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# Analysis of factors affecting algae removal from petrochemical industry wastewater using salts as biocide

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**Abstract.** Biocide was used in this study to remove algae in petrochemical wastewater. By using biocide, it has the advantage of producing a high quality effluent for reasonable operating and maintenance costs. Four independent factors, such as type of biocide, ratio of biocide mass to synthetic wastewater volume (B/W), agitation, and hydraulic retention time (HRT) were investigated. A two-level factorial design was used to investigate the effect of the independent factors as well as the interaction factors on the pH and reduction of algae mass (%). Some of the independent factors were shown to have a significant effect on both pH and reduction of algae mass (%). For pH, the order of significance was HRT > agitation > type of biocide > B/W ratio. It showed that the interaction factor of agitation \*HRT had the strongest effect (5.85%) on the pH value. For the reduction of algae mass (%), the order of significance was HRT > B/W ratio > agitation > type of biocide. It showed that interaction factor of agitation\*HRT had the strongest effect (8.79%) on the reduction of algae mass (%). From the analysis, the selected best conditions by Design Expert were determined at table salt as biocide, 3:2 B/W ratios, 100 rpm agitation rate, and HRT at 15 hours. It can be concluded that HRT was strongly influencing the application of salt in algae removal from petrochemical industry wastewater and the process could remove up to 85% of algae.

## 1. Introduction

The wastewater from the petrochemical industry is mostly contains toxic pollution [1]. Lagoon system is one of the most popular methods for wastewater treatment around the world and one of the simplest and least expensive. Lagoons are in-ground earthen basins used for the treatment of industrial wastewater by a natural process involving the use of algae and bacteria [2]. However, excessive algae growth can cause algae blooms. Algae blooms in lagoons affect on rising the pH level due to excessive photosynthesis by algae that cause more carbon dioxide (CO<sub>2</sub>) removed than added each night by respiration. As a result, pH may rise to abnormally high levels during the afternoon and may even remain high through the night [3]. So, one of wastewater treatment in the industry is to remove algae.

There are several techniques that have been practiced to remove algae from petrochemical wastewater such as chlorination, centrifugation, micro strainers, and others [4]. Chlorination has been



used successfully for the removal of algae. However, the major problems associated with this process are toxic to aquatic life and may require dechlorination even at low concentration [5]. Thus, one of the strategies to remove algae in petrochemical wastewater was by using biocide treatment. Biocides are chemicals designed to kill all sizes and life stages of organisms, especially microorganisms, and the effectiveness of biocides varies with the concentration of a biocide and duration of the exposure [6].

There are many types of biocides have been used in wastewater treatment nowadays. Oxidizing and non-oxidizing biocides have been used widely in petrochemical wastewater treatment [7,8]. Biocides can be added to wastewater to protect them against biological infestation and growth. For example, certain types of quaternary ammonium compounds are added to pool water or wastewater to act as an algicide, protecting the water from infestation and growth of algae. It is often impractical to store and use poisonous chlorine gas for wastewater treatment, so alternative methods of adding chlorine are used. These include adding compounds like salt [9]. Thus, the future study of biocide applications in algae removal from wastewater treatment plant can be enhanced.

The main objective of this study was to determine the factors that affect the algae removal process from petrochemical industry wastewater by using salt as a biocide. There are four factors that give a contribution to algae removals such as type of biocide, B/W ratio, agitation and HRT. Two Level Factorial Analysis (TLFA) was the process to determine the most contributing factor for algae removal. An important aspect of TLFA was the design of experiments [10]. TLFA designs allow us to study many factors with relatively small run size. They are very useful for identifying important factors and are widely used in many areas of scientific investigation [11-14].

## **2. Materials and methods**

### *2.1 Materials*

The sample of wastewater was collected from facultative ponds at one of the petrochemical wastewater industries in Gebeng, Pahang. Sodium Hydroxide (NaOH) was obtained from Sigma-Aldrich. Table salt and rock salt were purchased from local grocery shop.

### *2.2 Synthetic wastewater preparation*

The synthetic wastewater was prepared according to the pH value of analyzed wastewater. To regulate the pH, sodium hydroxide (NaOH) solution was added into the synthetic wastewater. The design basis of pH value was 11 because pH value of algae-containing wastewater was around 10 to 11 [15]. The pH value was determined using a pH electrode (Mettler Toledo, USA).

### *2.3 Mass of algae analysis*

Mass of algae in wastewater was analyzed by using dry weight measurement method. This method was conducted by calculating the mass difference between empty aluminum pan and aluminum pan with algae after dry in an oven for 12 hours [16].

### *2.4 Experimental design*

Four selected factors were studied in this research to investigate their effects on pH and reduction of algae mass (%) by using two-level factorial design. The factors were type of biocide, B/W ratio, agitation, and hydraulic retention time (HRT). The B/W ratio is according to mass:volume ratio with the working volume of 100 ml. The agitation was controlled by using orbital shaker at 100 rpm. All runs were conducted in batch system at HRT range between 8 to 15 hr. Table 1 shows the design factors and levels were coded as -1 (low level) and +1 (high level) where low level indicates the lowest range of the factors and high level indicates the highest range of the factors. 16 runs of experiments were conducted in this study. The responses of the experimental design were analyzed by using ANOVA based on the p-value with 95% of confidence level.

**Table 1.** Experimental design for factorial analysis.

Run	Factor 1 A: Type of biocide	Factor 2 B: B/W ratio	Factor 3 C: Agitation	Factor 4 D: HRT	Response 1 pH	Response 2 Reduction of algae mass
		(mg : mL)	(rpm)	(hour)		(%)
1	-1	-1	-1	-1	10.17	13.33
2	+1	+1	+1	+1	9.46	87.5
3	-1	-1	-1	+1	9.94	66.67
4	-1	+1	+1	+1	9.53	85.19
5	+1	-1	+1	-1	9.93	62.5
6	+1	+1	-1	-1	9.99	70
7	+1	-1	+1	+1	9.54	78.78
8	-1	+1	-1	+1	9.94	82.35
9	+1	-1	-1	+1	9.88	77.78
10	-1	-1	+1	+1	9.54	64.3
11	+1	-1	-1	-1	10.14	21.05
12	-1	+1	+1	-1	9.9	64
13	-1	-1	+1	-1	9.95	56.52
14	+1	+1	-1	+1	9.93	86.67
15	-1	+1	-1	-1	10.16	52
16	+1	+1	+1	-1	9.92	75
Factor	Level					
	-1			+1		
Type of biocide	Table salt			Rock salt		
B/W ratio (mg:mL)	1:1			3:2		
Agitation (rpm)	Without agitation (0)			With agitation (100)		
HRT (hour)	8			15		

### 3. Results and discussion

#### 3.1 Screening of factors affecting on the pH and reduction of algae mass (%)

Two-level factorial design was used to study the effect of all factors on the pH and reduction of algae mass. The factors were type of biocide, B/W ratio, agitation, and HRT. The result in Table 1 shows that pH value was ranged from 10.17 to 9.46. While, the reduction of algae mass (%) was ranged from 13.33 to 87.5%.

#### 3.2 Analysis of variance (ANOVA) for pH and reduction of algae mass (%)

Table 2 and Table 3 show the ANOVA analysis of pH and reduction of algae mass, respectively. The p-value for both pH and decrease in algae mass showed that the model was significant. The R-Squared for pH and reduction of algae mass were 0.9844 and 0.9981, respectively. The R-Squared was higher than 0.9 for chemical process, so this model was accepted. It is necessary to examine any interactions that are important in the experimental design analysis; the 2-way interactions show their significance at 95% confidence level. Therefore, a higher order of regression model such as the quadratic model is

needed to fit the response for pH and reduction of algae mass (%). The regression equation Eq. (1) and Eq. (2) represent the best description after the elimination of non-significant parameters ( $p > 0.01$ ) from the results summarized in Table 2 and Table 3. The final empirical models in terms of actual parameters were determined as follows:

$$\text{pH} = +9.87 - 0.021*A - 0.016*B - 0.15*C - 0.15*D - 7.500E - 003*A* B + 0.012*A* C + 3.750E - 003*A*D - 2.500E - 003*B*C + 0.011*B*D - 0.054*C*D \text{ (Eq. 1)}$$

$$\text{Reduction of algae mass} = +65.23 + 4.68* A + 10.11* B + 6.5* C + 13.43* D - 0.23* A * B - 0.46* A* C - 0.65* A*D - 3.91*B*C - 3.34* B*D - 6.21*C*D - 2.14*A*B*D + 4.54*B*C*D \text{ (Eq. 2)}$$

\*\*where A is type of biocide, B is B/W ratio, C is agitation and D is HRT.

**Table 2.** ANOVA table for pH.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.77745	10	0.077745	31.47571	0.0007	significant
A-Type of biocide	0.007225	1	0.007225	2.925101	0.1479	
B- B/W Ratio	0.004225	1	0.004225	1.710526	0.2478	
C-Agitation	0.354025	1	0.354025	143.33	< 0.0001	
D-HRT	0.36	1	0.36	145.749	< 0.0001	
AB	0.0009	1	0.0009	0.364372	0.5724	
AC	0.0025	1	0.0025	1.012146	0.3606	
AD	0.000225	1	0.000225	0.091093	0.7749	
BC	1E-04	1	1E-04	0.040486	0.8485	
BD	0.002025	1	0.002025	0.819838	0.4067	
CD	0.046225	1	0.046225	18.71457	0.0075	
Residual	0.01235	5	0.00247			
Cor Total	0.7898	15				
R-Squared	0.984363					
Adj R-Squared	0.953089					

**Table 3.** ANOVA table for reduction of algae mass (%).

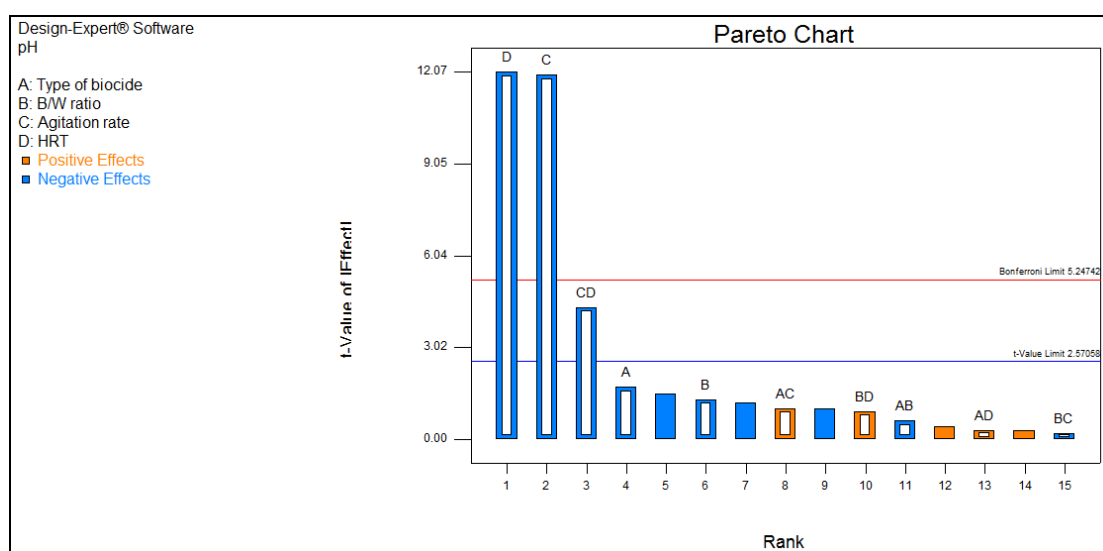
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	7001.265	12	583.4387	130.1145	0.0010	significant
A-Type of biocide	350.8129	1	350.8129	78.23587	0.0030	
B- B/W Ratio	1635.798	1	1635.798	364.8044	0.0003	
C-Agitation	675.2202	1	675.2202	150.583	0.0012	
D-HRT	2884.764	1	2884.764	643.3402	0.0001	
AB	0.837225	1	0.837225	0.186712	0.6948	
AC	3.404025	1	3.404025	0.759142	0.4477	
AD	6.8644	1	6.8644	1.530851	0.3040	
BC	244.9225	1	244.9225	54.62092	0.0051	
BD	178.356	1	178.356	39.77573	0.0081	
CD	616.7772	1	616.7772	137.5494	0.0013	
ABD	73.35923	1	73.35923	16.36007	0.0272	
BCD	330.1489	1	330.1489	73.62753	0.0033	
Residual	13.45213	3	4.484042			

Cor Total	7014.717	15				
R-Squared	0.998082					
Adj R-Squared	0.990411					

### 3.3 Effect on pH value

#### 3.3.1 Main effect and interaction effect between factors on pH value

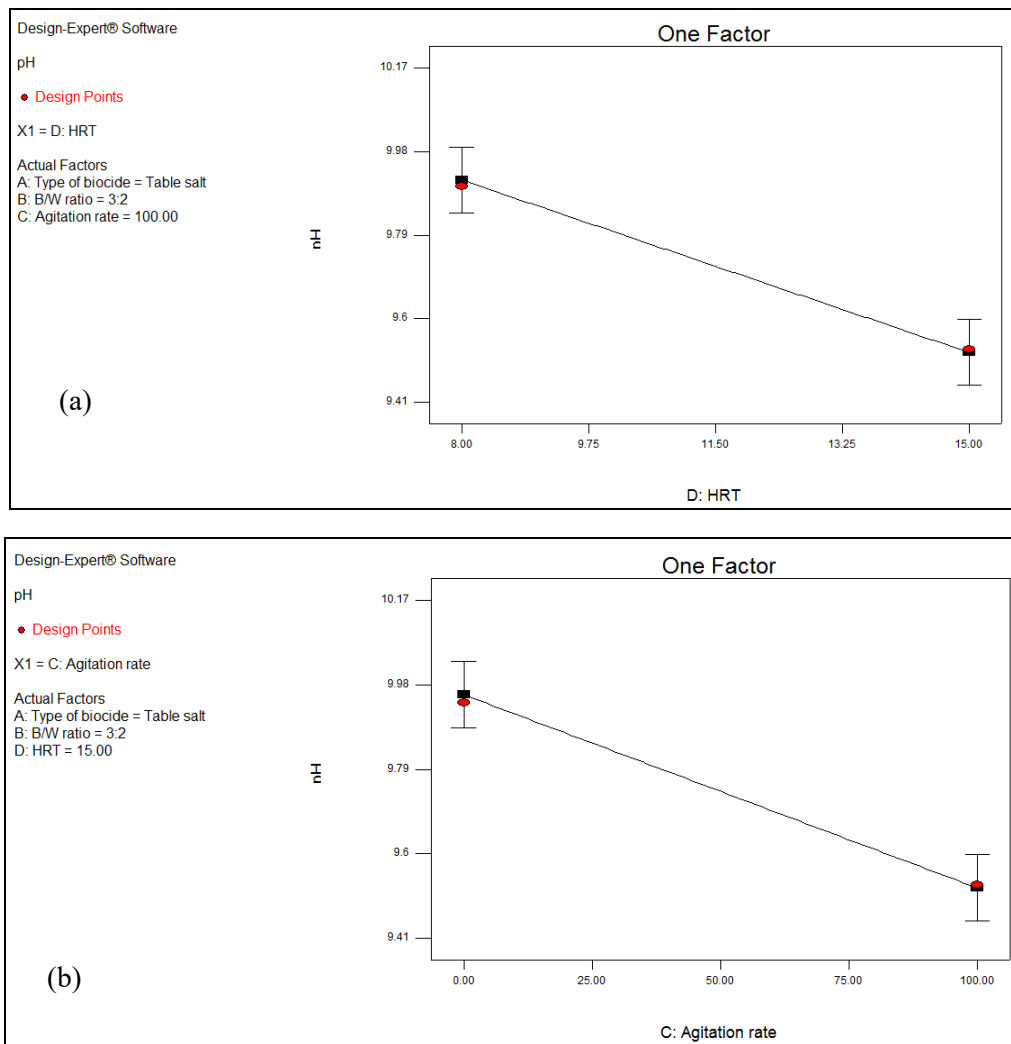
The Pareto Chart in Figure 1 shows the main effects and interaction effects of the factors for the pH value. For the main effect, it showed that HRT (D) gave the highest contribution for pH and followed by agitation factor (C). For interaction effects, the agitation and HRT (CD) gave the highest contribution to decrease the pH value rather than other interaction. Both of the highest contributions of main factor and interaction was in negative effect. The Negative effect was when the factor value not proportional to the response value. Therefore, pH was decreased as the value of the factors increased.



**Figure 1.** Contribution of independent and interaction effect to pH value.

#### 3.3.2 Effect of independent processing parameters on pH value

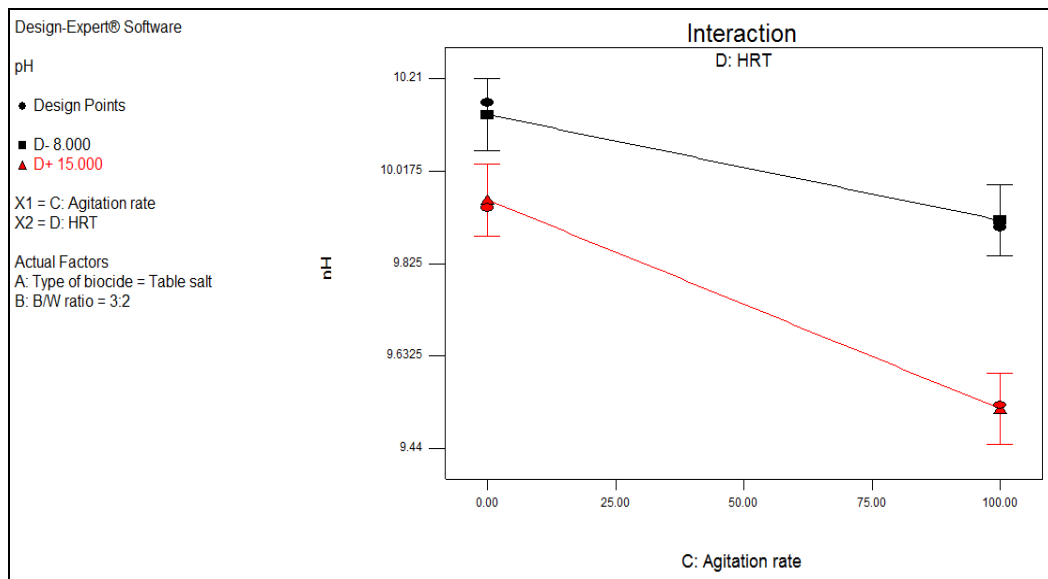
The effects of two independent variables on the pH were shown in Figure 2a and Figure 2b. Based on Figure 2a, pH level decreased with increasing of HRT. From the figure, pH was lower at 15h than at 8h. The pH value was 9.92 at 8h and 9.52 at 15h. It also showed there was no significant difference in pH between 8h and 15h. Chlorine ions ( $\text{Cl}^-$ ) from NaCl react with water producing hydrochloric acid (HCl) and lower the pH of the sample [17]. Hydrochloric acid (HCl) react with alkaline water and decreased pH rapidly at 0 h to 8 h. Meanwhile, the pH of sample started to be stable between 8h to 15h. Besides that, pH was decreased when the agitation was applied (100 rpm) as shown in Figure 2b. From the figure, the pH value at 0 rpm was 9.96 and 9.52 at 100 rpm. When the agitation was applied, the atmospheric carbon dioxide ( $\text{CO}_2$ ) reacts with the wastewater producing carbonic acid ( $\text{H}_2\text{CO}_3$ ) and cause the pH of water decreased [18].



**Figure 2.** Most effective independent parameters in pH value.

### 3.3.3 Interaction effects between factors on pH

According to the Design Expert Software analyzed data, the interaction gives a negative effect with the highest contribution was the interaction effect between agitation and HRT (CD) as shown in Figure 3. The figure shows that the pH level was lowest with agitation (100 rpm) and higher HRT value (15 hr). The pH level was 9.52 at 15 hours with 100 rpm agitation. However, the pH level was highest without agitation and low HRT (8 hr). The pH was 10.14 at 8 hours without agitation. Agitation increased the removal efficiency of algae [19]. Figure 1 shows that HRT is the most contributing effect in the pH reduction process. The blue coloured column in Pareto Chart (Figure 1) shows that HRT gives a negative effect to pH value, where the HRT value is inversely proportional to pH value. HRT is an important parameter in wastewater treatment, which directly affects reaction time, higher HRT value lead to longer reaction time. Therefore, higher HRT also has a significant effect on the mixing time for the wastewater and salt. As indicated in Figure 3, with the higher value of HRT (15 hr) the pH value becomes lower. This is aligned with the aim of this research to reduce the wastewater pH value.

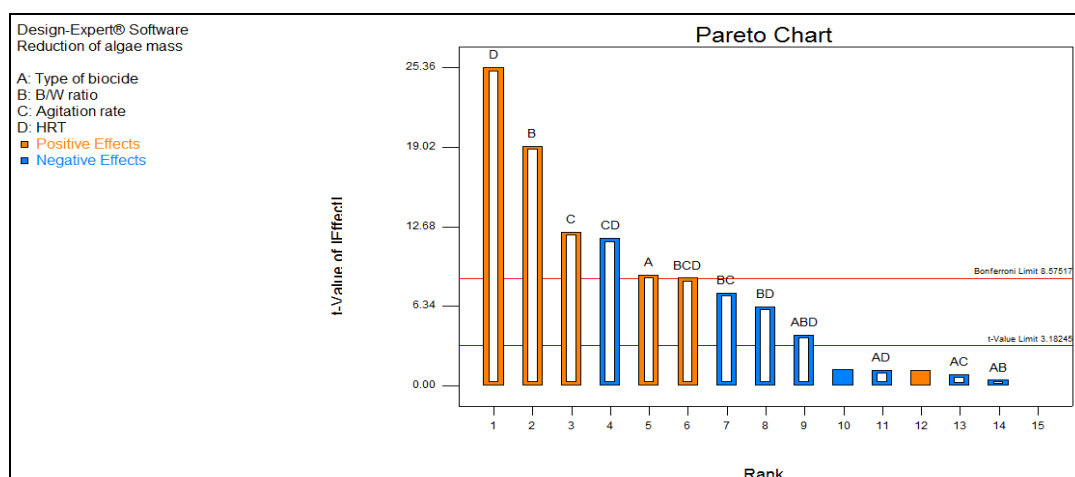


**Figure 3.** Interaction effect between factors on pH.

### 3.4 Effect on reduction of algae mass (%)

#### 3.4.1 Main effect and interaction effect between factors on reduction of algae mass (%)

The Pareto Chart in Figure 4 shows the main effects and interaction effects of the factors for the reduction of algae mass. For the main effect, it showed that HRT factor (D) gave the highest contribution for reduction of algae mass followed by ratio of biocide in synthetic wastewater factor (B) and agitation factor (C). For interaction effects, it showed the most contributing interaction effect between factors in the reduction of algae mass were agitation and HRT (CD). The effects were exceeding Bonferroni limit. Pareto charts establish t value of the effect by two limit lines, namely the Bonferroni limit line and t limit line. Coefficients with t value of effect above the Bonferroni line are designated as certainly significant coefficient while coefficients with t value of effect between Bonferroni line and t limit line are termed as coefficients likely to be significant. Then, t value of effect below the t limit line is statistically insignificant [20]. Therefore, effects found significant under Bonferroni are therefore more likely to be real.

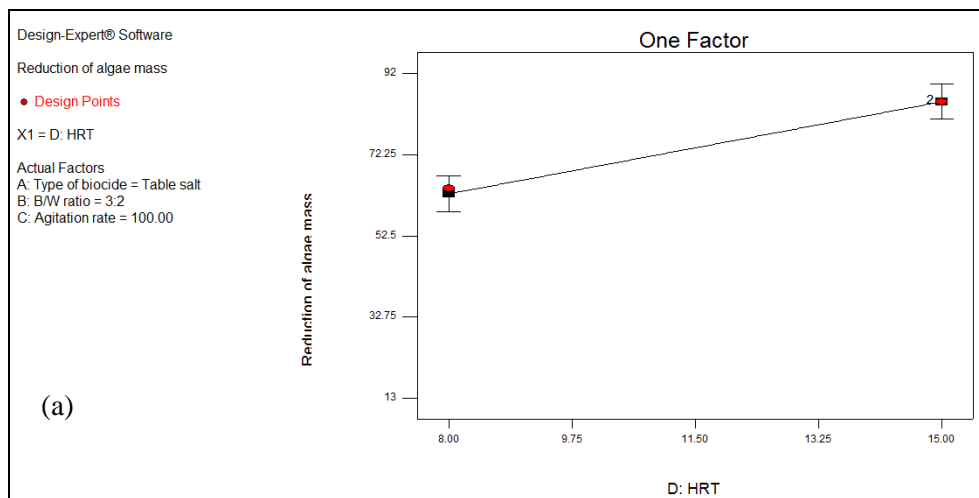


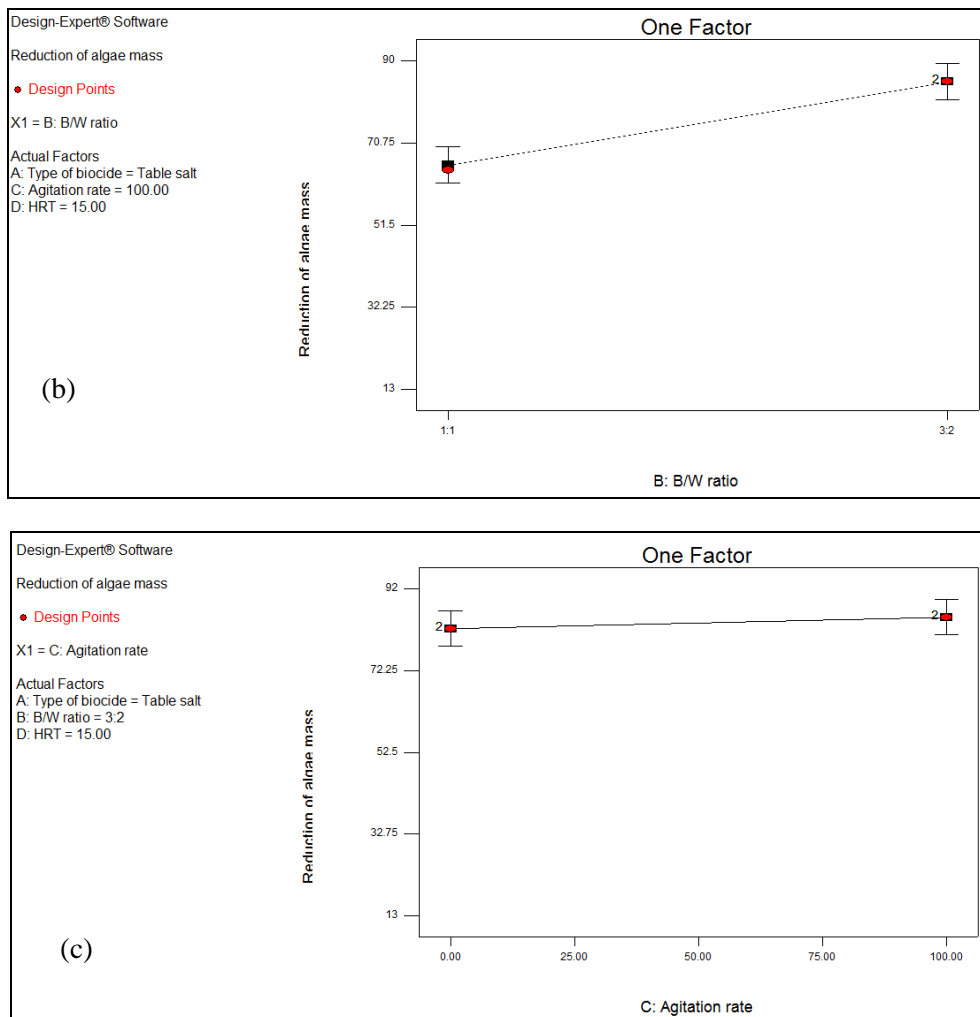
**Figure 4.** Contribution of independent and interaction effect to reduction of algae mass (%).



### 3.4.2 Effect of independent processing parameters on reduction of algae mass

Reduction of algae mass was significantly affected by HRT, B/W ratio, and agitation. The effect of HRT on the reduction of algae mass was shown in Figure 5a. It showed that the reduction of algae mass achieved 85.15% at 15 hours and was higher than 62.71% at 8 hours. The longer the HRT the better for the reduction of algae. After NaCl dissolves in water, algae are undergone osmosis process for several hours [21]. The longer reaction time means the longer osmosis process happen. Therefore, there is more algae cell removed. Figure 5b shows the B/W ratio gives significantly affect the reduction of algae mass (%). At 1:1 ratio, reduction of algae mass achieved 65.6% while increased to 85.15% at 3:2 ratios. It showed that the reduction of algae mass was proportional to the ratio. Salt concentration in water effects cells of algae [22]. High salt concentrations can cause osmosis happened and loss of cell activity. Algae cell needs to adjust osmotically between external solutes with a high concentration of NaCl and internal solutes in the cell cytoplasm [23]. Therefore, algae removal increased as the salinity of water increased [24]. Then, Figure 5c shows the agitation significantly affect the reduction of algae mass. It showed that the reduction of algae mass increased with agitation. Reduction of algae mass achieved 82.39% at 0 rpm while increased to 85.15% at 100 rpm agitation. Mixing is important in the wastewater treatment process which can be controlled by using agitation. Hence, application of agitation in the process resulted in significant improvement in the algae reduction due to the improvement in mass transfer fluxes. In addition, agitation also improved solubilization of particulate organic matter [25].

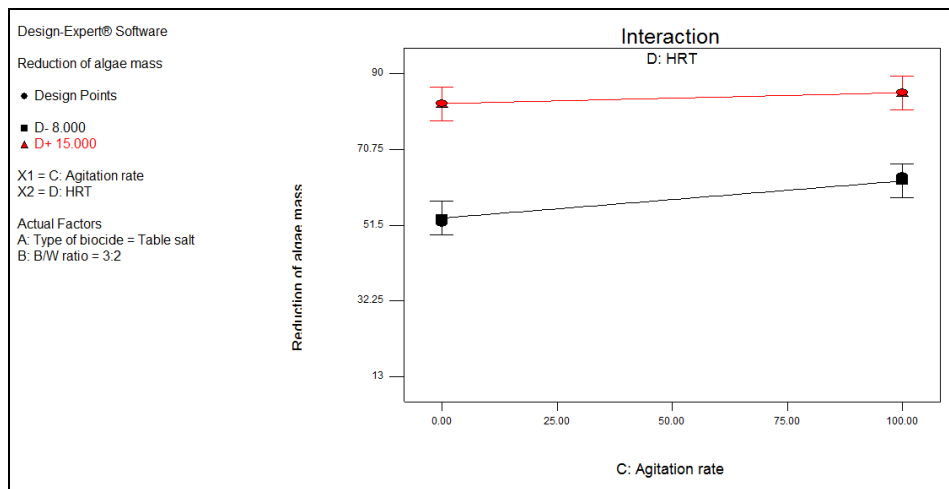




**Figure 5.** Most effective independent parameters in reduction of algae mass (%)

### 3.4.3 Interaction effect between factors to reduction of algae mass

Figure 6 shows the interaction effect between agitation and HRT (CD). The figure shows that the reduction of algae mass (%) was the highest at high agitation and HRT. The reduction of algae mass (%) was 85.15% at HRT of 15 hours and 100 rpm agitation. However, the reduction of algae mass was the lowest (53.29%) at the condition of no agitation and low HRT (8 days). This clearly shows that agitation is one of the most contributing factor in the algae reduction process as reported in section 3.4.1. Agitation is an important parameter since failures in the mixing process may result in non-uniform conditions of salt concentration, leading to poor system performance [26, 27]. Agitation has also been related to HRT which is influenced by algae concentration and morphology, rheological properties of the liquid phase and characteristics of the mixing system [28,29]. Therefore, longer HRT was better for reduction of algae mass.



**Figure 6.** Interaction effect between factors on reduction of algae mass (%).

### 3.5 Validation of experiment

The criteria set up to select the best processing condition is given in Table 4. Duplicate experiments were run to validate the suggested best condition and the result of the experiment is shown in Table 5. The experimental error was calculated for both pH and reduction of algae. The errors for pH were 0.7% and 1.12% for Run 1 and 2, respectively. Meanwhile, the errors for reduction of algae mass were 16.12% and 3.07% for Run 1 and 2, respectively. The best conditions from the experiment are by using salt as a biocide, 3:2 B/W ratios, 100 rpm agitation and HRT at 15 hours. 85% of algae removal was achieved at these conditions. There are a variety of ways to reduce growth of algae in the wastewater treatment pond. In some cases, coagulant and chlorine are applied where 96% of algae is removed using the coagulant and chlorine with dosages of approximately 20 mg/l and 4.0 mg/l, respectively [30]. Raman and Cook, (1988) had used treatment of wastewater with copper sulphate as an algacide and achieved 75% algae removal. Others have used buffered alum treatments to precipitate phosphate from the water column and reduce the loading of phosphorus that can reduce the algae [32]. Therefore, these results are comparable to the result obtained in this study with algae removal of 85%.

**Table 4.** Criteria for determination of best condition.

Name	Goal	Value
Type of biocide	is equal	Table salt
B/W ratio	is in range	1:1 – 3:2
Agitation	is in range	0 - 100
HRT	is in range	8 - 15
pH	minimize	-
Reduction of algae mass	maximize	-

**Table 5.** Comparison between predicted versus actual for pH and reduction of algae based on best condition

Factor		Value
A: Type of biocide		Table salt
B: B/W ratio		3:2
C: Agitation		100 rpm
D: HRT		15 h
Predicted pH		9.52
Experiment pH	Run 1	9.59
	Run 2	9.63
Error	Run 1	0.70%
	Run 2	1.12%
Predicted reduction of algae mass (%)		85.15
Experiment reduction of algae mass (%)	Run 1	73.33
	Run 2	82.61
Error	Run 1	16.12%
	Run 2	3.07%

#### 4. Conclusion

The result shows that HRT and agitation were the most contributing main factors as well as interaction factors for both pH and reduction of algae mass (%). For the reduction of algae mass, B/W ratio also gives significantly affect to decrease the algae mass. The best conditions for algae reduction were as follows: Table Salt as biocide, 3:2 B/W ratios, 100 rpm agitation and HRT at 15 hours. The experimental results at best condition for pH and reduction of algae mass were closed to the predicted values with 0.7% and 3.07% errors, respectively. As a conclusion, HRT was strongly influencing the application of salt in algae removal from petrochemical industry wastewater and the process could remove up to 85% of algae.

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#### Conflict of interest statement

The authors have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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