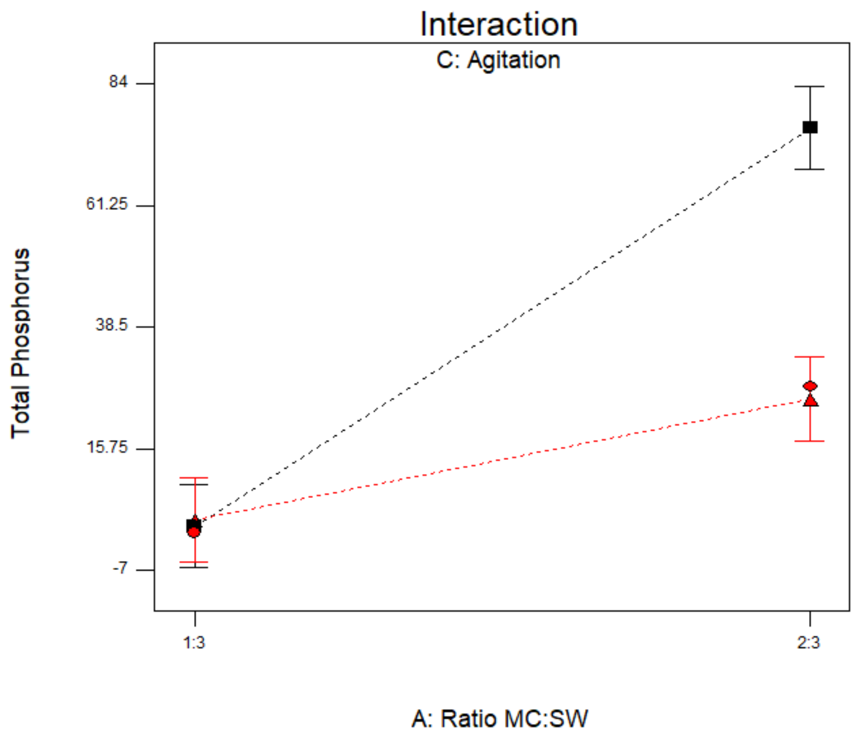



(c) Factor retention time

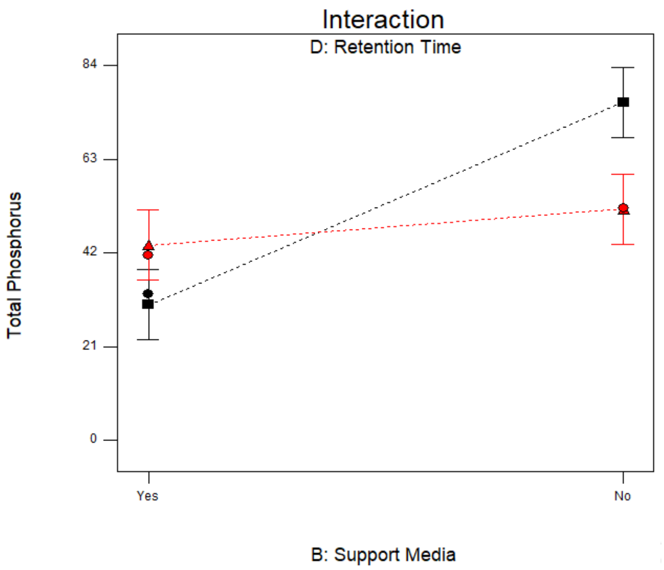
**Figure 6.** Analysis of most effective independent factor in total phosphorus removal

*3.10. Interaction effects between factors on total phosphorus removal*

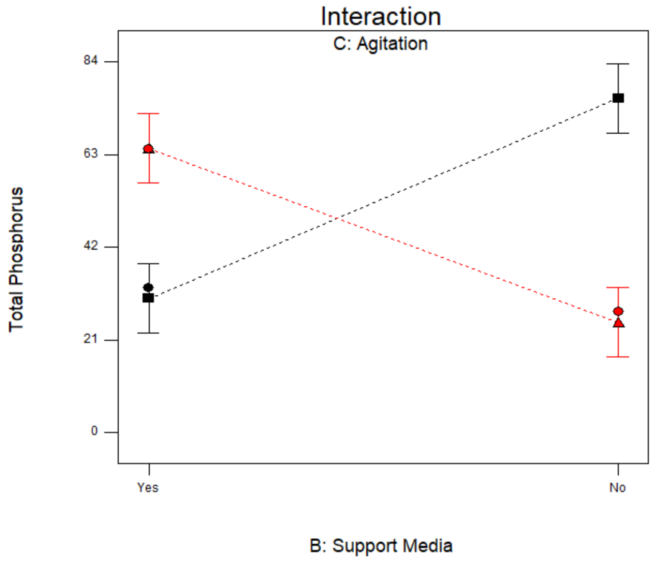
Figure 7a shows the interaction effect between the ratio AMC: SW and agitation (AC) on total phosphorus removal. When using ratio 2:3, for both 0 rpm (black line) and 100 rpm (red line) agitation, the total phosphorus removal value achieved 75.72% and 24.96%, respectively which was higher than ratio 1:3. Besides, there was no significant difference of agitation on total phosphorus removal when using ratio 1:3 but it showed a significant difference when using ratio 2:3. As indicated by Pareto Chart Figure 5, the most significant factor was ratio AMC: SW because it was above Bonferroni Limit while agitation was insignificant factor to total phosphorus removal since it was below than t-limit. Therefore, the ratio AMC: SW has important effect on total phosphorus removal. Total phosphorus removal achieved for ratio 1:3 was 1.26% at 0 rpm and become higher which was 2.39% at 100 rpm agitation. Figure 7(b) shows the interaction effect between support media and retention time (BD) on total phosphorus removal. When 4 days (black line) was applied, it gave a better performance on total phosphorus removal. There was a significant difference of present support media on total phosphorus removal because at present support media, total phosphorus removal achieved 30.37% while at absent support media, total phosphorus removal was 74.72%. When 8 days (red line) was applied, there was no significant difference of present of support media on total phosphorus removal because total phosphorus removal achieved 43.73% at present of support media, while at absent support media total phosphorus removal was 51.80%. Figure 7(c) shows the interaction effect between support media and agitation (BC) on total phosphorus removal. When 0 rpm (black line) was applied, it gave a better performance on total phosphorus removal. There was a significant difference of present support media on total phosphorus removal because at present support media, total phosphorus removal achieved 30.37% while at absent support media, total phosphorus removal was 75.72%. When 100 rpm agitation was applied, it shows a significant difference because the total phosphorus removal achieved 64.35% at present support media, while at absent support media total phosphorus removal was 24.96%.




(a) Factor ratio AMC: SW and agitation (AC)




(b) Factor ratio support media and retention time (BD)




(c) Factor support media and agitation (BC)

**Figure 7**: Interaction factor for total phosphorus removal

### *3.11. Best condition for COD removal*

For getting the highest COD removal, the best conditions for COD removal was determined during the experiments by Design Expert software (Table 10).

**Table 10.** Suggested best condition of COD for contaminated wastewater from hatchery industry

| | |
|---|---|
| Ratio AMC:SW | 2:3 |
| SM | No |
| Agitation | 100 rpm |
| Retention time | 8 days |

Three runs were conducted in order to compare the predicted result from Design Expert software and the actual result. Table 11 shows the comparison of predicted and actual data of total phosphorus removal. Run 1 was selected due to the highest value of COD removal compared to others. The highest total phosphorus removal achieved was 69%.

**Table 11.** Results from best condition for COD removal

| | COD Removal (%) | | |
|---|---|---|---|
| | Predicted | Actual | Error (%) |
| Run 1 | 74.59 | 69 | 8.10 |
| Run 2 | | 68 | 9.69 |
| Run 3 | | 68 | 9.69 |

### *3.12. Best condition for total phosphorus removal*

For getting the highest total phosphorus removal, the best conditions for total phosphorus removal was determined during the experiments by Design Expert software (Table 12).

**Table 12.** Suggested best condition of total phosphorus for contaminated wastewater from hatchery industry

| | |
|---|---|
| Ratio AMC:SW | 2:3 |
| SM | No |
| Agitation | 0 rpm |
| Retention time | 4 days |

Three runs were conducted in order to compare the predicted result from Design Expert software and the actual result. Table 13 shows the comparison of predicted and actual data of total phosphorus removal. Run 2 was selected due to the lowest value of error compared to others. The highest total phosphorus removal achieved was 75.68%.

**Table 13.** Results from best condition for total phosphorus removal

| | Total Phosphorus Removal (%) | | |
|---|---|---|---|
| | Predicted | Actual | Error (%) |
| Run 1 | 75.72 | 71.18 | 6.38 |
| Run 2 | | 75.68 | 0.05 |
| Run 3 | | 70.82 | 6.92 |

*3.13. Best condition for both highest COD and phosphorus removal*

Validation experiment was conducted based on the suggested of the best conditions by Design Expert software (Table 14). Three runs were conducted in order to compare the predicted result from Design Expert software and the actual result. The error was calculated by using equation (1). Table 15 shows the comparison of predicted and actual data on COD removal and total phosphorus. Run 3 was selected due to the lowest value of error compared to others. At condition to achieve the highest values for both COD and total phosphorus removal, we managed to achieve only 26.5% and 46.5% for COD and total phosphorus removal respectively.

$$\text{Error calculation} = \left|(\text{actual} - \text{predict})/\text{actual}\right| \times 100 \quad (1)$$

**Table 14.** Suggested best condition for contaminated wastewater from hatchery industry

| | |
|---|---|
| Ratio AMC:SW | 1:3 |
| SM | Yes |
| Agitation | 0 rpm |
| Retention time | 4 days |

**Table 15.** Results from suggested best condition

| | COD Removal (%) | | | Total Phosphorus (%) | | |
|---|---|---|---|---|---|---|
| | Predicted | Actual | Error (%) | Predicted | Actual | Error (%) |
| Run 1 | 24 | 25.58 | 6.18 | 53.60 | 42.86 | 25.07 |
| Run 2 | | 26.36 | 8.95 | | 41.57 | 28.95 |
| Run 3 | | 26.49 | 9.40 | | 46.50 | 15.28 |

## 4. Conclusion

The purpose of this study is to determine the factors affecting the removal of chemical oxygen demand, (COD) and total phosphorus in the contaminated wastewater from hatchery industry. For COD removal, the best condition was using 2:3 ratio AMC: SW, absent support media, 100 rpm agitation and 8 days retention time while the best condition for total phosphorus removal had the same best condition as COD removal except for the agitation and retention time because the agitation and retention time for total phosphorus removal were 0 rpm and 4 days respectively. The important factors for COD removal were ratio, agitation and retention time while for total phosphorus removal the important factor was ratio of AMC: SW only. Therefore, the best condition is different for COD removal and total phosphorus removal. Both processes have different requirements, which contribute to the different conditions for each removal. Further research on the treatment of hatchery wastewater using biological treatment is recommended. It would be best to investigate the other contaminants in the wastewater that could be treated using mixed culture and different factors.

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