Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2018.22886

129 (2018) 62–68
October

Evaluation of anaerobic stabilization pond for removal of pentachlorophenol from wastewater: response surface methodology

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Received 24 October 2017; Accepted 2 August 2018

ABSTRACT

Pentachlorophenol is one of the most important environmental pollutants that despite its dangerous nature are widely used in the industry. Therefore, its removal in aqueous solution is necessary because of toxicity, carcinogenicity, and undesirable health effects. To evaluate the efficiency of process of anaerobic stabilization pond with a volume of one cubic meter and the efficiency of removal of pentachlorophenol and organic matter from wastewater was studied. The effect of pentachlorophenol concentration (0.5, 2.5, and 5 mg/L) and the hydraulic retention time (24, 48, and 60 h) was evaluated on the process efficiency by the response surface methodology and the central composition design test at three levels and 13 runs. Also, to determine the relationship between high-performance liquid chromatography and spectrophotometric methods for measuring pentachlorophenol, SPSS software and *t*-test were used. The chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) levels in raw wastewater were 380 and 220 mg/L, respectively. The removal efficacy of pentachlorophenol, COD, and BOD_5 was in the range of 17.92%–77.84%, 12.35%–55.44%, and 21.82%–53.15%, respectively. The results showed a good correlation between laboratory values and predicted values of models ($P < 0.05$). Results proved that anaerobic stabilization pond may be a proper low-cost alternative for pentachlorophenol, COD, and BOD_5 removal from wastewaters.

Keywords: Anaerobic stabilization pond; Pentachlorophenol; RSM

1. Introduction

In recent decades, the increasing growth of industrial activities on the one hand and the non-compliance with environmental requirements on the other has led to large amounts of contaminants entering the environment. The accumulation of these pollutants in the environment is a serious threat to human health and the environment. Industrial wastewater, due to high pollution levels, with the presence of compounds that are not easily degraded in natural conditions and in some cases especially where the environment does not have the capacity to accept contamination is considered as an environmental problem [1]. Pentachlorophenol (PCP) is an organic compound and is a phenolic derivative that has received more attention from other phenolic derivatives due to the presence of five chlorine atoms on its benzene ring [2]. PCP is used in the production of toxins, wood preservatives, and herbicide compounds, to increase the strength

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of wooden bridges, fences, and textile [3]. This highly toxic compound with a half-life of 180–200 d is absorbed by the gastrointestinal tract, and its health effects can lead to damage to the central nervous process, immune process, digestive process, skin, kidney, liver, and blood. Removal of this substance is necessary because of high toxicity, carcinogenic potential, and undesirable health effects mentioned [4]. There are several methods for the removal of phenol and its compounds, most notably chemical oxidation [5], adsorption [6], and biological treatment [7], which is used alone or in combination with common methods. Choosing the proper technology and applying a process that can reach the desired targets for the treatment of a specific wastewater is subject to various conditions and parameters that need to be carefully considered. Paying attention to the anaerobic process for sewage treatment, especially for developing countries, is important because of the lack of appropriate tools to reduce the risks of pollutant discharge to environmental resources; they are subject to very serious problems [8].Considering the nature of the existing industrial process in these countries and the lack of methods for reducing waste generation and the presence of various pollutants in discharges into the environment, this should be considered more. With regard to flexibility, lack of need for lateral processes, high lifetime of the process, lack of heavy costs for sludge disposal, etc. in anaerobic process, it is necessary to study and apply it for the treatment of wastewater in developing countries [9]. Because a PCP removal study using the natural process of anaerobic stabilization pond (ASP) has not been performed so far, in this study, an ASP process has been studied for wastewater treatment with different concentrations of PCP. Stabilization ponds, the most commonly used wastewater treatment technology, are noteworthy with good features such as flexibility, ease of use, simplicity of operation and maintenance, cost effectiveness, fairly good efficiency, unreliability of technology, and their applicability to most sewage process [10]. This study was carried out on a real scale in a cement pond with continuous flow for wastewater and PCP solution, ambient condition and at hydraulic retention time less than the conventional. Organic loading rate of the process was adjusted by reducing the hydraulic retention time.

2. Materials and methods

2.1. Wastewater preparation

The sludge was taken from the wastewater treatment plant of Kermanshah, Iran. Samples were transferred to the laboratory under appropriate temperature conditions such that no change in the composition of the wastewater was generated. Chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), BOD_U, total suspended solid (TSS), and alkalinity in influent (mg/L) were 380.2 ± 28.46, 220.36 ± 26.14, 314.21 ±34, 200.28 ± 25.71, and 358.06 ± 54.3, respectively.

2.2. Anaerobic stabilization pond

The ASP was a cement rectangular in the earthen position, with 1 × 0.5 × 2 m (L × W × H) in dimension, volume of 1,000 L, 200-L volume feeding tank and equipped with a tap valve to regulate the flow (Fig. 1). The influent was 30 cm under the surface pond. In order to prevent phenol volatility, the surface of the pond was covered with a paraffin and plastic layer, and the results were evaluated in comparison with the open state. Considering the dimensions of the ASP to provide the volume loading in the standard range, HRT was 24, 48, and 60 h. In this experimental study, seeding and inoculation of ASP were performed before purifying. In order to adapt microorganisms to the wastewater, the pond was loaded for about 90 d until steady state condition regard to COD and BOD5 removal were achieved. PCP solution (Merck, Germany) with concentrations of 0.5, 2.75, and 5 mg/L was prepared. The bioreactor was conducted at ambient air. In order to ensure the anaerobic conditions, the concentration of sulfate was continuously measured in a range of 30–60 mg/L. It was also confirmed by measuring the oxidation reduction power that was less than 284 V. Pond management conditions were according to Almasi and Pescod [11].

2.3. Measurement

COD with a closed reflux method (5220 C) [12], and with the help of the COD meter (DR 5000, Hach, Jenway, USA), BOD_5 using the Winkler method according to 5210 C [13], and TSS according to the 2540 method [14] were measured. The pH meter (Digimed, DM-20, Brazil) was used to measure pH and oxidation–reduction potential. All experiments were carried out in accordance with standard methods for water and wastewater [15]. To measure PCP by spectrophotometry, 1 mL of hydrochloride acid and 10 mL of chloroform was added to 100 mL of the sample and was severely disrupted for 60 s. Subsequently, they separated from the two-phase doped decanter. The solution was again added to a 5 mL chlorine-solvent phase and 2 mL NaOH, 0.2N, and placed in centrifuge for 10 min at a speed of 5,000 rpm. The upper liquid of the centrifuge tube was then removed, and the absorbance of the sample at a wavelength of 254 nm was measured by a spectrophotometer [16]. In order to verify the accuracy of the method, 50% of the samples were simultaneously determined by high-performance liquid chromatography (HPLC) (Kneuer, Germany). In HPLC method, 5 mL of hexane solution was first added to 20 mL of filtered sample and was mixed for 10 min. After that, the two phases created were separated by a decanter, and a transparent portion containing solvent and PCP substance was injected into the device. Characteristics of the HPLC include acetyl

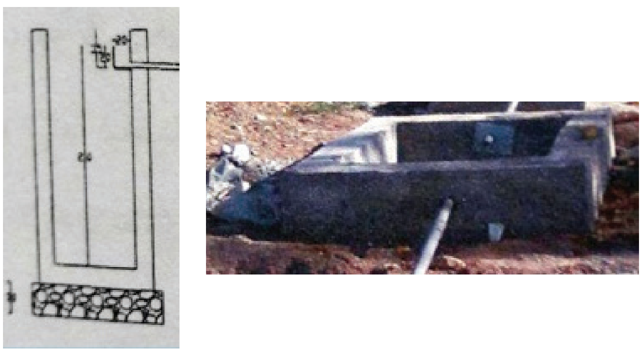

Fig. 1. The schematic and real figure of stabilization pond.

column C18, mobile phase acetonitrile 60%, distilled water 40%, contact time 3.81 min, pH 6, flow rate of mobile phase 1 mL/min, and detector UV-2600 with 254 nm [17]. All chemicals and reagents were of analytical grade and high purity from Merck, Germany. The standard PCP specification contains a boiling point of 309°C–310°C, a molecular weight of 288.28 g/mol. A solution of NaOH was used to accelerate the dissolution of PCP [18]. To prepare standard solutions, distilled water was used twice for distillation in the laboratory. All sampling and laboratory analysis procedures were carried out in accordance with standard methods of examination of water and wastewater [15]. The removal efficiency was calculated according to Eq. (1).

$$\text{Efficiency},\% = \left[\frac{C_i - C_e}{C_i}\right] \times 100 \tag{1}$$

where C_i and C_e values are the inflow and outflow concentrations of the pollutant.

2.4. Design experiments and computational modeling

The experimental runs were design of the experiments and according to response surface methodology with take benefit from Design Expert Software (version 7). Two independent variables, HRT and PCP concentrations, were selected for the design of the experiments. The ranges and levels of variables were considered at three levels, –1, 0, and +1, and 13 experiments (including 4 variable points, 4 axis points, 1 central point, and 4 repetitive points in the center) were designed (Table 1). The responses include removal of PCP, COD, BOD_5, and output alkalinity. The multi-degree coefficients of Eq. 2 were used to measure the coefficients [19].

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j + \ldots \tag{2}$$

where i and j are multi-degree and linear coefficients and β is the correlation coefficient. P value with 95% confidence level is used to evaluate the effect of model functions.

3. Result and discussion

3.1. Verification of spectrophotometric measurement with HPLC assay

In this study, two spectrophotometric and HPLC methods were used for measuring PCP. The results were analyzed using SPSS software (version 20; SPSS Inc. Chicago, IL). Initially, Shapiro–Wilk test confirmed the normality of the data. Then t-test was used to compare the difference between the two specimens obtained from spectrophotometric and HPLC methods, the results are given in Tables 2 and 3. Considering the t-test, there was no significant difference between the two samples obtained from the spectrophotometric and HPLC methods (P value > 0.05). So that spectrophotometry provides high accuracy data at a lower cost than HPLC.

3.2. Process analysis and modeling

Different responses have been investigated in this study. Linear (BOD_5) and quadratic models (COD and PCP) were used to match the data (Table 4). The models are obtained after eliminating the insignificant variables. Based on statistical analyzes, the models have low probability values and

Table 2
Parametric determination of pentachlorophenol data by spectrophotometric and HPLC methods

Methods	Shapiro–Wilk		
	Statistic	d*f*	*P*-value
HPLC	0.815	4	0.132
Spectrophotometric	0.809	4	0.120

Table 1
Laboratory conditions for measuring the efficiency of an anaerobic stabilization pond in pentachlorophenol removal

Run	Factors		Responses			
	A: concentration, mg/L	B: HRT, hr	PCP removal, %	COD removal, %	BOD_5 removal, %	Alkalinity output, mg/Lcaco$_3$
1	2.75	42	47.91	43.02	41.73	354.66
2	5	24	17.92	12.35	15.82	366.66
3	2.75	42	49.99	45.06	38.16	350.12
4	0.5	24	54.4	41.62	43.85	396.66
5	5	42	31.34	21.44	30.11	376.66
6	2.75	42	44.89	41.12	43.11	359.27
7	0.5	60	77.84	55.44	53.21	383.33
8	2.75	24	37.93	33.81	35.68	376.66
9	2.75	42	42.71	47.13	45.16	347.44
10	0.5	42	72.2	47	47.92	375.33
11	5	60	32.63	31.9	31.14	380
12	2.75	60	55.64	48.64	45.13	353.33
13	2.75	42	52.13	39.15	37.14	360.11

Table 3
Paired sample *t*-test to determine the significant of data

Method	Paired differences					*t*	d*f*	Significance (two-tailed)
	Mean	Standard deviation	Standard error mean	95% Confidence interval of the difference				
				Lower	Upper			
HPLC	0152	0.012527	0.006263	0.004683	0.035183	2.43	3	0.09
Spectrophotometric	0.5							

Table 4
ANOVA results for the studied responses

Response	Modified equations with significant terms	Model	Probability	R^2	Adjusted R^2	Adequate precision	S.D.	Coefficient of variation	Probability for lack of fit
COD removal	+42.56 – 13.06 A + 8.03 B – 7.6 A^2	Quadratic	<0.0001	0.9662	0.955	30.533	2.49	6.38	0.8737
BOD_5 removal	+39.9 – 11.32 A + 5.69 B	Linear	0.0001	0.8768	0.8521	19.248	3.68	9.41	0.4091
PCP removal	+47.5 – 20.43 A + 9.31 B	Linear	<0.0001	0.9595	0.9514	34.64	3.57	7.52	0.6101
Effluent alkalinity	+355.01 – 5.33 A + 19.26 A^2 + 8.26 B^2	Quadratic	0.0054	0.7393	0.6524	6.723	8.81	2.4	0.1128

high reliability levels. The *P* value for the studied responses was in the range of 0.000–0.0054. The *F* values and smaller *P* values represent the significance of the models, and all of the studied factors were significant (*P* value < 0.05). According to the results, lack of fit for all parameters was not significant (*P* > 0.05). Also, the appropriateness of the models was confirmed by correlation coefficient R^2 and adjusted R^2. The R^2 coefficient is defined as the ratio of the explained variable to the total variation and the degree of fitness of the model which was rationally high in all models (R^2 = 0.73–0.96). If the accuracy of the model is more than 4, it is desirable [19] which was achieved in all responses in the range of 6.72–34.64. In addition, standard deviation values (8.81–2.49) and variance coefficient (2.4–9.41) indicate significant reliability and high reliability of results. It should be noted that the coefficients of these equations and their mathematical symbols (–/+) represent the extent and direction of the effect of independent parameters on the efficiency of the ASP equation.

3.3. PCP removal

The three-dimensional graph of the response level on PCP removal is shown in Fig. 2(a).

As we can see, the efficiency of the pond increased with increasing HRT and lowering of the initial concentration of PCP. In general, the maximum and minimum PCP removal efficiency was 77.84% (HRT = 60 h, PCP concentration = 0.5 mg/L) and 17.92% (HRT = 24 h, PCP concentration = 5 mg/L), respectively. Regarding the linear equation, the effect of PCP concentration on the process efficiency over HRT and its inverse relationship with process performance were visible. Increased efficacy along with HRT can be caused by opportunity of biological availability in removing PCP [17]. The low efficiency of the stabilized pond can be attributed to the toxicity of PCP for the microbial population, the nature of the studied wastewater, and the low HRT of the pond. Reducing the PCP removal efficiency by increasing the concentration can be due to the effect of PCP on bacterial decomposition. However, given that the anaerobic pond in any of the different concentrations of PCP alone cannot remove organic pollutants to pass the environmental permits of discharges to surface water and groundwater. So, generally, it has been suggested to consider as a preliminary treatment step followed by facultative pond. In the study of Moussavi et al. [20], the efficacy of phenol removal by biological methods was reported to be 28.1% over a period of 5 d which was lower than this study. In a similar study by Dargahi et al. [21] on the removal of phenol from the oil refinery wastewater by a stabilized pond, the time had a dramatic effect on the efficiency of phenol removal as there was a statistically significant difference between the percentages of removal of compounds at different retention time. Also, the results of this study are consistent with the other study by Almasi et al. [10] regarding oil refinery, which has increased the efficiency of ASP in terms of removal of organic matter by increasing the contact time. Choudhary et al. [22] eliminated PCP from textile wastewater with the aid of wetland during 5.5 d and in pH of 7.7 and eliminated PCP concentrations from 67% to 100%. In the study of Asgari et al. [23], the removal of PCP by using a combination of microwave oven with persulfate/peroxide had the best removal efficiency at pH 11, persulfate concentration of 0.02 mol/L, 0.2 mol/L peroxide, and radiation intensity of 600 W, which is more expensive treatment methods relating to ASP. In the study by Shen et al. [24], the anaerobic PCP removal by upflow anaerobic sludge blanket (UASB) resulted in 99.9% removal, so that its output PCP concentration was 0.5 mg/L, which was much higher than this study by APS. Also, the anaerobic sludge efficiency gradually decreased with increasing PCP concentration, which is in consistent with the current results. Pang et al. [25] used chitin (pH = 6 and contact time 60 min) for adsorption of PCP and showed optimum efficiency was 57.9%.

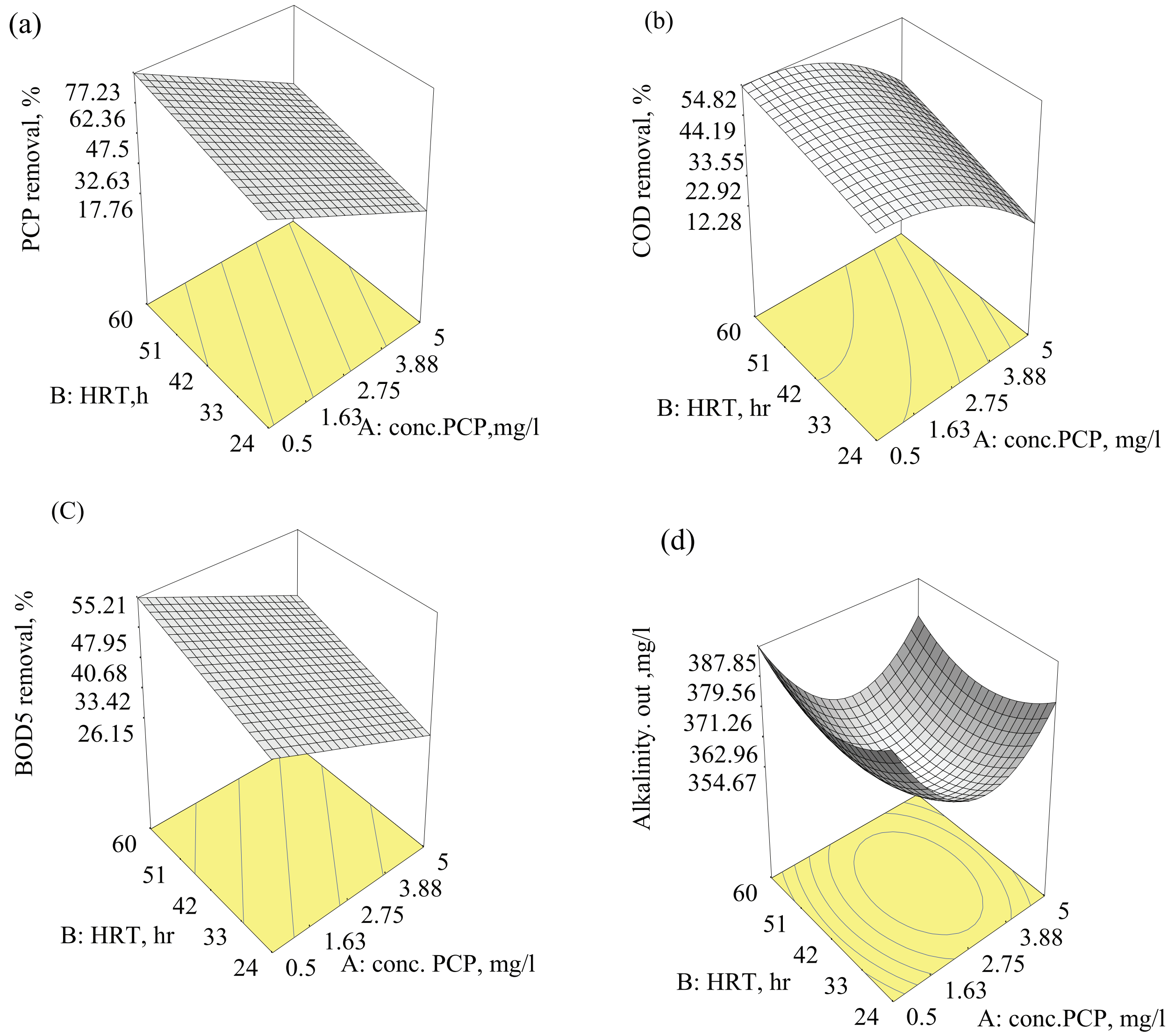

Fig. 2. Response surface plot for anaerobic stabilization pond: (a) PCP removal, (b) COD removal, (c) BOD_5 removal, and (d) effluent alkalinity.

3.4. BOD_5 and COD removal

In order to evaluate the effect of PCP toxicity on the stability of the ASP in removing organic matter, BOD_5 and COD parameters were studied. A quadratic (COD removal) and linear (BOD_5) models describe the changes. According to Table 1, A (concentrations of PCP), B (HRT), and A^2 are the most important model functions for the ASP. Figs. 2(b) and (c) show the effect of the variables on the removal of COD and BOD_5. It is observed from the figures that there is an increasing trend in removal of COD by increasing HRT and decreasing the concentration of PCP. The results showed that the maximum removal efficiency of COD (55.44%) and BOD_5 (53.21%) was obtained at a concentration of 0.5 mg/L PCP and 60 h of HRT. The effect of PCP concentration on COD removal efficiency was more effective than the effect of HRT. The results showed that the presence of PCP exerts an undesirable effect on the efficiency of BOD_5 and COD. The removal mechanism of COD and BOD_5 involves solid sedimentation. According to previous studies, the removal of BOD_5 could reach 92% in anaerobic ponds [26]. While most of the biodegradable materials are removed under anaerobic hydrolysis conditions, most organic suspended solids are converted into low-degradation intermediate compounds during the hydrolysis process in an anaerobic pond [27]. Also, the efficiency of the ASP is better than the results of the study by Abdel Aatty and Karnel [28]. Abdel-Aatty and Karnel [28] showed that the removal efficiency of COD and BOD_5 by anaerobic pond process was 28.89% and 22.21%, respectively. By increasing the concentration of PCP from 0.5 to 5 mg/L for 60 h, the COD and BOD_5 values decreased by 23.54% and 22.07%, respectively. The study by Papadopoulos et al. [29] showed that the removal rate of BOD_5 and COD of urban wastewaters by anaerobic pond process was 45% and 50%, respectively, which is similar to the results of this study. The study by Abdel Aatty and Karnel [28] showed that the removal efficiency of COD and BOD_5 by anaerobic pond process was 28.88% and 22.21%, respectively, which, despite the greater compatibility of municipal

Table 5
Validation experiment for optimal areas

Conditions		Response			
		COD removal, %	BOD_5 removal, %	PCP removal, %	Effluent alkalinity, mg/L
HRT = 60 h	Experimental values	55.44	53.21	77.84	354.66
Concentration of	Model values	56.0533	55.2162	77.2373	387.859
PCP = 0.5 mg/L	Standard error	1.76	4.34	2.29	5.38

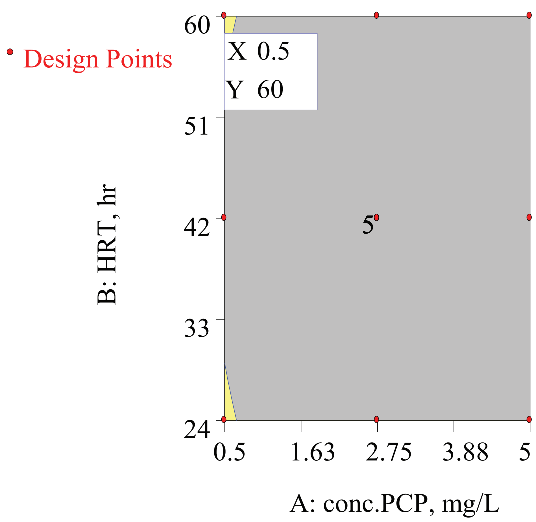

Fig. 3. Overlay plot for optimal region for removal pollutants from anaerobic stabilization pond.

wastewater with the microbial population yields less efficiency than this study. The study of Almasi et al. [10] shows that the efficiency of ASP in phenol removal from oil refinery effluent for a concentration of 100 mg/L with 2-d HRT is 89%, which is more than this research in the used wastewater. A study by Wang et al. [30] shows that removal efficiency of phenol at a concentration of 50 mg/L carried out by the UASB process was 84%. The study by Avelar et al. [31] showed that by increasing the phenol concentration in the wastewater, the efficiency of stabilization pond was reduced, so that at a concentration of 100 mg/L phenol, the removal efficiency was 81.88% and the lowest removal rate was at 400 mg/L. The results are similar to this study. In general, it can be concluded that ASPs have a relatively good performance in removing organic compounds at different concentrations of PCP for 60 h of HRT.

3.5. Process optimization and validation of the model

Graphical optimization is a multi-layered map to indicate the region in which the response values are obtained. Fig. 3 shows graphical optimizations, which represent the region that provide the desired response values yellow region). The optimal region is identified based on the four parameters for removal of PCP, COD, BOD_5, and alkalinity, which are considered as criteria. The yellow zone covers concentrations of PCP 0.5 mg/L for HRT of 24 and 60 h, respectively. In order to verify the accuracy of the models, one point is selected in optimal zone (conditions shown by the flag are shown in Fig. 3). The bioreactor was operated to compare actual values with predicted values of responses. Table 5 shows the results of the experiment in optimal areas. The correctness of the optimal conditions with design of experiment was examined by standard error for each response. Standard error for COD, BOD_5, PCP removal, and effluent alkalinity were 1.76, 4.34, 2.29, and 5.38.

4. Conclusion

Stabilization pond is a conventional process for treating organic and unconventional substances such as PCP. Therefore, its function was surveyed in this study. Optimum conditions were obtained at a concentration of 0.5 mg/L of PCP and 24 and 60 h of HRT. The results indicate a good relationship between the experimental results and the predicted data. Data were good fit to models of linear for BOD_5 and quadratic for COD and PCP. In this study, we observed a greater effect of PCP concentration on efficiency than on HRT. The efficiency of PCP, COD, and BOD_5 removal was 77.84%, 55.44%, and 53.21%, respectively. The results showed a high removal of PCP. Therefore, the existing process can be used to remove PCP from industrial wastewater with good acceptability.

Acknowledgment

The authors would like to acknowledge the financial support by Kermanshah University of Medical Sciences.

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