ORIGINAL RESEARCH PAPER

Optimal conditions for the biological removal of ammonia from wastewater of a petrochemical plant using the response surface methodology

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ABSTRACT: High concentrations of nitrogen compounds, such as ammonia observed in the petrochemical industry, are the major environmental pollutants. Therefore, effective and inexpensive methods are needed for its treatment. Biological treatment of various pollutants is a low cost and biocompatible replacement for current physico-chemical systems. The use of aquatic plants is an effective way to absorb the nutrient pollutants. In this study, the optimal operating conditions in the biological removal of ammonia from the urea-ammonia wastewater of Kermanshah Petrochemical Company by *Lemna gibba* were determined using the response surface methodology. *Lemna gibba* was collected from the ponds around Kermanshah and maintained in a nutrient medium. Effect of the main operational variables such as ammonia concentration, residence time and *Lemna gibba* to surface ratio on optimal conditions of ammonia removal from wastewater has been analyzed using the Box-Behnken model design of experiments. Model numerical optimization was performed to achieve the maximum amount of ammonia removal from wastewater. The ammonia removal percentage varied from 13% to 88%, but the maximum amount of ammonia removal was determined at ammonia concentration of 5 ppm and *Lemna gibba* residence time of 11 days in wastewater based on the quadratic model. *Lemna gibba* to surface ratio of 2:5 was measured at 96.449%. After optimization, validation of ammonia removal was performed under optimum conditions and measured at 92.07%. Based on the experimental design and the predicted under model conditions, the maximum amounts of ammonia removal percentage in the experiments were 82.84% and 88.33% respectively, indicating the high accuracy of the model to determine the optimum conditions for the ammonia removal from wastewater.

Keywords: *Ammonia removal; Lemna gibba; Optimization; Petrochemical wastewater; Response surface methodology.*

INTRODUCTION

In recent years, the increasing demand for energy has led to the generation of various pollutants and, consequently, clean water crisis (Pal *et al.,* 2016). Petrochemical complexes are among the most important polluting industries and the effluent produced in different plants has high levels of organic and inorganic pollutants (Hodges *et al.,* 2017; Cechinel *et al.,* 2015). Uncontrolled release of nitrogen compounds to the environment leads to many environmental problems. According to the studies yet have been done, many countries have a stringent environmental legislation to control nitrogen release into the environment (Zimmo *et al.,* 2004). Petrochemical wastewater treatment is carried out using various physical, chemical and biological methods (Singh *et al.,* 2017). Phytoremediation is a biological treatment in which plants are used to remove pollutants from the medium (Lee 2013). Conventional nitrogen removing

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methods employed in the wastewater treatment plants are effective but expensive. Because of the nitrogen conversion to gas, it is not possible to reuse it (Toyama *et al.,* 2014). Phytoremediation is cost-effective and easy to operate and transforms the wastewater nutrients into reusable biomass (Zhao *et al.,* 2014). The aquatic plants directly absorb nutrient pollutants from wastewater and that is why they are more effective than terrestrial plants (Cheng and Stomp 2009). Various aquatic plants such as canna lily, water lettuce, water hyacinth, reed, pennywort and duckweed have been used for wastewater treatment (Zhao *et al.,* 2014). Duckweeds are small aquatic floating plants belonging to the Lemnaceae family. These plants have a rapid growth rate and high nutrient uptake from wastewaters. In addition, they have a high potential for ammonium adsorption from wastewater and converting it into proteins. In recent years, many researches have focused on duckweed and its role in wastewater treatment (Cheng *et al.,* 2002; El-Shafai *et al.,* 2007; Xu & Shen 2011). Among the various plant-based wastewater treatment systems, duckweed ponds have been successfully used for the removal of heavy metals and nutrients in many countries. Recent studies have shown that these systems can also be used to remove organic micropollutants such as pesticides and pharmaceutical wastewater (Gatidou *et al.,* 2017). High level of ammonia in urea-ammonia units is one of the major problems in petrochemical wastewaters. A variety of physical, chemical and biological methods (such as activated sludge, ammonia anaerobic oxidation, ion exchange, contact membrane, etc) have been proposed to improve ammonia removal from wastewater. Compared to these methods, phytoremediation has significant benefits, including low operating costs and nutrient recycling (Krishna *et al.,* 2012). In order to increase the efficiency of bioremediation, it is essential to know the optimal operating conditions in wastewater treatment. Classical optimization methods are performed by changing one factor at time and keeping others constant (Moghadam *et al.,* 2008; Moghadam *et al.,* 2009; Moghadam *et al.,* 2010; Khorram *et al.,* 2015). These methods are time-consuming, expensive and unable to examine the interactions among various factors simultaneously. Some influential variables in biological processes have interactional effects. Eexperimental design methods, such as response surface methodology (RSM), have been successfully used to optimize various processes. RSM consists of mathematical and statistical techniques

for the development of functional relationships between the response of interest and a number of controllable input variables in a complex process. In this method, the optimum experimental conditions are obtained by analyzing the minimum number of the tests, and the process can be formulated. However, statistical methods are used for biological treatments in many studies (Amini *et al.,* 2009; Mona *et.al.,* 2011; Shahriari Moghadam *et al.,* 2014; Titah *et al.,* 2014; Choinska *et al.,* 2018). To the best of our knowledge, there is no report on the optimization of ammonia removal (AR) from petrochemical wastewater using *limna gibba* by the statistical analysis of the operating conditions. In the present work, RSM was used as a statistically-based design of experiment (DOE) to study the effects of main operating variables, including ammonia concentration (X_C), *Lemna gibba* to free surface fluid ratio (X_{LR}), and residence time of *Lemna gibba* in wastewater (X_t), on the removal of ammonia. Moreover, the optimal operating conditions for biological removal of ammonia from the urea-ammonia wastewater of Kermanshah Petrochemical Company were determined. This study has been carried out in the Research Laboratory of Kermanshah University of Technology of Iran in 2017.

MATERIALS AND METHODS

Materials and culture

Lemna gibba (order Arales), which belongs to the Lemnaceae family, was collected from a watershed around Kermanshah in Iran. The plant was verified by taxonomy experts using the identification keys, and the purified species was replicated in 3 glass aquariums containing culture medium with a length of 100 x 40 x 30 cm in the laboratory. Table 1 shows the composition of the culture medium used in the

Table 1: Composition of the culture medium

Compounds	Value $\frac{mol}{m^3}$
$CaCl_2$	1.00
$MgSO_4$	1.65
NaH_2PO_4	0.65
K_2SO_4	0.50
K_2CO_3	0.16
$Fe - EDTA$	27×10^{-3}
H_3BO_3	5.77×10^{-3}
$MnCl_2$	1.13×10^{-3}
$ZnSO_4$	0.19×10^{-3}
$CuSO_4$	0.08×10^{-3}
Na_2MoO_4	0.05×10^{-3}

aquariums. The medium was replaced every three days with 300 ml of nutrient solution (Cedergreen *et al., 2002*). The auxiliary light for growth was provided by a plant LED growth lamp (25 *W* –12 *V*) in a 16-hour light cycle and 8-hour darkness. The optimized culture medium was used in pH = 7.2 and the environment temperature was maintained at 25 °C (Cedergreen *et al., 2002*).

Optimization experiments

Optimization tests were carried out in 15 glass aquariums with dimensions of 50 x 25 x 30 cm. Different treatment aquariums were randomly placed in the laboratory and 25 liters of the petrochemical wastewater was added to each. Operational conditions of each treatment were specified according to Table 4, and the environment temperature was maintained at 25°C. Before the optimization process, the capacity and ability of the plant for the removal of ammonia were examined under Run No. 4 (medium level of operational variables).

Ammonia measurement

Sampling of wastewater (diluted in required concentrations) was performed in different operating conditions. An ammonia measurement device, namely I733 Checker HC (Handheld Colorimeter– HANA Ind.), was used to determine the amount of ammonia dissolved in water based on the device protocol. Characteristics of the wastewater containing ammonia are shown in Table 2. The general characteristics of the wastewater (such as pH, TH, Ca^{+2}, conductivity, MA, PA, SS, TDS and Cl reported in Table 2) were investigated in the research laboratory of Kermanshah Petrochemical Company based on the protocol (Patnaik, 2010).

Design of experiments and optimization

According to the previous studies, the removal of ammonia depends on various variables and it is necessary to reduce costs of experiments and economize the time (Goncalves *et al., 2011*; Li *et al., 2007*; Peng *et al., 2006*). These goals can be achieved using RSM to obtain the suitable data from the experimental runs. This method has been reported in the literature as away to reduce the production process costs. In the present study, the Box-Behnken experimental design method was used to identify the optimal conditions for the removal of ammonia from petrochemical wastewater using *Lemna gibba*. In this regard, DOE was carried out using Minitab 18 software. Effects of the main operating variables, including ammonia concentration (C), *Lemna gibba* to free surface fluid ratio (LR), and residence time of *Lemna gibba* in wastewater (t) on the removal of ammonia from wastewater were investigated. The range of levels of variables (low (-1), middle (0), and high (+1)) were determined based on the Box-Behnken experimental design method and listed in Table 3. These values were considered for the ammonia concentrations of 5, 10, and 15 ppm, *Lemna gibba* to free surface fluid ratios of 1:5, 3:10, and 2:5, and *Lemna gibba* residence times of 3, 7, and 11 day. Using this method, 13 tests with two duplicate tests were done at a central point to determine the errors. The experimental design and results of the tests are shown in Table 4. The *Lemna gibba* to free surface fluid ratio was measured by dividing the water surface. For example, when the aquarium surface is completely covered with the plant, and when half

Table 2: Characteristics of the wastewater containing ammonia

Characteristics	Value
pH	9.9
TH (*ppm as* $CaCO_3$)	290
Ca^{+2} (*ppm as* $CaCO_3$)	45
Conductivity ($\frac{\mu s}{cm}$)	19600
Ammonia (*ppm*)	15
MA (*ppm as* $CaCO_3$)	950
PA (*ppm as* $CaCO_3$)	380
Cl (*ppm*)	4900
TDS ($\frac{mg}{L}$)	11750
SS. ($\frac{mg}{L}$)	52

Table 3: The range of levels in variables

Factors	Symbol	Range of levels		
		−1	0	+1
Ammonia concentration (*ppm*)	C	5	10	15
Residence time (*day*)	t	3	7	11
Lemna gibba to free surface fluid ratios	LR	1:5	3:10	2:5

Table 4: Experimental design for three independent variables and the responses

Run No.	Manipulated variables			Response
	X_C	X_t	X_{LR}	AR (%)
1	0	1	-1	31.17
2	-1	0	-1	39.41
3	0	-1	1	32.72
4	0	0	0	29.19
5	0	-1	-1	13.36
6	-1	1	0	78.24
7	1	0	1	44.22
8	1	-1	0	20.43
9	0	0	0	28.90
10	0	1	1	88.03
11	0	0	0	28.89
12	1	0	-1	32.71
13	-1	0	1	56.63
14	1	1	0	83.63
15	-1	-1	0	62.28

the water surface is covered with the plant, $X_{LR} = 1:2$.

Analysis of variance (ANOVA) is a reliable method to analyze and define the degree of certainty in the experimental data (Montgomery, 2017; Ryan *et al.,* 2011). Further statistical analysis was performed on the model using the ANOVA. The response variable was fitted with a full quadratic model in order to relate the removal of ammonia % to this variable. The form of the mathematical model is shown in Eq. 1.

$$P = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_{ij} \quad (1)$$

Where, P is removal of ammonia %, X_i, and X_{ij} are uncoded independent variables, β_0 is offset term, and β_i, β_{ij}, and β_{ij} are regression coefficients. The mathematical experimental model was tested via ANOVA with a significance level of 5%. ANOVA was used to determine the significance of the second-order models. The statistical significance of the second-order models was determined by F-value. The calculated F-value is defined as the mean squares regression (including linear, square, and interaction) and the mean-square residual as can be seen in Eq. 2:

$$F - value = \frac{MS_{regression}}{MS_{residual}} \quad (2)$$

In which, mean squares regression and mean square residual can be calculated by Eqs. 3 and 4 respectively.

$$MS_{regression} = \frac{SS_{regression}}{DF_{regression}} \quad (3)$$

$$MS_{residual} = \frac{SS_{residual}}{DF_{residual}} \quad (4)$$

RESULTS AND DISCUSSION
Measurement of ammonia removal

Results of the percentage of ammonia removal from petrochemical wastewater with different levels of operational variables are shown in Table 4. During the experiment, despite the death of a part of the plant due to the toxicity of wastewater, no new *Lemna gibba* was added to the wastewater. The biomass of the dead plant was collected from the surface of the water.

Response variance analysis

A quadratic model for the removal of ammonia was obtained using the least square of error method, which is shown in Eq. 5.

$$AR \% = 28.99 - 6.95\, X_C + 19.03\, X_t$$
$$+13.12\, X_{LR} + 17.04\, X_C^2 + 15.11\, X_t^2$$
$$-2.79\, X_{LR}^2 + 11.81 X_C.X_t$$
$$-1.43 X_C.X_{LR} + 9.38 X_t.X_{LR} \quad (5)$$

The measured and predicted values of ammonia removal from the wastewater and also the values predicted by the quadratic model can be seen in Fig. 1. R^2 and R^2_{adj} in the ammonia removal model were 0.958 and 0.883, respectively. Standard deviation analysis for the quadratic model of ammonia removal is reported in Table 5. It can be seen that the total degree of freedom is 14. Furthermore, regression and residual error degrees

of freedom are 9 and 5, respectively. A comparison between F-values presented in Table 5 shows that the F-value calculated for the model is greater and has a higher significance level. The p-values less than 0.05 indicate that model terms are significant. According to this case, $X_t, X_{LR}, X_C^2, X_t^2$ and $X_C.X_t$ are the significant regression terms. The p-values greater than 0.05 indicate that the regression terms are not significant (such as the terms of, $X_t. X_{LR}$, X_{LR}^2 and $X_C. X_{LR}$).

The empirical model for ammonia removal is

plotted in contour diagrams using Minitab tools (Figs. 2a, b and c). In Fig. 2a, contour diagram of ammonia removal is plotted based on X_C and X_{LR} (coded residence time is at the intermediate level: $X_t = 0$). In Fig. 2b, contour diagram of ammonia removal is plotted based on X_t and X_{LR} (coded ammonia concentration is at the intermediate level: $X_C = 0$). Fig. 2c displays contour diagram of ammonia removal based on X_t and X_C (coded *Lemna gibba* to surface ratio is at the intermediate level: $X_{LR} = 0$). As it can be seen in the contour diagram
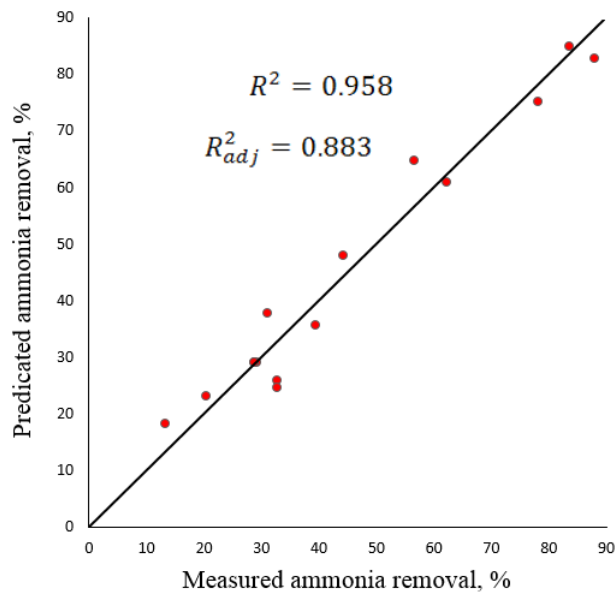

$$R^2 = 0.958$$
$$R_{adj}^2 = 0.883$$

Fig. 1: Comparison of the measured and predicted values of ammonia removal

Table 5: Standard deviation analysis for the quadratic model of ammonia removal

| Sources	DF	SS	MS	F − value	p − value	Degree of significance
Regression	9	7455.10	828.34	12.78	0.006	Highly significant
X_C	1	386.00	386.00	5.95	0.059	Not significant
X_t	1	2898.65	2898.65	44.71	0.001	Highly significant
X_{LR}	1	1376.81	1376.81	21.24	0.006	Highly significant
X_C^2	1	1071.74	1071.74	16.53	0.010	Significant
X_t^2	1	843.51	843.51	13.01	0.015	Significant
X_{LR}^2	1	28.70	28.70	0.44	0.535	Not significant
$X_C.X_t$	1	557.90	557.90	8.61	0.033	Significant
$X_C.X_{LR}$	1	8.15	8.15	0.13	0.737	Not significant
$X_t.X_{LR}$	1	351.56	351.56	5.42	0.067	Not significant
Residual error	5	324.16	64.83	-	-	-
Lack-of-fit	3	324.10	108.03	3721.02	0.060	Not significant
Pure error	2	0.06	0.03	-	-	-
Total	14	7779.26	-	-	-	-
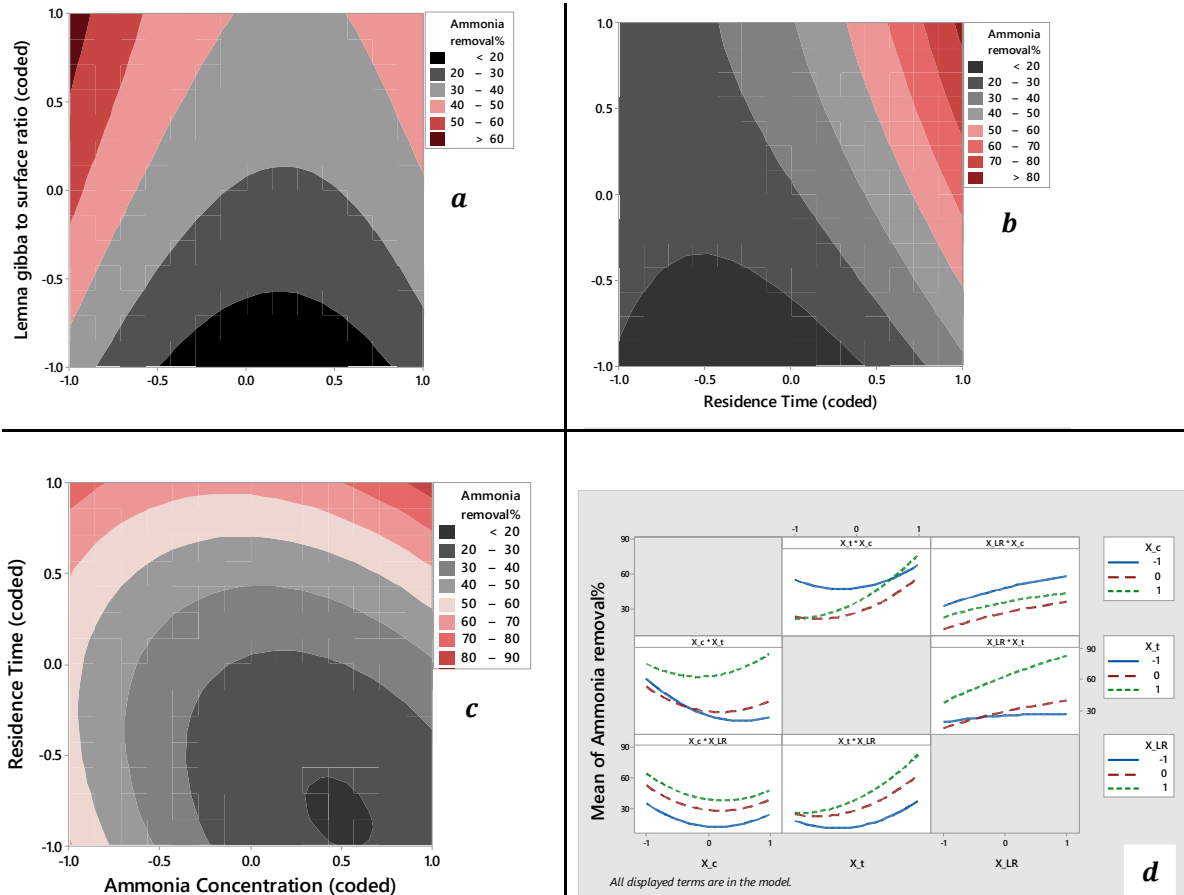
Fig. 2: (a, b, c): Contour plot of ammonia removal percents based on coded ammonia concentration, coded residence time, and
Lemna gibba to surface ratio, and (d): Plot of main and interaction effects of variables on ammonia removal in percent

(Fig. 2a: coded residence time at the intermediate level), the ammonia removal percent increased with the increase of *Lemna gibba* to surface ratio, while its amount and its increasing rate in lower concentrations of ammonia were higher. The results of the contour diagram (Fig. 2b: coded ammonia concentration at the intermediate level) showed that the ammonia removal percent increased with the increase of residence time and its rate at higher levels of factor X_{LR} was higher. According to the results of the contour diagram (Fig. 2b; $X_{LR} = 0$), the ammonia removal percent was negligible at the low and intermediate levels of coded residence time and at the intermediate and high levels of coded ammonia concentrations. According to the results of interaction with the horizontal axis X_C in the first column, the ammonia removal percent reached its

maximum amount at all levels of and low level of X_t (Fig. 2d). This trend declined in the intermediate level and ascended again. However, at low level of factor X_t, decrease of the ammonia removal percent was also observed at high concentrations of ammonia. Based on the results of interaction with the horizontal axis X_t at the second column, the ammonia removal percent was increased at all levels of X_{LR} and X_C. Considering the interaction with the horizontal axis X_{LR} in the third column, the ammonia removal percent was increased at all levels of X_{LR} and X_C was increased. However, at low level of factor X_t, change of levels in factor X_{LR} did not have a significant effect on the process of ammonia removal from the wastewater. Model numerical optimization was performed to achieve the maximum amount of ammonia removal from the wastewater. Based

on the quadratic model, the maximum percentage of ammonia removal was determined at ammonia concentration of 5 ppm and *Lemna gibba* residence time of 11 days in wastewater. *Lemna gibba* to surface ratio of 2:5 was measured at 96.449%. After optimization, validation of ammonia removal was performed under optimum conditions and measured at 92.07%. Based on the experimental design and the predicted under model conditions (Run No. 10), the maximum amounts of ammonia removal percent in the experiments were 82.84% and 88.33% respectively, indicating the high accuracy of the model to determine the optimum conditions for the removal of ammonia from wastewater. Although the removal of ammonia by plants has been investigated in previous studies (Fang *et al.,* 2007; Ojoawo *et al.,* 2015), there is no report on the optimization of ammonia removal from petrochemical wastewater using plants. In addition, the effects of factors such as ammonia concentration, *Lemna gibba* to surface ratio and its residence time in ammonia removal yet have not been investigated. However in previous studies, other factors (such as pH) have been effective in ammonia removal (Huang *et al.,* 2008; Zorpas *et al.,* 2010; Juliet Selvarani *et al.,* 2015). In the current study, all the experiments were carried out at pH of the petrochemical wastewater (9.9) according to actual environmental conditions (Table 2). According to results of previous studies, the amount of ammonia removal in a wastewater with a lower pH would be higher (Afkhami *et al.,* 2010; Rahmani *et al.,* 2010; Wang *et al.,* 2008).

CONCLUSION

In this study, the optimal operating conditions in biological removal of ammonia from the urea-ammonia wastewater of Kermanshah Petrochemical Company by *Lemna gibba* were determined using the response surface methodology. According to the obtained results, the maximum percentage of ammonia removal was 96.449% based on the quadratic model. This amount was determined at ammonia concentration of 5 ppm, *Lemna gibba* residence time of 11 days in wastewater, and *Lemna gibba* to surface ratio of 2:5. Based on the experimental design and the predicted values under model conditions, the maximum amounts of ammonia removal were 82.84% and 88.33% respectively. The slight difference between these values indicates the high accuracy of the model to determine the optimum conditions for the removal of ammonia from the petrochemical wastewater. Results of this study demonstrate that the phytoremediation by *Lemna gibba* in optimum conditions can be used as a practical, biocompatible and reliable method for the removal of ammonium from the urea-ammonia wastewater of petrochemical companies.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

ABBREVIATIONS

ANOVA	Analysis of variance
AR	Ammonia removal
Ca^{+2}	Calcium
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
Cl	Chlorine
cm	Centimeter
$CuSO_4$	Copper(II) sulfate
DF	Degrees of freedom
DOE	Design of experiment
Fe-EDTA	Ferric sodium EDTA
Fig.	Figure
Eq.	Equation
H_3BO_3	Boric acid
K_2CO_3	Potassium carbonate
K_2CO_4	Potassium sulfate
L	Liter
MA	Methyl orange alkalinity
mg	milligram
$MgSO_4$	Magnesium sulfate
ml	milliliter

$MnCl_2$	Manganese(II) chloride
mol	mole
MS	Mean square
$MS_{regression}$	Mean squares regression
$MS_{residual}$	Mean square residual
m^3	Cubic meter
NaH_2PO_4	Sodium phosphate
Na_2MoO_4	Sodium molybdate
No.	Number
PA	Phenol alkalinity
pH	Potential of hydrogen
ppm	Part per million
RSM	Response surface methodology
R^2	Root squared (Coefficient of determination)
R^2_{adj}	Adjusted R-Squared
SS	Sum of squares
SS.	Suspended solids
TDS	Total dissolved solids
TH	Total hardness
V	Volt
W	Watt
X_C	Ammonia concentration
X_{LR}	*Lemna gibba* to free surface fluid ratio
X_t	Residence time
Zn_2SO_4	Zinc sulfate
µs	Microsiemens
°C	Celsius degree
%	Percentage

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