



Biological treatment of phenol from petroleum refinery wastewater using mixed indigenous cultures in a rotating biological contactor: experimental and statistical studies

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ABSTRACT

In the present study, the removal performance of phenol and Chemical Oxygen Demand (COD) from a petroleum refinery wastewater (PRW) in a Rotating Biological Contactor (RBC) was investigated. The impact of temperature of wastewater (T) (35–45°C), rotational speed (ω) (2–14 rpm) and disc submergence (Ω) (30–50%) on phenol and COD removal was examined. The response surface methodology (RSM) was applied to minimize the number of runs and investigate the optimum operating conditions. Seventeen runs were carried out and the optimum conditions for phenol and COD removals were statistically obtained at temperature of 35°C, rotational speed of 11 rpm and disc submergence of 46%. These predicted data were in good agreement with the observed ones, as well. The result indicated that, the removal efficiency of phenol, cyanide, ammonia nitrogen, hydrogen sulfide, COD, biological oxygen demand (BOD₅), total dissolved solid (TDS), total suspended solid (TSS), total organic carbon (TOC) and turbidity respectively were under the 99%, 82%, 40%, 93%, 89%, 87%, 76%, 85%, 55% and 58%, in the optimum conditions that mentioned above. The major group of microbes (for the phenol microbial treatment) isolated from effluent of American Petroleum Institute (API) separator identified as *Bacillus* and *Pseudomonas* species. Furthermore, indigenous bacteria no need to acclimate. It also produced low sludge compared with the activated sludge in the RBC. Therefore, this type of bacteria was successfully applied in this research.

Keywords: COD; Optimization; Petroleum refinery wastewater; Phenol biodegradation

1. Introduction

Petroleum refining utilizes large quantities of water for desalting, distillation, thermal cracking, catalytic and treatment processes to produce useful products [1]. Refining process generates wastewater (0.4–1.6 times the volume of crude oil processed) [2]. Discharge of untreated petroleum refining wastewater into water bodies results in environmental and human health effects due to release of toxic contaminants (hydrocarbons, phenol and dissolved minerals) [3].

Phenol and its derivatives are common water pollutants and include wide variety of organic chemicals. As it adversely affects the aquatic biota, phenol is one of the 129 specific priority chemicals that is considered toxic under the 1977 Amendments to the Clean Water Act and for which the US Environment Protection Agency (EPA) has issued water quality criteria [4]. Phenol at concentration as low as 5 mg/L imparts typical smell upon chlorination and the World Health Organization (WHO) prescribed 1 mg/L as the maximum permissible concentration of phenol in drinking water [5].

Microbial degradation is a useful strategy to eliminate organic compounds and detoxify wastewaters and polluted environment [6]. Phenol is degraded by diverse

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microorganisms including yeasts, fungi and bacteria (*Pseudomonas putida* [7], *Kelibiella*, *Citrobacter* and *Shigella* [8], *Pseudomonas aeruginosa* and *Pseudomonas fluorescense* [9], *Acinetobacterbaumannii* [10], *Pseudomonas stutzeri* [11], *Bacillus brevis* [12]).

A rotating biological contactor is an aerobic and anaerobic attached growth fixed film bioreactor that offers an alternative technology to the conventional activated sludge process [13].

Rotating biological contactor (RBC) systems have several advantages such as usable for the secondary treatment of industrial wastewater and biodegradable materials removal. The advantages offered by RBC are relatively small land requirement, easy construction and expansion, compact design with separate compartments, simple process control and monitoring, low operating and maintenance cost, short hydraulic retention time (HRT), high oxygen transfer efficiencies (OTE), high biomass concentration per volume reactor, low sludge volume index (SVI) values in the second clarifier, no requirement of sludge recirculation, resistance to shock and toxic loads, no problem with malodors and flies [14]. Estimation reveal that RBCs require about 40–50% of the energy requirements of an activated sludge system and 70–80% of a trickling filter (TF) system [15].

Over the years a number of studies have been carried out on RBC systems. The previous studies relevant to the present study have been summarized in Table 1.

Yang and Humphrey [16] reported that it was possible to reduce phenol concentration in wastewater up to 1 to 2 mg/L by a batch first stage RBC.

Choung et al. [17] treated synthetic phenolic wastewater by a RBC. They reduced phenol concentration below 2 mg/L while phenol concentration was around 314.5 mg/L in the initial effluent.

Tyagi et al. [18] investigated the efficiency of the RBC-PUF system for the treatment of petroleum refinery wastewater at various hydraulic loading. They found removal efficiency of TCOD, oil, ammonia nitrogen, phenol more than 87, 80, 99 and 85% respectively.

Banerjee [19] decomposed phenol in a RBC. He investigated that an increase in the hydraulic loading increases phenol removal rate.

Israni et al. [20] showed that a RBC behaves similar to a nine well-mixed tanks in series during their modeling.

Alemzadeh et al. [21] studied the phenol removal performance in a RBC. According to this research, optimum phenol removal was at 99.90% for COD concentration of 800 mg dm⁻³. Furthermore, the optimal removal was found at the end of second stage while the third stage had not significant effect on the phenol removal. Moreover, the effect of temperature on improving the removal efficiency was significant in the temperature range of 13–43°C although the optimum operating temperature was at 36°C.

Melo et al. [22] improved phenol biodegradation in a RBC by availability of oxygen and stirring speed.

Anupama and Hampannavar [23] applied a RBC for 99% phenol removal when phenol initial concentration was in the range of 40–180 mg/L.

Mirbagheri and Mirkhalili [24] examined the performance of RBC reactor for the treatment of petroleum refin-

Table 1
Showing details of various experimental data in previous works

Type of scale	Mode of working	Staging	Reactor	Disc	T (°C)	ω (rpm)	Ω (%)	HRT (h)	References
Lab	Continuous	Four	Acrylic	–	25	20	–	–	[17]
Lab	Batch	Four	Plexiglass $A_T = 0.49 \text{ m}^2$ $V_W = 6.2 \text{ lit}$	PUF D = 25 cm No = 12 Space = 2 cm	–	10	42.5	7.6 3.8 2.53 1.89	[18]
Bench	Continuous	Four	Aluminum Thk = 5 mm $A_T = 1.98 \text{ m}^2$	PS D = 41 cm No = 60	20.5 27	1 24	–	–	[19]
Bench	Continuous	Single	Steel $V_W = 0.5 \text{ dm}^3$	Thk = 2 mm No = 9	20 30	40 175	50	–	[20]
Bench	Continuous	Three	Plexiglass Thk = 5 mm $A_T = 1.5 \text{ m}^2$ $V_T = 7.5 \text{ lit}$	Plexiglass Thk = 3 mm D = 21 cm No = 72	13 43	15	–	–	[21]
Bench	Batch	Single	Glass $V_T = 7.5 \text{ lit}$ $V_W = 3 \text{ lit}$	Steel D = 9 cm No = 9	–	40 70 100 125	–	–	[22]
Lab	Batch	Single	Glass Thk = 5 mm $V_W = 10 \text{ lit}$	PMMA D = 18 cm No = 6	27 32	50 75 100	30 35 40	24 28 36	[23]
Pilot	Continuous	Three	$A_T = 0.39 \text{ m}^2$ $V_W = 12.5 \text{ lit}$	Plexiglass Thk = 1 cm D = 25 cm No = 12	–	10	40	–	[24]

ery wastewater. According to this research, 96% and 91% for COD and suspended solid (SS) were respectively obtained when the average initial COD was at 620 mg/L.

It can be seen from Table 1 that in most cases the range of parameters covered in the previous works is narrow and the effects of variation in temperature, rotational speed and disc submergence have been studied at ambient temperature range (20–32°C), high rotational speeds (40–175 rpm) and disc submergence less than 40%. Also, the initial concentration of phenol were studied only in high concentrations (40–400 mg/L). In addition, active sludge has always been used to grow microorganisms and to form biofilms on discs. The aim of the present study was to address these shortcomings in previous works.

In this research, a single stage RBC with 24 plexi-glass discs was fabricated and used for phenol biodegradation obtained from a real petroleum refinery wastewater (most important environmental pollutants found in the wastewater of refinery) using isolated indigenous bacteria instead of activated sludge. Several parameters (such as temperature, rotational speed and percentage submergence of discs) effect on phenol and COD removal was carefully studied.

2. Materials and method

2.1. Wastewater sampling and characterization

The applied wastewater used in this research was collected from the effluent of an API separator of Imam Khomeini Oil Refinery Company (IKORC, Arak, Iran). The fresh wastewater was collected in a 20 l container and immediately stored in a refrigerator (with temperature of 4 °C). The wastewater characteristics are given in Table 2.

2.2. Isolation and identification

In order to isolate indigenous bacteria from effluent of the API oil separator (phenolic wastewater), the streaking technique [25–27] was applied. Therefore, a semi-solid surface was used to produce discrete colonies. These colonies

can be used to identify the organism, purify the strain free of contaminants and produce a pure genetic clone.

In the second stage, the gram staining method [25–27] was exploited to identify the bacteria that had the capability of growth and reproduction in polluted conditions. It was found that the degrading bacteria are component species of gram-positive *Bacillus* (blue-violet and rod-shaped) and gram-negative *Pseudomonas* (pink-red).

2.3. RBC design and experimental set-up

According to the Karia et al.'s technique [28], a RBC was designed (total surface area required: 1.5 m², the total surface area of each disc: 0.0628 m², numbers of discs: 24, width of each reactor: 52 cm, and length of shaft: 60 cm) by some parameters assumption (size: bench-scale, diameter of discs: 20 cm, thickness of discs: 0.5 cm, disc spacing: 1.5 cm, disc submergence: 40%, flow rate: 0.03 m³/d, and hydraulic loading rate: 0.02 m³/m²·d). The accuracy of design was carefully checked by calculating the other parameters (3 ≤ organic loading rate: 5.4 g BOD₅/m²·d ≤ 10).

A single-stage bench-scale system with 24 discs was used in the present research (as shown in Fig. 1). The tank (in which the discs rotated in it) was made of iron (with 5 mm thickness) covered by fiber-glass with semi-circular shape and total liquid volume of 16 l. The discs were made of plexi-glass (with 5 mm thickness) and were mounted on a 50 mm diameter rigid stainless steel shaft at a spacing of 15 mm. Since a mild temperature similar to the natural wastewater lagoon was needed (36 °C), a suitable heater was also installed into the reactor. The desired rotational speeds were obtained with a motor and gearbox. The specifications of experimental set-up are illustrated in Table 3.

The RBC was inoculated with a mixed culture of *Bacillus* and *Pseudomonas* species (isolated from effluent of the API) that were able to degrade phenol. The cultures were aerobically incubated in nutrient broth on an orbital shaker (90 rpm) at 37°C for 24 h. The reactor was inoculated with 1.5 l of the mixed cultures (around 10% of wastewater) every week and the biomass was allowed to grow on the discs for 3 weeks. A visible growth of biomass on the discs was noticed after 20 days of inoculation. A healthy biomass growth was indicated by the golden color of solution. Biofilm thickness (L_f) was measured with a micrometer (± 0.1 mm). Measurements were carried out on the removable section before and after biofilm attachment. $L_f = 0.9$ mm was observed after the biofilm formation. The disc biomass index (DBI) is defined

Table 2
Wastewater characteristics

Parameter	Value
T (°C)	36
pH	6–8
Tur (FTU)	18.73
Phenol (mg/L)	7–13
CN ⁻ (mg/L)	0.01–0.06
NH ₃ -N (mg/L)	14–68
H ₂ S (mg/L)	0.1–10
COD (mg/L)	300–600
BOD ₅ (mg/L)	132
TOC (mg/L)	58
TDS (mg/L)	654
TSS (mg/L)	34

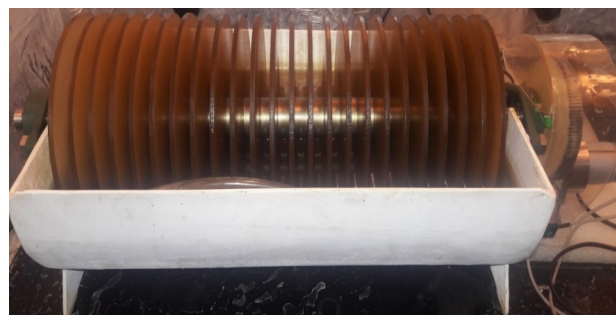


Fig. 1. Fabricated rotating biological contactor (RBC).

Table 3
Bench-scale RBC reactor set-up

Specification	RBC tank	Discs	Shaft
Number	1	24	1
Shape	Semi circular cylindrical	Circular	Cylinder
Fabrication	Iron plate+fiberglass	Plexi-glass	Stainless Steel
Length (cm)	52	–	60
Diameter (cm)	28	20	5
Thickness (mm)	5	5	–
Spacing discs (mm)	–	15	–
Surface area (m ²)	–	1.44	–
Total volume (l)	16	–	–
Working volume (l)	8.5–14.5	–	–

as the ratio of total volatile solids to total solids of the disc biomass [(gTVS/m²)/(gTS/m²)]. It represents the amount of active biomass on the disc. 34.214 g/m² of TS, 29.424 g/m² of TVS and DBI = 0.86 was obtained in this research. Furthermore, the mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) concentration raised from minimal concentration to 2.773 and 2.387 g/l, respectively.

2.4. Analytical procedures

The treated samples were filtered through the Whatman filter papers (0.45 μm). According to the standard methods [29], phenol concentration was measured using 4-aminoantipyrine method at 470 nm using a spectrophotometer [DR 5000, version 1.10 (401517) Germany] and COD was estimated by COD Reactor [BOX389.LOVELAND.COLO.U.S.A].

2.4.1. Phenol analysis

The 4-aminoantipyrine method measures all ortho- and meta-substituted phenols. These phenols react with 4-aminoantipyrine in the presence of potassium ferricyanide to form a colored antipyrine dye. The dye is then extracted from the aqueous phase with chloroform and the color is measured at 460 nm. The sensitivity of the method varies with the type of phenolic compound. Because water samples may contain various types of phenolic compounds, the test results are expressed as the equivalent concentration of phenol.

2.4.2. COD analysis

The mg/L COD results are defined as the mg of O₂ consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr₂O₇²⁻) to green chromic ion (Cr³⁺). When the 0.7–40.0 or the 3–150 mg/L colorimetric method is used, the amount

of Cr⁶⁺ remaining is determined. When the 20–1500 mg/L or 200–15,000 mg/L colorimetric method is used, the amount of Cr³⁺ produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences. Test results for the 0.7 to 40.0 mg/L range are measured at 350 nm. Test results for the 3 to 150 mg/L range are measured at 420 nm. Test results for the 20 to 1500 and the 2000 to 15,000 mg/L COD range are measured at 620 nm.

2.5. Experimental design

Design Expert software (version 7.0.0) was applied for design, mathematical modeling and optimization. The parameters (independent variables) used in this study were temperature (X₁), rotational speed (X₂) and disc submergence (X₃). Removal of phenol (Y₁) and COD (Y₂) were considered as the dependent factors (response). Since pH was not significant variable in this research and based on batch studies (for phenol and COD removal with certain initial concentrations and at different times) pH (6.5–7.5) and HRT (8 h) were fixed to reduce the number of variables and simplify the experimental design. Three independent variables were converted to dimensionless ones. Central Composite Design (CCD) was applied in this work. For statistical calculations, the selected independent variables were converted into the dimensionless codified data. Table 4 shows independent variables and their levels for the CCD used in the present study. According to the statistical analysis by the software (DoE), the experiments were conducted in eight factorial, six axial and three central points.

3. Results and discussion

3.1. Regression models and statistical analysis

According to the software, Analysis of Variance (ANOVA) can be used for the data analysis. The quality of the fit polynomial model was expressed by the coefficient of determination R². Furthermore, the statistical significant was checked by the student t-test in the same program. The model terms were also evaluated by the probability value. One factor and 3D surface plots were obtained based on the effects of three factors at three levels.

Eqs. (1) and (2) show two correlations for phenol and COD removals:

$$\begin{aligned} \text{Phenol Removal Efficiency}(\%) \\ = +85.08 - 5.18A + 0.34B + 0.70C - 0.81AB \\ - 0.16AC - 0.11BC + 5.83A^2 - 0.69B^2 \end{aligned} \quad (1)$$

$$\begin{aligned} \text{COD Removal Efficiency}(\%) \\ = +73.51 - 4.54A + 0.44B + 0.67C - 0.28AB \\ + 0.052AC - 0.20BC + 4.18A^2 - 0.33B^2 \end{aligned} \quad (2)$$

where A, B, C are temperature, rotational speed and disc submergence, respectively. On the basis of the coefficients in Eqs. (1) and (2), it can be seen phenol and COD removals increased with the rotational speed and disc submergence while the temperature decreased them. A considerable

Table 4
Independent variables and their levels for the CCD

Symbol	Factor	Coded levels of variables		
		−1	0	1
A	Temperature (°C)	35	40	45
B	Rotational speed (rpm)	2	8	14
C	Disc submergence (%)	30	40	50

effect of temperature on both of phenol and COD removal was observed and influence of the interaction between the variables wasn't significant.

Tables 5 and 6 illustrate variance analysis for response surface quadratic model terms for phenol and COD removal, respectively. The model F-values of 18.99 and 59.92 imply the models are significant for percentage phenol and COD removals. There is only a 0.10% and 0.01% chance that model F-values occurs due to noise. Values of Prob > F less than 0.0500 indicate model terms are significant. For the phenol removal model, A, A² and For the COD removal model, A, C, A² are significant model terms.

The quadratic model statistical results for COD and color removals are summarized in Table 7. They indicate a high reliability in the estimation of Phenol and COD removal efficiencies ($R^2 = 0.9620$ and 0.9876 , respectively). A high R^2 coefficient ensures a satisfactory adjustment of the quadratic model to the experimental data. In optimizing a response surface, an adequate fit of the model should be achieved to keep away from poor outcome. The Predicted R-Squared of 0.6196 is not as close to the Adjusted R-Squared of 0.9114 for phenol removal as one might normally expect. The Predicted R-Squared of 0.8652 is in reasonable agreement with the Adjusted R-Squared of 0.9712 for COD removal. The adequate precision (AP) value is a measure of the signal to noise ratio and was found to be 11.226 and 20.600 for phenol and COD removal respectively, which indicates an adequate signal (Table 7). AP values higher than four are desirable and confirm that the predicted models can be used to navigate the space defined by the CCD.

Figs. 2(a) and (b) show a good agreement between the predicted data (for the responses) and the observed ones, the data points are distributed relatively close to the straight line. In fact, the models could navigate the design space.

The residuals from the least squares fit are important for judging the model adequacy. Fig. 3 (a) and (b) clearly present studentized residuals vs. normal percent of probability as a straight line.

3.2. Effects of operating parameters on RBC performance

For the graphical explanation of the interactions, the three-dimensional plots of the regression models were used. The corresponding response surface plots obtained from Eqs. (1) and (2) are illustrated in Figs. 4 and 5. The response surface plots obtained from the software provides a three-dimensional view of the phenol (a) and COD (b) removals surface with combinations of various independent variables. Moreover, reduction in the removal efficiencies occurs by moving away from the peak points. It means

Table 5
Analysis of variance for response surface quadratic model terms for phenol removal

Source	Sum of squares	df	Mean squares	F-value	P-value
Model	331.29	8	41.41	18.99	0.0010
A	268.43	1	268.43	123.10	< 0.0001
B	1.16	1	1.16	0.53	0.4940
C	4.94	1	4.94	2.27	0.1829
AB	5.20	1	5.20	2.38	0.1735
AC	0.21	1	0.21	0.095	0.7679
BC	0.090	1	0.090	0.041	0.8455
A ²	33.99	1	33.99	15.59	0.0076
B ²	0.47	1	0.47	0.22	0.6591
C ²	0.000	0	–	–	–
Residual	13.08	6	2.18	–	–
Lack of Fit	13.08	4	3.27	–	–
Pure Error	0.000	2	0.000	–	–
Cor Total	376.80	16	–	–	–

Table 6
Analysis of variance for response surface quadratic model terms for COD removal

Source	Sum of squares	df	Mean squares	F-value	P-value
Model	238.35	8	29.79	59.92	< 0.0001
A	205.75	1	205.75	413.84	< 0.0001
B	1.89	1	1.89	3.81	0.0989
C	4.52	1	4.52	9.08	0.0236
AB	0.61	1	0.61	1.22	0.3122
AC	0.022	1	0.022	0.044	0.8402
BC	0.31	1	0.31	0.63	0.4584
A ²	17.47	1	17.47	35.14	0.0010
B ²	0.11	1	0.11	0.21	0.6611
C ²	0.000	0	–	–	–
Residual	2.98	6	0.50	–	–
Lack of Fit	2.98	4	0.75	–	–
Pure Error	0.000	2	0.000	–	–
Cor Total	253.85	16	–	–	–

Table 7
ANOVA results for phenol and COD removals

Variable	Phenol removal	COD removal
Standard deviation	1.48	0.71
Mean	87.04	75.04
Coefficient of variance	1.70	0.94
PRESS	130.99	32.53
R-Squared	0.9620	0.9876
Adjusted R-Squared	0.9114	0.9712
Predicted R-Squared	0.6196	0.8652
Adequate precision	11.226	20.600

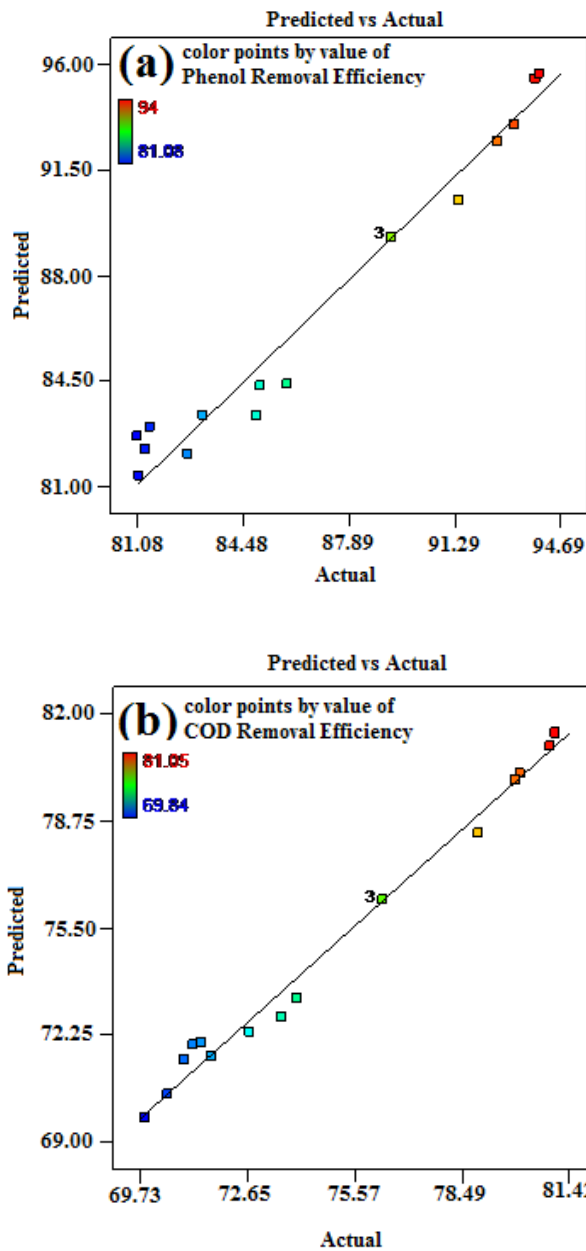


Fig. 2. Predicted vs actual values for a) phenol removal, b) COD removal.

that neither increase nor decrease in any of the tested variables is desired.

3.2.1. Effect of temperature on phenol and COD removals

The temperature is the main factor which directly affects and controls the rate of biological process. In fact, temperature affects the viscosity of RBC sludge [30]. In this research, effect of temperature was studied in a mild range of 35–45°C. Figs. 4 and 5 show that temperature had a negative effect on phenol and COD removal. An increase in the temperature over the optimum range leads to a decrease in the microbial activity. The maximum phenol and COD removal was

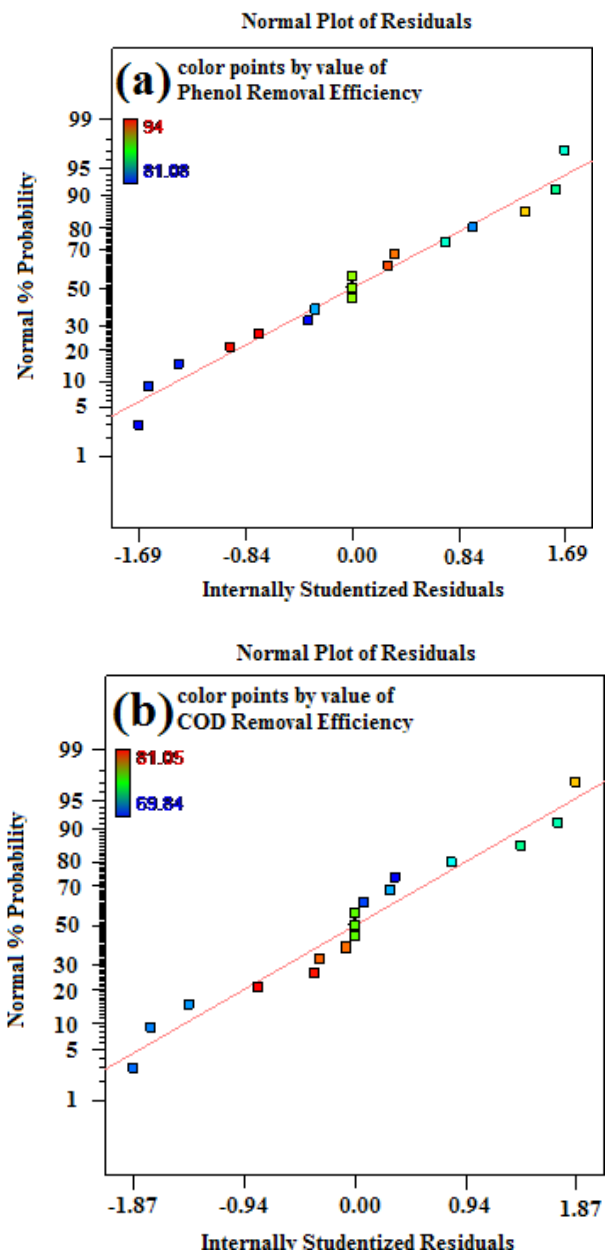


Fig. 3. Normal probability vs internally studentized residual values for a) phenol b) COD.

obtained at temperature of 35°C. Phenol and COD removal respectively decreased from 94.00% to 85.02% and from 81.05 to 72.71% when the temperature increased from 35 to 45°C. According to a similar work on phenol removal, optimum point was obtained at temperature of 36°C [21].

3.2.2. Effect of rotational speed on phenol and COD removals

The speed of rotation assists oxygen transfer and nutrient required for growth of microorganism in the biofilm. The film thickness and the dissolved oxygen can be controlled by adjusting the rotational speed of the discs [31].

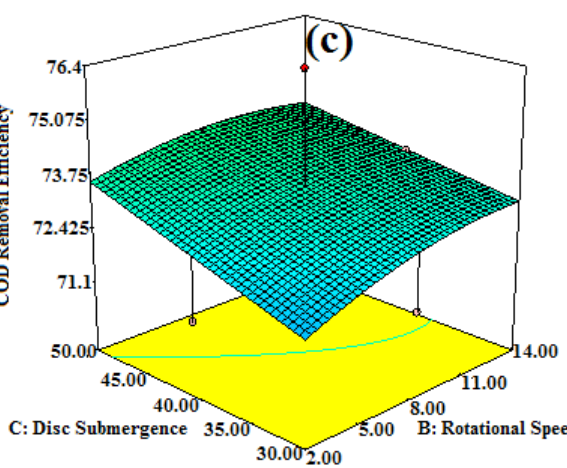
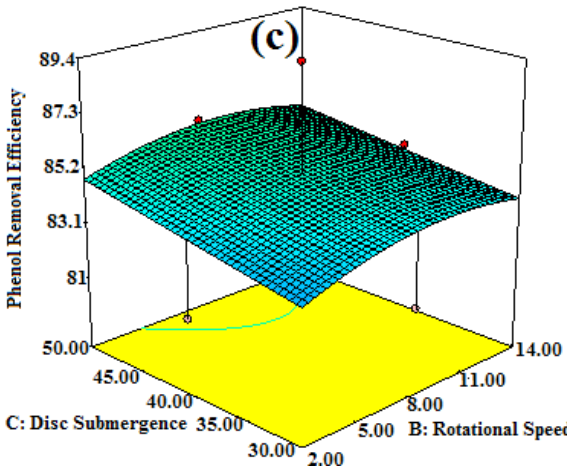
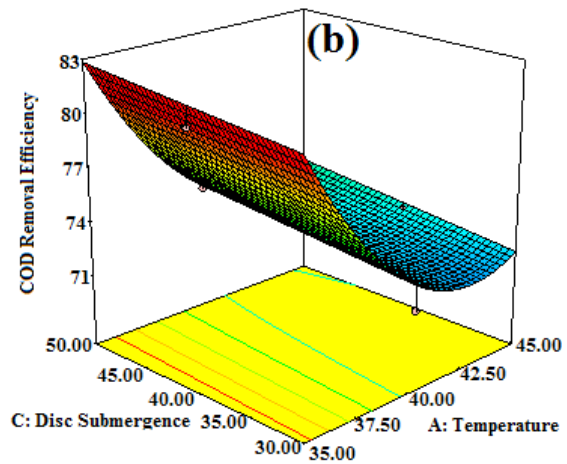
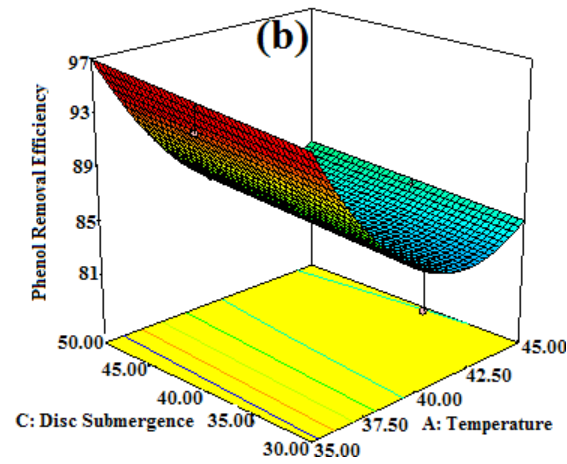
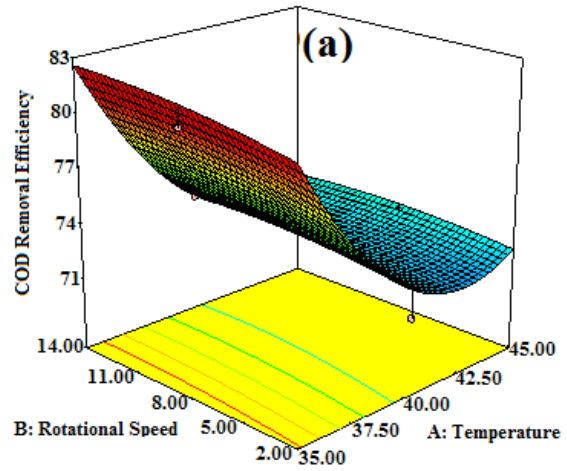
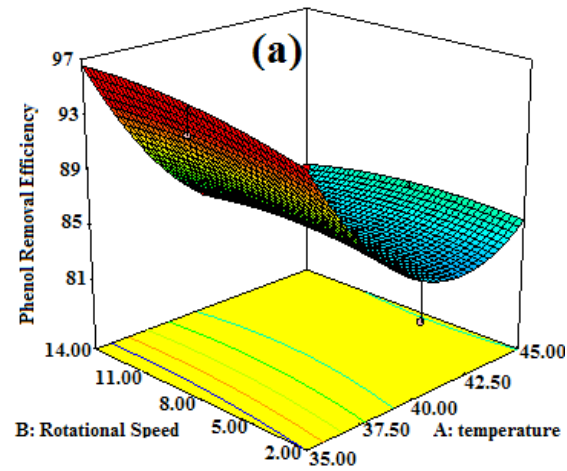


Fig. 4. Three-dimensional surface of phenol removal as a function of a) T & ω , b) T & Ω and c) ω & Ω .

Fig. 5. Three-dimensional surface of COD removal as a function of a) T & ω , b) T & Ω and c) ω & Ω .

In this research, the effect of rotational speed was studied in the three values (2, 8 and 14 rpm). Phenol and COD removal increased with the rotational speed of discs increment (up to 11 rpm). The phenol and COD removal respectively increased from 81.08% to 89.27% and from 71.17% to

76.34% when rotational speed increased from 2 to 8 rpm although the phenol and COD removal decreased to 84.91% and 73.58% when rotational speed increased from 8 to 14 rpm. According to a similar work on phenol removal, optimum point was obtained at rotational speed of 10 rpm [19].

3.2.3. Effect of percentage of submergence of disc on phenol and COD removals

Disc submergence is the other factor that effect on RBC performance. Partial submergence is used for aerobic RBC and submergence more than 50% is not practically possible as the bearing holding the shaft will be immersed in wastewater and can deteriorate the shaft work [32].

In this research, the experiments were carried out with three submergence levels (30, 40 and 50%). As shown in Figs. 4 and 5, the rate of phenol and COD removal was in maximum up to discs submergence of 46%. Phenol and COD removal was respectively improved from 81.47% to 89.27% and from 71.40% to 76.34% when discs submergence increased from 30% to 40% although they decreased 85.89% and 74.00% when discs submergence increased from 40% to 50%. According to a similar work on phenol removal, the removal increased with the submergence percentage increment [23].

3.2.4. Effect of phenol concentration

Effect of initial concentration of phenol and COD on the removal was studied by changing in wastewater type. Phenol and COD removal respectively decreased from 99.82% to 98.14% and from 89.18% to 88.30% when phenol and COD concentration respectively increased from 11.6 to 97 mg/L and from 305 to 342 mg/L (at optimum conditions and HRT of 24 h) although variation of feed phenol concentration (10–100 mg/L) did not change the reactor effluent phenol concentration (0.02–2 mg/L). According to a similar work on phenol removal, the removal increased with the phenol concentration enhancement. It is due to growing the cultures [18].

3.3. Optimization and validation

The software can automatically find the optimum conditions. It is necessary to validate these conditions with comparing the theoretical removal data with the experimental ones. Numerical optimization was used to determine the optimum parameters for maximum removal efficiency. The optimum conditions for phenol and COD removal was obtained based on the response surface and desirability function. In this case, all variables were targeted to be in range.

In order to confirm the accuracy of the predicted models and the reliability of the optimum conditions, an additional experiment was carried out. Table 8 illustrates the observed data (obtained from the experiment) under the optimum conditions compared with the predicted ones [obtained from the models (Eqs. (1) and (2))]. Low errors between the observed and predicted data validated both models.

4. Conclusions

In the present work, a petroleum wastewater containing phenol was treated using a RBC with 24 discs. It was concluded that isolated indigenous bacteria (*Bacillus* and *Pseudomonas* species) from a phenolic wastewater can successfully remove phenol. The effect of temperature, rotation speed and discs submergence on the phenol and COD removal rate were carefully investigated. The rotational speed and discs submergence had positive effect (up to optimum point) on the removal although they had negative effect on the removal after that point. A mild temperature (similar to the lagoon natural temperature) can assist the removal. The optimum conditions [(temperature of 35.01 and 35.04°C), (rotational speed of 11.53 and 11.16 rpm) and (discs submergence of 46.56% and 46.43%)] were found for phenol and COD removal, respectively.

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Nomenclature

A	—	Surface area
D	—	Diameter of disc
L_f	—	Biofilm thickness
T	—	Temperature
V	—	Volume

Greek letter

ω	—	Rotational speed
Ω	—	Disc submergence

Subscripts

T	—	Total
W	—	Working

Abbreviations

API	—	American Petroleum Institute
ANOVA	—	Analysis of Variance
BOD ₅	—	Biological Oxygen Demand
CCD	—	Central Composite Design
COD	—	Chemical Oxygen Demand

Table 8

Optimum conditions obtained from the software and experiment for phenol and COD removal

Response	T (°C)	ω (RPM)	Ω (%)	Observed	Predicted	Error
Phenol	35.01	11.53	46.56	94.25	97.01	2.93
COD	35.04	11.16	46.43	81.09	82.74	2.03

DBI	—	Disc Biomass Index
DoE	—	Design of Experiment
EPA	—	Environment Protection Agency
HRT	—	Hydraulic Retention Time
MLSS	—	Mixed Liquor Suspended Solid
MLVSS	—	Mixed Liquor Volatile Suspended Solid
OTE	—	Oxygen Transfer Efficiencies
PMMA	—	Poly Methyl Methacrylate
PRW	—	Petroleum Refinery Wastewater
PS	—	Poly Styrene
PUF	—	Poly Urethane Foam
RBC	—	Rotating Biological Contactor
RPM	—	Round per Minute
RSM	—	Response Surface Methodology
SS	—	Suspended Solid
SVI	—	Sludge Volume Index
TDS	—	Total Dissolved Solid
TF	—	Trickling Filter
Thk	—	Thickness
TOC	—	Total Organic Carbon
TS	—	Total Solids
TSS	—	Total Suspended Solid
TVS	—	Total Volatile Solids
WHO	—	World Health Organization

References

- 1] M. Bagajewicz, A review of recent design procedures for water networks in refineries and process plant, *Comput. Chem. Eng.*, 24 (2000) 2093–2113.
- 2] A. Coelho, V.A. Castro, M. Dezotti, Treatment of petroleum refinery wastewater by advanced oxidation processes, *J. Hazard. Mater.*, 137 (2006) 178–184.
- 3] B.H. Diya'uddeen, W.M.A. Daud, D.R. Abdul, Treatment technologies for petroleum refineries effluents, *Process Saf. Environ. Protect.*, 89 (2011) 95–105.
- 4] A. Singh, V. Kumar, J.N. Srivastava, Assessment of bioremediation of oil and phenol contents in refinery wastewater via bacterial consortium, *J. Petrol. Environ. Biotechnol.*, 4 (2013) 1–4.
- 5] P. Saravanan, K. akshirajan, P. Saha, Growth kinetics of an indigenous mixed microbial consortium during phenol degradation in a batch reactor, *Bioresour. Technol.*, 99 (2008) 205–209.
- 6] A. Gallego, M.S. Fortunato, J. Foglia, Biodegradation and detoxification of phenolic compounds by pure and mixed indigenous cultures in aerobic reactors, *Int. Biodeterior. Biodegrad.*, 52 (2003) 261–267.
- 7] S. Seker, H. Beyenal, B. Salih, A. Multi-substrate growth kinetics of *pseudomonas putida* for phenol removal, *Appl. Microbiol. Biotechnol.*, 47 (1997) 610–614.
- 8] F. Kafizadeh, M.S. Farhangdoost, Y. Tahery, Isolation and identification of phenol degrading bacteria from Lake Parisian and their growth kinetic assay, *Afr. J. Biotechnol.*, 29 (2010) 6721–6726.
- 9] S.E. Agarry, B.O. Solomon, S.K. Layokun, Kinetics of batch microbial degradation of phenols by indigenous binary mixed culture of *pseudomonas aeruginosa* and *pseudomonas fluorescense*, *Afr. J. Biotechnol.*, 7 (2008) 2417–2423.
- 10] S.B.C. Prasad, R.S. Babu, R. Chakrapani, Kinetics of high concentrated phenol biodegradation by *acinetobacterbaumannii*, *Int. J. Biotechnol. Biochem.*, 6 (2010) 609–615.
- 11] A. Viggiani, G. Olivieri, L. Siani, A. Di Donato, A. Marzocchella, P. Salatino, P. Barbieri, E. Galli, An airlift biofilm reactor for the biodegradation of phenol by *pseudomonas stutzeri ox1*, *J. Biotechnol.*, 123 (2006) 464–477.
- 12] V. Arutchelvan, V. Kanakasabai, R. Elangovan, Kinetics of high strength phenol degradation using *bacillus brevis*, *J. Hazard. Mater.*, 129 (2006) 216–222.
- 13] Metcalf, Eddy, *Wastewater Engineering Treatment and Reuse*. 4th edition. McGraw-Hill, US (2005).
- 14] S. Cortez, P. Teixeira, R. Oliveira, Rotating biological contactors: a review on main factors affecting performance, *Rev. Environ. Sci. Biotechnol.*, 7 (2008) 155–172.
- 15] R.L. Droste, *Theory and Practice of Water and Wastewater Treatment*, New York, John Wiley, US (1997).
- 16] R.D. Yang, A.E. Humphrey, Dynamics and steady state studies of phenol biodegradation in pure and mixed cultures, *Biotechnol. Bioeng.*, 17 (1975) 1121–1235.
- 17] Y.K. Choung, B.H. Bae, K.H. Ahn, Treatment of phenolic wastewater using rotating biological contactor, *Water. Pollution Control. Asia*, (1988) 685–690.
- 18] R.D. Tyagi, F.T. Tran, A.K.M. Chowdhury, Biodegradation of petroleum refinery wastewater in a modified rotating biological contactor with polyurethane foam attached to the discs, *Water Res.*, 27 (1993) 91–99.
- 19] G. Banerjee, Treatment of phenolic wastewater in RBC reactor, *Water Res.*, 31 (1997) 705–714.
- 20] S.H. Israni, S.S. Koli, A.W. Patwardhan, J.S. Melo, S.F. D'souza, Phenol degradation in rotating biological contactors, *J. Chem. Technol. Biotechnol.*, 77 (2002) 1050–1057.
- 21] I. Alemzadeh, M. Vossoughi, M. Houshmandi, Phenol biodegradation by rotating biological contactor, *Biochem. Eng. J.*, 11 (2002) 19–23.
- 22] J.S. Melo, A.W. Patwardhan, S.F. D'Souza, Effect of oxygen transfer limitations in phenol biodegradation, *Process Biochem.*, 40 (2005) 625–628.
- 23] P.N.V. Anupama, U.S. Hampannavar, Biodegradation of phenol using rotating biological contactor, *Int. J. Environ. Sci.*, 2 (2011) 105–113.
- 24] A. Mirbagheri, H. Mirkhalili, Refinery wastewater treatment by rotating biological contactors, *Proceedings of the 2nd National Conference on Health, Environment and Sustainable Development (HESD)*, Bandarabas, Iran (2012).
- 25] S. Mahesh, P. Aleem Basha, B. Kavitha, Isolation and characterization of bacteria isolated from municipal sewage water of Nandyal, Kurnool, A.P., India, *Asian J. Microbiol. Biotech. Env. Sci.*, 19 (2017) 772–777.
- 26] W.M.F. Wan Ishak, S. Jamek, N.A. Jalanni, N.F. MohdJama-ludin, Isolation and identification of bacteria from activated sludge and compost for municipal solid waste treatment system, *Proceedings in the International Conference on Biology, Environment and Chemistry*, IACSIT Press, Singapore, 24 (2011) 450–454.
- 27] M.K. Sharifi-Yazdi, C. Azimi, M.B. Khalili, Isolation and identification of bacteria present in the activated sludge unit, in the treatment of industrial waste water, *Iranian J. Publ. Health*, 30 (2001) 91–94.
- 28] G.L. Karia, R.A. Christian, *Wastewater treatment: concept and design approach*. 2nd edition. PHI Learning Pvt Ltd (2013).
- 29] APHA-AWWA-WEF (American Public Health Association, American Water Works Association, Water Environment Federation), *Standard Methods for the Examination of Water and Wastewater*, 18th edition. Washington DC, US (1999).
- 30] B. Abu-Jdayil, F. Banat, M. Al-Sameraiy, Steady rheological properties of rotating biological contactor sludge, *J. Water. Resource. Protect.*, 2 (2010) 1–7.
- 31] M. Del Borghi, E. Palazzi, F. Parisi, Influence of process variable on modeling and process design of a rotating biological surface, *Water Res.*, 19 (1985) 573–580.
- 32] P. Teixeira, R. Oliveira, Denitrification in a closed rotating biological contactor: effect of disk submergence, *Process Biochem.*, 37 (2001) 345–349.