Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2017.21064

87 (2017) 199–208
August

Phenol removal from oil refinery wastewater using anaerobic stabilization pond modeling and process optimization using response surface methodology (RSM)

Abdollah Dargahi[a], Mitra Mohammadi[b], Farhad Amirian[c], Amir Karami[b], Ali Almasi[b,]*

[a]*Department of Environmental Health Engineering, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran, Tel. +989141597607, email: a.dargahi29@yahoo.com (A. Dargahi)*
[b]*Department of Environmental Health Engineering, School of Public Health, Kermanshah University of Medical Sciences, Kermanshah, Iran, Tel. +989188573064, email: m.mohamadi725@gmail.com (M. Mohammadi), Tel. +989183598931, emal: amir_karami119@yahoo.com (A. Karami), Tel. +989181317314, email: alialmasi@yahoo.com (A. Almasi)*
[c]*Department of Pathology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran, Tel. +989339755662, email: farhad_amirian@yahoo.com (F. Amirian)*

Received 10 October 2016; Accepted 24 June 2017

ABSTRACT

Oil refinery process releases toxic pollutants into aqueous environment. Phenol and its derivations as the most important pollutants pose severe environmental concern. In this study, the rectangle anaerobic stabilization pond (ASP) consisting of feed tank with workload of 60 Lit (1 × 0.2 × 1) meter of phenol was used. This study evaluated the interactive effect of phenol concentration (200–400 mg/l), temperature (8–24°C) and Hydraulic retention time of (HRT) (2–5 d) on the efficiency of anaerobic stabilization pond for oil refinery wastewater treatment. In this study, experiments were carried out based on central composite design (CCD) and analyzed and modeled by response surface methodology (RSM) aimed at demonstrating the operating variables and also the interactive effect of three independent variables on 7 responses. The maximum removal efficiency of SCOD, TCOD, SBOD and TBOD were 66.26, 68.95, 65.3 and 67.02%, respectively, at phenol concentration of 200 mg/L, HRT of 2 d, and temperature of 24°C. Generally, the ratio of N/P varied between 6.69–9.12 and 7.04–12.93, respectively, in influent and effluent of anaerobic stabilization pond. The maximum phenol removal efficiency reached 70.53% and 81.63% at phenol concentration of 200 mg/L, temperature of 24°C with HRT (2 and 5 d), respectively. The phenol removal efficiency was significantly influenced by increasing the temperature compared to decreasing the phenol concentration. The result indicated that the anaerobic stabilization pond was a capable biological treatment process that could achieve the moderate removal of oil refinery wastewater.

Keywords: Phenol; Oil refinery; Wastewater; Anaerobic stabilization pond; RSM

1. Introduction

Crude oil refinery plants are considered among the environmental polluting industries that discharge annually large volume of effluent from various processing units into wastewater treatment plants [1]. In Petroleum refinery, crude oil is processed and transformed into 2500 more useful refined products including, diesel fuel, gas oil, gasoline, kerosene, petroleum oils, etc [2]. The quantity and characteristics of generated wastewater in oil refinery plant depends on the type of processing unit that regularly contains the pollutants such as phenol, benzene, heavy metal, nutrient , etc [3]. Industrial effluent contains toxic pollutants that are introduced into aqueous environment and wastewater treatment plant, repeatedly [4]. So far water pollution through organic and inorganic substances as a result of industrial activities in oil refinery has posed severe difficulties and challenges for public health [5]. Among these pollutants, phenol is of great importance, given that phenol and its derivations have been considered as serious environmental concern that causes extreme toxicity impact to human beings, air and

---

*Corresponding author.

soil ecology as well [6]. Phenol inhibited the synthesis and replication of DNA in cell, and additionally as noted in the studies, it prevented from the reparation of DNA in diploid human fibroblast [5]. Phenol has been placed in the list of the most important pollutants according to Environmental Protection Agency (EPA) and European Agency (EU) due to its high toxicity, persistence, bio accumulative and improper organoleptic characteristics [7]. Such a practical and environmental-friendly technology should be considered for phenol removal before discharging to the environment due to actually numerous threats of phenol compounds to human health and environment [8]. Currently, petrochemical refinery industry emphasizes techniques particularly cost-effective and high-efficiency wastewater treatment technology [9]. It should be taken into account that toxicity of residual petroleum products, significant quantities of industrial effluent, poor biodegradability of oil compounds and leaching base on environmental standard have created the major concern [10]. The result of various studies revealed that microorganisms in biological systems are developed as bacterial community against the environmental destructive effects, like biomass against the wastewater toxicity [11].

Stabilization pond has been regarded as one of the most efficient biological treatment processes, it is especially appropriate in tropical and subtropical regions [12]. Generally, the efficiency of stabilization ponds depends on the environmental condition such as ample sunlight and temperature as the key factors and precipitation, evaporation and presence of toxic pollutants as well [13]. Optimization of the process variables is necessary to attain the optimal removal efficiency. The usual experimental design needs a large number of experiments resulting in some time and economical problems. Therefore, it can be administered using the statistical experiments design that minimizes the number of experiments [14]. The objective was to optimize the responses which were affected by independent variables and their interaction, given the experimental design. In this study, the experiment was designed based on central composite design (CCD) and was analyzed by response surface methodology (RSM), that provided proper statistical tools aimed at designing and analyzing experiments for process optimization [15].

Present study, modeled and analyzed oil refinery wastewater treatment by anaerobic stabilization pond using RSM, that analyses the simultaneous effect of three independent variables (phenol concentration, HRT and temperature) on 7 parameters as responses (SCOD, TCOD, $SBOD_5$, TBOD, $N\text{-}NH_3$, $P\text{-}PO_4$ and Phenol.

2. Method and materials

2.1. Chemicals

All chemicals and reagents were prepared with purity of 99.99% purchased from (Merck Co. Germany). It should be noted that deionized water which was procured in laboratory was used to prepare the desired standard solution.

2.2. Type of wastewater

Wastewater was procured from separator unit effluent in oil refinery process plant, Kermanshah, Iran.

2.3. Start up and operation of anaerobic stabilization pond

A pilot scale of anaerobic stabilization pond was used in this study, composed of feed tank with V workload of 60 L and equipped with valve disk aiming at flow adjustment. The rectangle stabilization pond with dimensions of 1 × 0.2 × 1 m, was completely sealed to prevent from oxygen entrance. The wastewater was fed into stabilization pond with HRT (2 and 5 d), based on 95 and 40 L/d of influent, respectively. The stabilization pond was seeded with 1.5 L of sludge seed taken from municipal wastewater plant that had been equalized and mixed. The microorganism acclimation to achieve the steady state of system was lasted for about 90 d. The stabilization pond was fed in bath process by combination of molasses and refinery oil wastewater aimed at regulating the organic load rate of system. The molasses wastewater was provided by sugar production factory, Kermanshah, Iran. The molasses wastewater contains the COD and BOD_5 levels of 2400 mg/L and 1680 mg/L, respectively.

2.4. Chemical analysis

In present study, composite sampling was done every 2 h, which was immediately transferred to laboratory and was kept in refrigerator (2–4°C). Analytical laboratory test was done on collected samples. Chemical oxygen demand (COD) was measured based on closed reflex method (Jenway, Hach USA DR 5000), (Method 5220C). Biological oxygen demand (BOD_5) was measured for five days according to titrimetric method (Method 5210B). pH was analyzed using pH meter (Digimed model DM-20, Digicron AnalíticaLtda, São Paulo, Brazil). $N\text{-}NH_4$ was analyzed by direct Nesslerization (Method 4500 C), Colorimetric method was used to determine phosphate (Method 4500 P), and photometric method was used for phenol analysis (Method 5530 C). Nitrate, phosphate and phenol were determined by spectrophotometer (Varian UV-120-02 California, USA). UV probe software was used to control the system and obtain results of experiment. It should be noted that sample collection, transfer and all chemical analysis were performed according to standard method for water and wastewater examination [16].

2.5. Experimental design

Central composite design, Box-Behnken design, Hybrid design and three level factorial design are the different classes of response surface design. RSM design is the most frequently used Central composite design (CCD). Aimed at analyzing the data, RSM was used as a technique for collecting mathematical and statistical data in order to evaluate three independent variables, that is, phenol concentration (A), temperature (B) and HRT (C), surrounded by phenol concentration of (200–400 mg/L), temperature (8–24°C), and HRT (2, 5 d) to assess the 7 different responses; removal efficiency of TCOD (total chemical oxygen demand), SCOD (soluble chemical oxygen demand), $TBOD_5$ (total biochemical oxygen demand), $SBOD_5$ (soluble biochemical oxygen demand), $N\text{-}NH_3$, $P\text{-}PO_4$ and phenol). Accordingly, 13 experiments were designed. The design consisted of (4 variable points, 4 axial points, 1 central point and 4 repetition points in center). The result obtained based on CCD, was analyzed using ANOVA variable analysis software, and multiple regression analysis. Also the result can be demonstrated by 3-D plot regarding the simultaneous effect of independent variables on the responses. In this study, the relationships among response, input and quadratic equation model were determined to predict the optimal variables identified:

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j + \ldots \quad (1)$$

Y, i, j, b, X are process response, linear coefficient, quadratic coefficient, regression coefficient and coded independent variables, respectively.

3. Result and discussion

3.1. Statistical analysis

Central composite design (CCD) was selected to explore the correlations between variables and responses. The experimental data obtained from the 7 responses (Y_1–Y_7) are demonstrated in (Tables 1 and 2). Based on experimental design (Tables 1 and 2), the response surface analysis was fitted properly to the experimental result. Factor coded models as well as analysis of variance (ANOVA) are represented for all responses in (Tables 3 and 4). The different degree polynomial models are used for data fitting. Fitting the experimental data was conducted by higher degree polynomial equation i.e Quadratic vs 2FI and linear. The final model terms were obtained after removing insignificant variables and their interaction. The significance of

Table 1
Experimental conditions and results (HRT: 2 days)

Run	Temp, °C	Phenol. Conc. mg/l	Rem. SCOD, %	Rem. TCOD, %	Rem. SBOD5, %	Rem. TBOD5, %	Rem. N-NH$_3$, %	Rem. P-PO$_4$, %	Rem. Phenol, %	N/P out
1	24	400	49.41	55.63	49.67	53.5	33.66	41.15	55.86	8.71
2	16	200	50.6	51.83	49.87	50.36	27.4	36.17	47.96	12.5
3	24	300	57.14	62.8	59.83	61.69	39.03	46.41	63.47	8
4	16	300	41.3	45.1	43.22	42.69	23.1	29.15	41.19	8.75
5	16	300	39.2	47.79	45.52	44.99	24.8	31.45	44.89	8.65
6	16	300	43.1	42.17	44.91	40.2	21.2	27.05	36.98	8.51
7	16	300	37.6	45	46.87	39.29	23	28.11	40.89	8.36
8	8	200	37.71	38.72	35.18	36.94	15.92	17.92	31.39	12.16
9	8	300	29.14	31.41	28.6	29.7	13.13	15.9	24.39	9.12
10	16	300	41.8	47.1	41.87	44.98	19.9	30.02	42.98	8.37
11	24	200	66.26	68.95	65.3	67.02	43.39	58.23	70.53	12.93
12	8	400	21.4	24.21	20.83	22.83	8.43	10.88	18.22	7.04
13	16	400	34.4	38.41	33.81	36.61	19.3	23.01	34.04	7.68

Table 2
Experimental conditions and results (HRT: 5 days)

Run	Temp, °C	Phenol. Conc. mg/l	Rem. SCOD, %	Rem. TCOD, %	Rem. SBOD5, %	Rem. TBOD5, %	Rem. N-NH$_3$, %	Rem. P-PO$_4$, %	Rem. Phenol, %	N/P out
1	24	400	58.67	62.12	56.96	59.13	40.21	49.73	61.12	8.71
2	16	200	54	57.54	54.3	53.95	36.4	41.38	54.77	9.2
3	24	300	68.84	72.47	66.85	70.52	49.12	53.16	69.38	8.15
4	16	300	51.66	52.67	50.36	51.06	30.1	35.24	45.61	8.75
5	16	300	49.16	54.17	52.66	53.16	32.3	37.34	44.31	8.53
6	16	300	54.16	51.1	49.63	48.86	27.8	32.84	46.98	8.39
7	16	300	51.6	52.1	52.33	51	31.3	35.12	44.8	8.74
8	8	200	40.22	42.65	38.54	39.86	21.64	25.57	35.92	7.5
9	8	300	34.49	36.87	33.87	35.61	16.89	21.65	29.82	9.12
10	16	300	52	53.6	48.2	53.2	29.1	32.51	46.61	8.58
11	24.	200	73.51	76.44	71.84	73.32	56.22	65.19	81.63	10.8
12	8	400	27.36	26.63	26.61	28.38	12.66	16.74	21.24	7.06
13	16	400	42.98	43.4	40.87	41.57	24.3	31.12	39.18	7.9

Table 3
ANOVA results for the equations of the Design Expert 6.0.6 for studied responses, HRT= 2 day

Response, %	Modified equations in terms of code factors	Type of model	F value	Adeq precision	R^2	Adj. R^2	Pred. R^2	S.D.	C.V	PRESS	Probability for lack of fit
Rem. SCOD	$Y_1 = +40.9 – 8.23A + 13.59 B + 2.4A^2$	Quadratic	174.93	46.02	0.983	0.977	0.974	1.71	4.07	44.59	0.8911
Rem. TCOD	$Y_2 = +46.09 – 6.88A + 15.51\ B$	Linear	303.83	55.28	0.983	0.98	0.976	1.69	3.66	40.65	0.8992
Rem. SBOD5	$Y_3 = +44.39 – 7.67A + 15.03 B – 1.95A^2$	Linear	275.55	56.73	0.989	0.985	0.982	1.44	3.32	30.2	0.9684
Rem. TBOD5	$Y_4 = +42.40 – 6.90A + 15.46 + 2.88B^2$	Quadratic	207.66	48.14	0.985	0.981	0.978	1.67	3.83	38.32	0.9535
Rem. $N-NH_3$	$Y_5 = +22.67 – 4.22A + 13.10B + 2.92B^2$	Quadratic	197.82	44.58	0.985	0.98	0.975	1.4	5.83	29.28	0.9508
Rem. $P-PO_4$	$Y_6 = +29.28 – 6.21A + 16.85 B + 2.47B2 – 2.51 AB$	Quadratic	189.6	46.03	0.989	0.984	0.96	1.62	5.31	78.19	0.5785
Rem. Phenol	$Y_7 = +41.28 – 6.96A + 19.31 B +2.70B^2$	Quadratic	215.88	47.72	0.986	0.981	0.98	1.98	4.67	50.1	0.9996

Table 4
ANOVA results for the equations of the Design Expert 6.0.6 for studied responses, HRT = 5 day

Response, %	Modified equations in terms of code factors	Type of model	F value	Adeq precision	R^2	Adj. R^2	Pred. R^2	S.D.	C.V	PRESS	Probability for lack of fit
Rem. SCOD	$Y_1 = +50.67 – 6.45A + 16.49 B$	Linear	256.08	49.83	0.98	0.977	0.969	1.92	3.78	58.82	0.4247
Rem. TCOD	$Y_2 = +52.79 – 7.41A + 17.48B – 2.48A^2 + 1.72B^2$	Quadratic	588.75	34.4	0.996	0.994	0.993	0.96	1.84	15.3	0.8905
Rem. SBOD5	$Y_3 = +50.56 – 6.71A + 16.10 B – 2.37A^2$	Quadratic	310.76	58.47	0.99	0.987	0.982	1.41	2.84	32.24	0.9370
Rem. TBOD5	$Y_4 = +51.92 – 6.34A + 16.52 B – 2.55A^2$	Quadratic	196.28	45.89	0.984	0.979	0.968	1.8	3.54	61.43	0.5223
Rem. $N-NH_3$	$Y_5 = +30.19 – 6.18A + 15.73 B + 2.60B^2 – 1.76 AB$	Quadratic	263.05	54.82	0.992	0.988	0.987	1.29	4.11	21.64	0.9915
Rem. $P-PO_4$	$Y_6 = +34.65 – 5.76A + 17.35 B + 4.02B^2$	Quadratic	121.29	35.03	0.975	0.967	0.942	2.38	6.51	122.07	0.4011
Rem. Phenol	$Y_7 = +45.89 – 8.46A + 20.86 B + 3.96B^2 – 1.46 AB$	Quadratic	499.8	75..94	0.996	0.994	0.985	1.25	2.61	45.93	0.2571

each model was identified using P value and F value. The smaller amount of (P-values < 0.05) and great amount of F-value show the greater significance of corresponding model [19]. The statistical analysis showed the significance of each model (P-value < 0.0001) for all responses at HRT of (2, 5 days) that indicated the significance of the corresponding model.

As it is shown in Tables 3 and 4, the F-values were 215.88, 189.6, 197.82, 207.66, 275.55, 303.83 and 174.93 at HRT of 2 d, and 499.8, 121.29, 263.05, 196.28, 310.76, 588.75 and 256.08, respectively, at HRT of 5 d, for 7 responses (Y_1–Y_7). Lack of fit (LOF) indicated the variation of data around the fitted models, based on the result. In this study, the LOF was not significant for all the models. It represents a proper model predicted. Furthermore, the fitting of model verified by Pred R-Squared, adjusted R^2 and R^2 between the experimental and the model predicted values. In this study, Pred R-Squared, Adjusted R^2 and R^2 are very close to each other and nearly 1. The R^2 in all models was remarkably more than ($R^2 > 0.98$), in other words, about 0.98 of variation for $TBOD_5$, SBOD5, TCOD, SCOD, $N-NH_3$, $P-PO_4$ and phenol removal was explained by independent variables appropriately. The validity and reliability of analysis identified through the adequate values of 4 or more [14]), were generally based on the result and the p-value for all responses were significantly over 4 and between 44.58–56.73 and 34.4–75.94, respectively at HRT (2, 5 d). It showed the proper value for analysis validated. Moreover, the low amount of standard deviation at HRT (2, 5 d) were 3.32–5.83 and 1.84–6.51, respectively, and also coefficient of variation (CV) were 1.4–1.98 and 0.96–2.37 at HRT (2, 5 d), respectively, representing the considerable reliability and proper precision of the experiments [17]. It should be mentioned that the coefficient and mathematical symbol (+/-) of resulted equation showed the most effective variable in anaerobic stabilization pond performance.

3.2. Process performance

Oxidation reduction potential (ORP) was monitored continuously in anaerobic stabilization pond in order to ensure the anaerobic condition in this study. The average of (ORP < –246) confirmed the anaerobic condition in stabilization pond.

3.2.1. Carbon removal

Effects of different phenol concentrations and temperatures of stabilization pond at different HRT (2, 5) days on responses (TBOD5, TCOD, $SBOD_5$, and SCOD removal) are presented in Tables 3 and 4. The removal efficiency trend of $SBOD_5$, SCOD changed when the phenol concentration rose from 200 to 400 mg/L, and also the temperature from 8 to 24°C, at HRT of 5 d (Figs. 1 and 2). The removal efficiency of $SBOD_5$, SCOD increased considerably by raising temperature and declining phenol concentration. The result showed that temperature was a more effective factor in terms of carbon removal in stabilization pond compared to phenol concentration. It must be mentioned that variation of removal efficiency was more apparent at high sys-

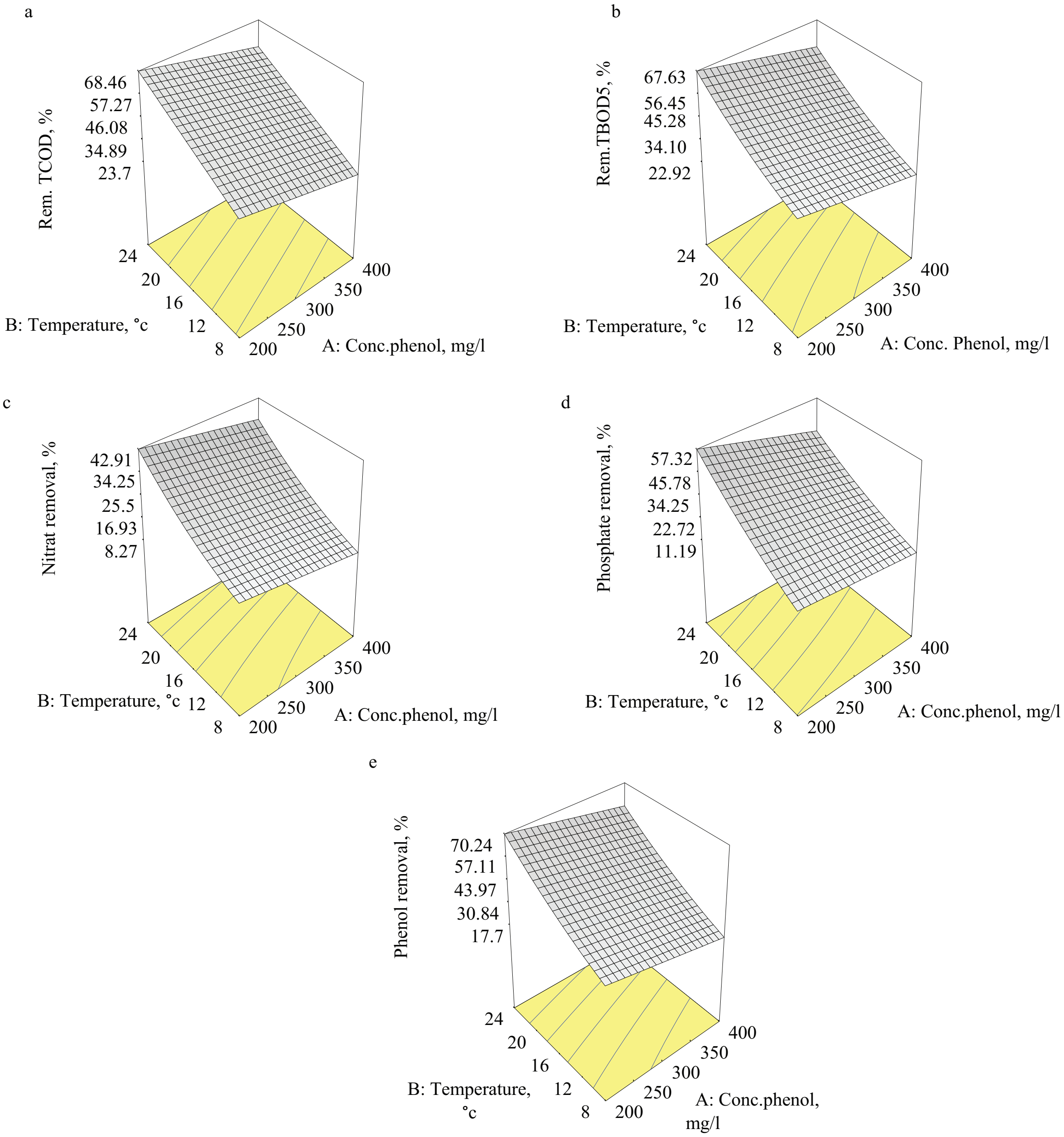

Fig. 1. 3D Surface plots for a: TCOD removal , b: TBOD5 removal, c: Nitrate removal, d: Phosphate removal and e: Phenol removal in HRT: 2 days.

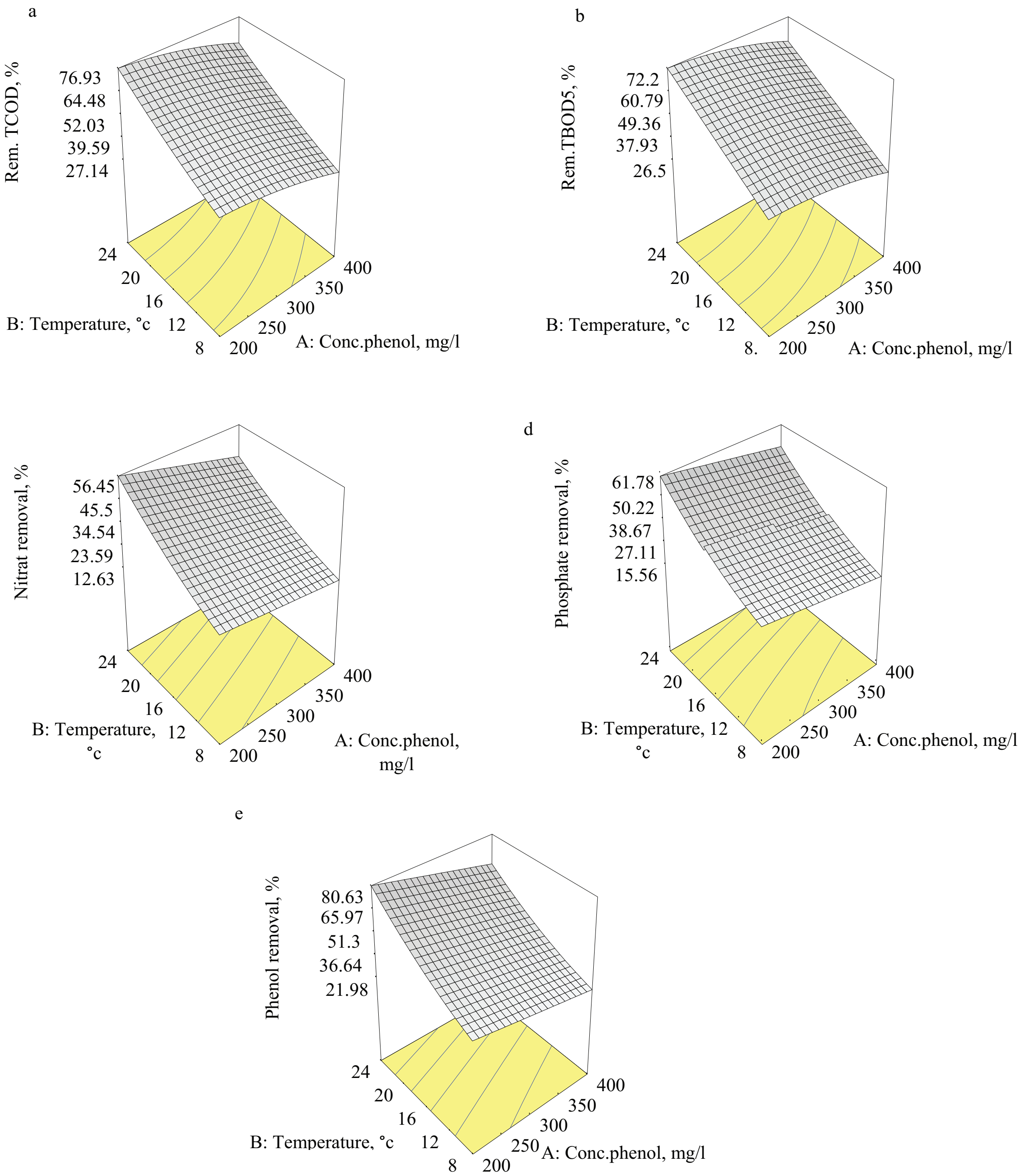

Fig. 2. 3D Surface plots for a: TCOD removal , b: TBOD5 removal, c: Nitrate removal, d: Phosphate removal and e: phenol removal in HRT: 5 days.

tem temperatures. It was found that SCOD, TCOD, $TBOD_5$ and $SBOD_5$ removal efficiency increased 28.55, 13.32, 30.08 and 30.12%, respectively, when temperature increased from 8 to 24°C at HRT of 2 d. However, the removal efficiency of SCOD, TCOD, TBOD and SBOD grew 16.85, 30.23, 13.52 and 15.63%, respectively, when phenol concentration went down from 400 to 200 mg/L. The maximum removal efficiency of SCOD, TCOD, SBOD and TBOD reached 66.26, 68.95, 65.3 and 67.02%, respectively (HRT of 2 d, temperature of 24°C and phenol concentration of 200 mg/L). It was observed that removal efficiency of SCOD, TCOD, SBOD and TBOD reached 27.36, 26.63, 26.61 and 28.38%, respectively (HRT of 5 d, temperature of 8°C and phenol concentration of 400 mg/L). It should be mentioned that volumetric loading of stabilization pond in phenol concentration of 200, 300 and 400 mg/l, were 118.55, 131.74 and 143.48 $g/BOD_5/m^3{\cdot}d$, respectively, at temperature of 24°C and 121.54, 136.01 and 148.12 $g/BOD_5/m^3{\cdot}d$, respectively, at temperature of 8°C that leads to low efficiency of pond by raising phenol concentration and lowering system temperature. When HRT rose from 2 to 5 d, the removal efficiency of SCOD, TCOD, $SBOD_5$ and TBOD went up 11.7, 9.67, 7.02 and 8.83%, respectively (at 24°C and phenol concentration of 400 mg/L), although this removal efficiency was not significant. Less removal efficiency was observed at lower temperature of anaerobic stabilization pond. It can be attributed to lower microorganism growth and slower degradation rate of dissolved substance. Although vast majority of biodegradable substances are removed by hydrolysis in anaerobic digestion, most suspended organic solids are converted into soluble intermediate compounds with poor biodegradability by hydrolysis in anaerobic stabilization pond [18]. The anaerobic stabilization pond achieved higher COD removal efficiency than BOD_5 removal which is not used for municipal and industrial wastewater. It may be attributed to multi-phase process of oil refinery waste due to volatile characteristics of upper layer, and also separation of some layers owing to hydrophobic characteristics of oily wastewater that stays or precipitates in the column inside the reactor. Another notable reason for degradation of non-biodegradable compounds converted into biodegradable fragments such as Catechol, aldehydes and acidic materials and is higher COD removal efficiency compared to BOD_5. It was in contrast with the study carried out by [19]. As result of surplus dissolving of the biodegradable compound (SCOD) in the depth of anaerobic stabilization pond, the overall decline of TCOD was observed. Also the anaerobic stabilization pond was found to be more capable for the treatment of oil refinery wastewater containing different concentrations of phenol compared with the study conducted by Mahssen et al. It showed that removal efficiency of COD and BOD_5 reached 28.89% and 22.21%, respectively [20]. Effect of temperature on biological reaction rate is completely obvious. Several studies have proved the appropriate performance of stabilization pond in warm weather [21].

3.2.2. Nutrient removal

Almost since the mid-1970, the use of algae to mitigate the nutrients N and P in wastewater treatment has been considered for combatting the Eutrophication [22]. Microalgae are a very diverse group of photosynthetic organisms that absorb N and P during their growth, then the generated biomass can be converted into energy or more raw materials depending on appropriate processing. Microalgae are able to adjust the nutrient based on surrounding concentration [23]. However, N concentration in microalgae depends on P concentration. Accordingly, present work evaluated the ratio of N/P. Generally, the ratio of N/P varied between 6.69–9.12 and 7.04–12.93, respectively, in influent and effluent that are presented in Tables 1 and 2. In general, the investigation showed that P removal was higher than N removal, leading to increase in N/P ratio in effluent than influent of stabilization pond, (Tables 1, 2). it was in contrast with Whitton et al., study that evaluated the influence of microalgae's N and P composition on wastewater nutrient remediation, and the result showed that the N/P ratio ranged from 2.03 to 15 from the third to tenth day [24]. According, the ratio of N/P declined from 12.16 to 7.04 in influent of system while phenol concentration increased from 200 to 400 mg/L, in HRT of 2 d and temperature of 24°C. The maximum and minimum ratio of N/P were observed at temperature of 8 and 24°C, respectively and HRT of 2 d. In other words, the lowest phenol concentration was observed at low system temperature ; therefore, increase in temperature resulted in improvement in the P removal efficiency. It can be attributed to mesophilic microorganisms and algae that provide the optimum condition for their growth [24].

Phosphate removal efficiency ranged from 2% to 14.48% and from 4.08% to 8.97%, respectively by HRT of 2, 5 d that was in contrast with Whitton el al. study. Therefore, N concentration is considered as a limiting agent in terms of raising the removal efficiency of stabilization pond. Overall, the phosphate removal is associated with N removal in cellular metabolism [25]. Mostly, in microalgae, N integrated into protein for ribosome production and ribosomal RNA (rRNA) while most P uptook is stored in rRNA. Therefore,enough N is needed to ensure no limitation for synthesis of protein. Based on studies carried out, in low N environment, the P uptake into biomass remains low, regardless of the P concentration in the biomass [26]. In another study, it was revealed that the uptake rate is associated with the ability of microalgae to store available phosphate via luxurious uptake pathway which accumulates the polyphosphate within the cell [1]. In general, based on the results of experiments a similar trend was observed in terms of N and P removal efficiency at HRT 2, 5 d. Hence, increasing phenol concentration and lowering temperature led to decrease in the removal efficiency trend. As it is seen in Table 3, the coefficient of (A, B) in N removal were 4.22 and 13.1, respectively, this means that temperature has more positive effect than phenol concentration, and the same trend for phenol removal efficiency was observed. It should be taken into account that the different effects of microalgae in terms of nutrient removal depends on the type of wastewater, wastewater treatment technology, temperature and the condition of operation system [27]. Orumieh et al. [27] indicated that maximum removal efficiency of N and P were 33% and 25%, respectively in stabilization pond. But the nutrient removal efficiency was more satisfying in this study compared to Orumieh's study result. Generally, the experiment result revealed that temperature variation was a more effective factor in terms of nutrient removal in stabilization pond. N concentration decreased due to cellular uptake at high system temperature [27].

3.2.3. Phenol removal

In regard with transforming the waste into simple end product, the use of biological treatment is increasing nowadays [5]. The surface plot for phenol removal in anaerobic stabilization pond is depicted in (Figs. 1 and 2), indicating the interaction effect of phenol concentration and temperature. As it can be seen, raising temperature and lowering phenol concentration enhanced phenol removal efficiency. For instance, removal efficiency decreased from 20.51% to 14.67% and phenol concentration rose from 200 to 400 mg/L, at the same temperature of 24°C at HRT 2, 5 d, respectively. The result showed that phenol removal efficiency varied between 18.22–70.53% and 21.24–81.63%, respectively, at HRT 2 and 5 d. The maximum removal efficiency of phenol concentration was observed in phenol concentration of 200 mg/l and temperature of 24°C. It was found that the lowest removal efficiency was observed in maximum phenol concentration and lowest system temperature. It was evident that phenol removal efficiency was more affected by temperature than phenol concentration. In consequence, the anaerobic digestion of organic compounds such as phenol, produced the nutrient rich sludge which can be used as fertilizer in agricultural soil with low level of pathogen. Azbar et al. studied the phenol removal in anaerobic hybrid reactor. The result revealed that phenol removal efficiency ranged from 39 to 80% under different conditions that is consistent with the study by [28]. Shabita et al. evaluated the microbial degradation of various phenols and their derivations. In this condition 4-n- propylphenol and phenol are degraded [29]. In the first stepwise of anaerobic pathway, phenol is carboxylated in the para position to 4-hydroxybenzonate, the enzyme involved was the 4-hydrobenzonate carboxylase [5]. This study represented that removal efficiency increased slightly when HRT went up. The maximum removal efficiency were 70.53% and 81.63 (at phenol concentration of 200 mg/L, temperature of 24°C and HRT of 2 and 5 d, respectively. The phenol removal efficiency was more significantly influenced by high temperature than decrease in the phenol concentration. According to Table 2, it was found that the phenol removal efficiency enhanced 39.14% (Runs 8, 11) when temperature increased from 8 to 24°C, and phenol concentration declined from 400 to 200 mg/L at HRT of 2 d. And also phenol removal efficiency increased 14.67%, (runs 1, 11). The effect of increase in temperature and decrease in phenol concentration at HRT of 5 days was significantly apparent (Table 2).

3.3. Process optimization and verification

The experiment was carried out based on CCD to attain process optimization. In this study, 3 independent variables of phenol concentration, temperature and HRT were evaluated aiming at processing the optimization of the anaerobic stabilization pond. Graphical optimization produces an overlay plot expressing the feasibility response value in the factor space. Overlay plot represents the region which meets the proposed criteria. The optimum area relative to 7 responses was determined (Fig. 3). The yellow area shows the region that satisfies the responses. And the shaded region is related to variables of space. According to the response, the optimum region in terms of carbon, nutrient and phenol removal were identified 60%, 40% and 70%, respectively at HRT of 2 d and in terms of total carbon, soluble carbon, nitrate, phosphate and phenol removal were 70%, 60%, 50%, 60% and 80%, respectively, at HRT of 5 d. Aimed at confirming the adequacy and reliability of the model, a point among optimum area was selected which is depicted in Fig. 3 and the actual and predicted values of model were compared. Table 5 shows the results of experiments within the optimum region. The accuracy of optimum condition was determined for each response from the DOE experiments that was tested through applying standard deviation. The results revealed that experimental findings were very close to predicted values by the model.

4. Conclusion

The results of experiment revealed raising phenol concentration led to decrease in the performance of anaerobic stabilization pond due to increasing the phenol toxicity on purifying bacteria in oil refinery wastewater treatment. In order to analyze the resulting data, RSM was used to demonstrate the effect of operating variables and interaction effect on the response as well. The results demonstrated the significant effect of HRT, temperature and phenol concentration on anaerobic stabilization pond efficiency in pilot scale. Temperature was more effective on Phenol removal efficiency compared with phenol concentration. In general, anaerobic stabilization pond was found to be a successful biological treatment process for moderate removal of organic and inorganic compounds using appropriate operations. The applicable properties of pond stabilization such as flexibility, simplicity, performance and also operation

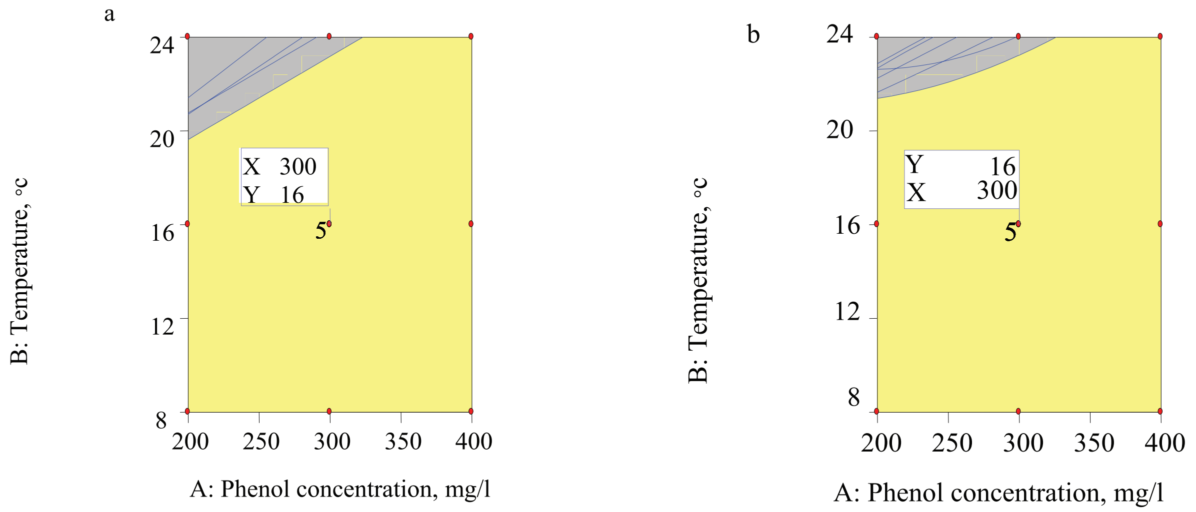

Fig. 3. Overlay plots for the optimal region, a: HRT: 2 days, b: HRT: 5 days.

Table 5
Verification experiments at optimum conditions

HRT, d		Conditions	Responses, %						
			TCOD removal	SCOD removal	TBOD5 removal	SBOD5 removal	N-NH$_3$ removal	P-PO$_4$ removal	Phenol removal
2	Experimental values	Phenol concentration: 300 mg/l Temperature, 16°C	46.0862	42.2354	43.8938	43.4838	22.6317	30.4192	42.5223
	Model response with Cl 95% Error		45.04	40.95	42.47	42.42	21.13	29.09	41.02
	Standard deviation		1.75	2.15	2.40	1.78	1.66	2.20	2.52
5	Experimental values	Phenol concentration: 300 mg/l Temperature, 16°C	52.7903	50.6654	51.3452	49.4631	30.1962	34.5738	45.791
	Model response with Cl 95% Error		51.83	49.48	49.85	48.29	28.84	32.74	44.58
	Standard deviation		1.06	1.99	1.65	1.97	1.49	2.03	1.34

led to applying stabilization pond rather than complex and expensive wastewater treatment technology such as activated sludge, etc.

References

[1] L. Wang, M. Min, Y. Li, P. Chen, Y. Chen, Y. Liu, Y. Wang, R. Ruan, Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant, Appl. Biochem. Biotechnol., 162 (2010) 1174–1186.

[2] T. Misiti, U. Tezel, S.G. Pavlostathis, Fate and effect of naphthenic acids on oil refinery activated sludge wastewater treatment systems, Water Res., 47 (2013) 449–460.

[3] Y.A. Yavuz, S. Koparal, U. Öğütveren, Treatment of petroleum refinery wastewater by electrochemical methods, Desalination, 258 (2010) 201–205.

[4] V. Naddeo, A. Cesaro, D. mantzavinos, D. Fatta-kassinos, V. Belgiorno, Water and wastewater disinfection by ultrasond irradiation - A critical review, Glob NEST J., 16 (2014) 561–577.

[5] N.V. Pradeep, S. Anupama, J.M. Arunkumar, K.G. Vidyashree, K. Ankitha, P. Lakshmi, J. Pooja, Treatment of sugar industry wastewater in anaerobic down flow stationary fixed film (DSFF) reactor., Sugar Tech, 16 (2014) 1–9.

[6] A. Almasi, A. Dargahi, A. Amrane, M. Fazlzadeh, M. Mahmoudi, A.H. Hashemian, Effect of the retention time and the phenol concentration the stabilization pond efficiency in the treatment of oil refinery wastewater, FEB., 23 (2014) 2541–2548.

[7] A. Dargahi, A. Almasi, M. Soltanian, P. Zarei, A. Hashemian, H. Golestanifar, Effect of molasses on phenol removal rate using pilot-scale anaerobic reactors, J. Water Wastewater., 25 (2014) 2–12.

[8] Z. Cheng, F. Fu, Y. Pang, B. Tang, J. Lu, Removal of phenol by acid-washed zero-valentaluminium in the presence of H_2O_2, Chem. Eng. J., 260 (2015) 284–290.

[9] A. Moslehyani, A.F. Ismail, M.H.D Othman, T. Matsuura, Design and performance study of hybrid photocatalytic reactor-PVDF/MWCNT nanocomposite membrane system for treatment of petroleum refinery wastewater, Desalination, 363 (2015) 99–111.

[10] S.O. Rastegar, S.M. Mousavi, S.A. Shojaosadatia, S. Sheibani, Optimization of petroleum refinery effluent treatment in a UASB reactor using response surface methodology, J. Hazard. Mater., 197 (2011) 26– 32.

[11] A. Almasi, A. Dargahi, M.H. Ahagh, H. janjani, M. Mohammadi, L. Tabandeh, Efficiency of a constructed wetland in controlling organic pollutants, nitrogen, and heavy metals from sewage, J. Chem. Pharm. Sci., 9 (2016) 2924–2928.

[12] N. Mburu, S.M. Tebitendwa, J.J.A. Van Bruggen, D.P.L Rousseau, P.N.L. Lens, Performance comparison and economics analysis of waste stabilization ponds and horizontal subsurface flow constructed wetlands treating domestic wastewater: A case study of the Juja sewage treatment works, J. Environ. Manage., 128 (2013) 220–225.

[13] V. Del Nery, M.H.Z. Damianovic, E. Pozzi, I.R. de Nardi, V.E.A. Caldas, E.C. Pires, Long-term performance and operational strategies of a poultry slaughterhouse waste stabilization pond system in a tropical climate, J. Resour. Converserv. Recy., 71 (2013) 7–14.

[14] A. Almasi, A. Dargahi, M. Mohamadi, H. Biglari, F. Amirian, M. Raei, Removal of Penicillin G by combination of sonolysis and Photocatalytic (sonophotocatalytic) process from aqueous solution: process optimization using RSM (Response Surface Methodology). Electron Physician., 8 (2016) 2878–2887.

[15] E. Ghasemi, M. Sillanpää, Optimization of headspace solid phase micro extraction based on nano-structured ZnO combined with gas chromatography-mass spectrometry for pre concentration and determination of ultra-traces of chloro benzenes in environmental samples, Talanta, 130 (2014) 322–327.

[16] W. Apha, AWWA, Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC, 2008.

[17] A.M. Antonopoulou, V. Papadopoulos, I. Konstantinou, Photocatalytic oxidation of treated municipal wastewaters for the removal of phenolic compounds: optimization and modeling using response surface methodology (RSM) and artificial neural networks (ANNs), J. Chem. Technol., 87 (2012) 1385–1395.

[18] A. Almasi, A. Dargahi, M. Pirsaheb, The effect of different concentrations of phenol on anaerobic stabilization pond performance in treating petroleum refinery wastewater, J. Water Wastewater, 1 (2013) 61–68.

[19] M. Pirsaheb, M. Mohamadi, A.M. Mansouri, A.A.L. Zinatizadeh, S. Sumathi, K. Sharafi, Process modeling and optimization of biological removal of carbon, nitrogen and phosphorus from hospital wastewater in a continuous feeding and intermittent discharge (CFID) bioreactor, Korean J. Chem. Eng., 32 (2015) 1340–1353.

[20] A.M. Abdel-Aatty, M. Karnel, Performance evaluation of a waste stabilization pond in a rural area in Egypt, Am. J. Environ. Sci., 4 (2008) 316–325.

[21] M.A.I. Al-Hashimi, H.T. Hussain, Stabilization pond for wastewater treatment, Eur. Sci. J., 9 (2013) 67–74.

[22] H. Kocyig, A. Ugurlu, Biologycal decolorization of rective Azo dye anarobic/aerobic sequencing batch reactor system, Glob. NEST J., 17 (2015) 210–215.

[23] H.J. Choi, S.M. Lee, Effect of the N/P ratio on biomass productivity and nutrient removal from municipal wastewater, Bioprocess Biosyst. Eng., 38 (2015) 761–766.

[24] R. Whitton, A.L. Evel, P. Marc, F. Ometto, R. Villa, B. Jefferson, Influence of microalgal N and P composition on wastewater nutrient remediation, Water Res., 91 (2016) 371–378.

[25] I. Loladze, J.J. Elser, The origins of the Redfield nitrogen-to-phosphorus ratio are in a homoeostatic protein-to-rRNA ratio, Ecol. Lett., 14 (2011) 244–250.

[26] A. Beuckels, E. Smolders, K. Muylaert, Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment, Water Res., 77 (2015) 98–106.

[27] H.R Orumieh, R. Mazaheri, Efficiency of stabilization ponds under different climate condition in Iran, Indian J. Fundam. Appl. Life Sci., 5 (2015) 794–805.

[28] N. Azbar, F. Tutuk, T. Keskin, Biodegradation performance of an anaerobic hybrid reactor treating olive mill effluent under various organic loading rates, Int. Biodeterior. Biodegrad., 63 (2009) 690–698.

[29] A. Shibata, Y. Inoue, A. Katayama, Aerobic and anaerobic biodegradation of phenol derivatives in various paddy soils, Sci. Total Environ., 367 (2006) 979–987.