Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2015.1004112

57 (2016) 6044–6050
March

Taylor & Francis
Taylor & Francis Group

Application of factorial experimental design methodology for the removal of phenol from water by innovate hybrid bioprocess

Wassila Lazli, Dalila Hank, Saliha Zeboudj, Abdelkader Namane, Amina Hellal*

Laboratoire des Sciences et Techniques de l'Environnement, Ecole Nationale Polytechnique, 10 Avenue Hacen Badi, BP182, El Harrach, 16200 Alger, Algeria, Tel. +21 321525301; Fax: +21 321522979; emails: wass-76@hotmail.fr (W. Lazli), d.hank@hotmail.fr (D. Hank), salihazeboudj@g.enp.edu.dz (S. Zeboudj), namaneaek@yahoo.fr (A. Namane), amina.hellal@g.enp.edu.dz (A. Hellal)

Received 9 February 2014; Accepted 27 December 2014

ABSTRACT

The purpose of this work was to study the phenol elimination by immobilized bacteria cells on a combined material. The immobilization by inclusion of *Pseudomonas aeruginosa* in calcium alginate beads with powdered activated carbon (PAC) allows the adsorption and biodegradation of phenol simultaneously. The full factorial design methodology was carried out to estimate the influence of the considered parameters (initial phenol concentration, amount of PAC, and diameter of beads) on the time of phenol removal. The adsorbent amount was the most significant parameter followed by the initial phenol concentration, while the effect of beads diameter was insignificant. The minimum time of phenol elimination obtained from the optimization procedure was 22.6 h. This optimum was achieved at 5 g/L of PAC and an initial phenol concentration of 100 mg/L.

Keywords: Phenol; Adsorption; Biodegradation; Powder activated carbon; Immobilized bacteria; Full factorial design

1. Introduction

Phenol is frequently found in the wastes from many industrial processes such as resin manufacturing, oil refineries, pharmaceuticals, dyes, paper-processing plants, coal liquefaction, textiles, and plastic industries [1,2]. It is typically found in concentrations up to 1.5 g/L, but this can rise to 4.5 g/L in much polluted waters [3]. Phenol and its higher homologous are listed as priority pollutants by the US Environmental Protection Agency (EPA) because they are considered to have toxic effects on human health even if present in small concentrations. In fact, blood changes, liver, kidney and serious gastrointestinal damage, cardiovascular disease, and even death have been reported in humans acutely exposed to phenol [4]. Therefore, the World Health Organization has set a limit level of 1 mg/L to regulate the phenol concentration in drinking waters, and the EPA calls for lowering phenol content in the wastewater to less than 1 mg/L [5]. Phenol is also very toxic to fish and has been lethal at concentrations between 5 and 25 ppm, while concentrations as low as 0.1 ppm in surrounding waters can taint the taste of fish [3,6]. For strict compliance of environmental regulations, it is

*Corresponding author.

Presented at the 3rd Annual International Conference on Water (CI.EAU2013), 18–20 November 2013, Algiers, Algeria

imperative to remove phenol from industrial effluents before discharging into freshwater streams to protect the aquatic life and allow the use of the water downstream [7].

The various existing removal technologies for phenol can be divided into two categories: physicochemical and biological methods. Physicochemical methods such as chemical oxidation, solvent extraction, adsorption, membrane process, coagulation, and flocculation often suffer from serious drawbacks including high cost and formation of hazardous byproducts [8–10]. On the other hand, biodegradation techniques have the potential to completely mineralize a large number of organic compounds at a relatively low cost. Thus, the biological methods have turned out to be a favorable alternative for the removal of phenolic pollutants present in the industrial effluents [11,12]. In spite of their toxic properties, many aerobic bacteria such as *Pseudomonas aeruginosa* are capable of using phenolic compounds under aerobic conditions as the carbon and energy sources. A typical pathway for metabolizing phenol is to dihydroxylate the benzene ring to form a catechol derivative and then to open the ring through ortho- or meta-oxidation [13,14]. However, the use of free cells for biodegradation of phenol involves many serious problems such as substrate inhibition of microbial growth at higher concentrations of phenol and maintenance of correct cell concentration [9,15]. These constraints can be eliminated by immobilizing microorganisms in a porous solid matrix. The polymer gels are able to protect the microorganisms from xenobiotic toxicity and help in increasing the biodegradation rate through a higher cell concentration. In addition, the bioprocess can be controlled more easily, and the continuous process can take place at a high dilution rate without washout [15–17]. The entrapment of cells in polymeric gel beads of calcium alginate has received much attention due to their low toxicity to cells, usability, and low cost [9,12]. Recently, the combination of physicochemical and biological processes is increasingly used. The most reported combined treatments are: photocatalytic–biological, electrochemical–biological, ozonization–biological, and UV–biological treatment [18].

In this study, the combined adsorption–biodegradation treatment of phenol has been proposed by immobilization of *P. aeruginosa* in calcium alginate beads supplemented with powdered activated carbon (PAC). This combination allows a more rapid removal of the substrate [10,19]. The factorial design (2^3) allows estimating the main effects of parameters and their interactions of the response model through a relatively smaller number of experiments [20].

2. Material and methods

2.1. Bacterial strain and culture medium

A pure strain of *P. aeruginosa* ATCC27853, provided from Pasteur Institute of Algiers, was maintained on nutrient agar at 4°C. Mineral salt medium (MSM), which contained the following components (g/L): KH_2PO_4 (1.5), K_2HPO_4 (0.5), NaCl (0.5), $MgSO_4{\cdot}7H_2O$ (0.5), NH_4NO_3(3.0), $FeSO_4{\cdot}7H_2O$ (0.02), and $CaCl_2{\cdot}2H_2O$ (0.02) [21], was used for bacterial culture. Media and apparatus were autoclaved at 121°C for 20 min. Phenol was filtered through a 0.45 μm membrane filter and added to the sterilized MSM as the sole carbon source. The pH for all solutions was adjusted to 7.0 with 0.1 M NaOH.

2.2. Inoculum development

Pure *P. aeruginosa* culture was cultivated into 100 mL of MSM at 37°C. Bacterial cells were then harvested at the stationary phase by centrifugation (6,000 rpm at 4°C for 10 min), washed three times, and suspended in sterile MSM.

2.3. Immobilization of bacterial cells and PAC in Ca-alginate beads

A known amount of PAC was first added to a 3% (w/v) sodium alginate solution and then the mixture was homogenized and autoclaved for 15 min at 120°C. After that, the bacterial cells suspension was added to the previous mixture with a ratio of 1/10 (v/v). These alginate-PAC-cells suspensions were extruded as drops into a 3% (w/v) $CaCl_2$ solution [12] using a burette connected to different tips in order to produce beads with different diameters. The composite beads, in the salt solution, were incubated for 1 h at 37°C for complete replacement of sodium ions by calcium ions. Finally, the beads were washed three times with sterile distilled water to eliminate $CaCl_2$ residues and stored at 4°C. The detail characteristics of powdered activated carbon F400 used in this study are mentioned in Table 1.

Table 1
Characteristics of powdered activated carbon

Characteristic	Value
Origin	Bituminous oil
Iodine number (mg/g)	1,050
Specific surface area (m^2/g)	1,050–1,200
Function of acid surface (mEq/g)	0.23

2.4. Phenol determination

The residual phenol concentration in the medium was determined by a spectrophotometric method based on the condensation of 4-aminoantipyrine with phenol in the presence of an oxidizing agent, potassium ferricyanide, to form a colored antipyrine dye under alkaline conditions [12]. The absorbance was read at a 510 nm wavelength using Shimadzu UV–VIS 1240 spectrophotometer.

2.5. Degradation experiments

All the degradation experiments were carried out in batch mode in 1,000 mL conical flasks containing 500 mL of MSM supplemented with phenol at different initial concentrations. Fifty milliliters of alginate beads containing immobilized PAC and *P. aeruginosa* was suspended in the reactor medium. Experiments were carried out at 37°C on a rotary shaker at 150 rpm under aseptic and aerobic conditions. Samples were taken at preset time intervals until complete degradation of phenol was achieved.

2.6. Full factorial design experiments

The principle steps of statistically designed experiments are: determination of the response which reflects the aim of the study; factors and their levels; and choice of the experimental design and statistical analysis of the data [22,23]. A full 2^3 factorial design was performed to evaluate the importance of three factors: the initial phenol concentration (X_1), the amount of PAC (X_2), and the diameter of composite beads (X_3) on the response (Y), which is the time, expressed in hours, required for total phenol removal. The number of experiments conducted is considered as 2^3. The two levels which correspond to each variable are taken in coded form as +1 and −1. The low and high levels for the factors were selected according to some preliminary experiments which showed that an increase in concentration of activated carbon increases the efficiency on elimination of the phenol, taking into account the economic aspect. On the other hand, it is interesting to treat high concentrated phenolic solutions. The results showed that the immobilization of bacteria can treat solutions in which the concentration of phenol is greater than the inhibitory concentration (500 mg/L) observed when freely suspended bacteria are used.

Finally, the bead size factor is considered to control if an increase of the diameter limits or not the diffusion of the phenol. Table 2 summarizes these factors and their respective levels.

The statistical designs (*t*-tests and analysis of variance (ANOVA)) were performed using the software JMP release 7.0.

3. Results and discussion

3.1. Design of experiments by FFD

The design matrix of coded values for factors and the response (Y) measured in each factorial experiment is shown in Table 3. The order, in which the experiments were made, was randomized to avoid systematic errors [24]. The results were analyzed using JMP release 7.0, and the main effects and interaction between factors were determined. The effect of a factor is defined as the change in response produced by a change in the level of a factor from a lower to higher level. This is frequently called a main effect as it refers to the major factors of interest in the experiment [25]. The coded mathematical model utilized for 2^3 factorial designs can be given as:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{23}X_2X_3 + a_{13}X_1X_3 + a_{123}X_1X_2X_3 \quad (1)$$

Table 2
Factors and levels used in the 2^3 factorial design study

Factors	Levels	
	−1	+1
X_1: Initial phenol concentration (C), (mg/L)	100	500
X_2: Amount of PAC (m), (g)	1	5
X_3: Diameter of composite beads (d), (mm)	3	5

Table 3
Design matrix and the results of the 2^3 full factorial design (FFD)

Experiment	X_1	X_2	X_3	Y (h)
1	−1	−1	−1	47
2	+1	−1	−1	72
3	−1	+1	−1	23
4	+1	+1	−1	46
5	−1	−1	+1	48
6	+1	−1	+1	76
7	−1	+1	+1	22
8	+1	+1	+1	46
9	0	0	0	51
10	0	0	0	50

Table 4
Estimated regression coefficients for the time of phenol elimination

Term	Estimate	Std. error	*t*-value	*p*-value
Intercept	48.1	0.863134	55.73	0.0003
a_1	12.5	0.965013	12.95	0.0059
a_2	−13.25	0.965013	−13.73	0.0053
a_3	0.5	0.965013	0.52	0.6560
a_{12}	−0.75	0.965013	−0.78	0.5184
a_{13}	0.5	0.965013	0.52	0.6560
a_{23}	−0.75	0.965013	−0.78	0.5184
a_{123}	−0.25	0.965013	−0.26	0.8198

where Y is the time of total phenol elimination; a_0 is the global mean; a_i, a_{ij}, and a_{123} represent the regression coefficients relating to the main factor effects, 2-way, and 3-way interactions respectively; and X_1, X_2, and X_3 stand for initial phenol concentration, amount of adsorbent, and diameter of the composite beads, respectively. The regression coefficients, standard errors, *t*-values, and *p*-values appear in Table 4.

By substituting the coefficients a_i, a_{ij}, and a_{123} in Eq. (1) with their values from Table 4, the time of phenol elimination (Y) can be expressed as:

$$Y = 48.1 + 12.5X_1 - 13.25X_2 + 0.5X_3 - 0.75X_1X_2 + 0.5X_1X_3 - 0.75X_2X_3 - 0.25X_1X_2X_3 \quad (2)$$

Fig. 1 shows the actual (observed) vs. predicted plot for the model. The predicted model presents an adjusted square correlation coefficient R^2 (adj) of 97.5%, fitting the statistical model quite well.

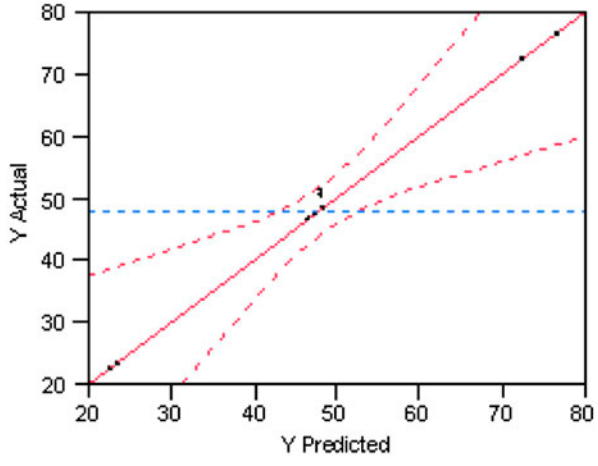

Fig. 1. Observed versus predicted response plot.

3.2. Student's t-test

The significance of the regression coefficients was determined by applying a Student's *t*-test. With a 95% confidence level and two degrees of freedom, the value of *t*-critic is equal to 2.92. The coefficient of the regression is statistically significant if the corresponding *t*-value is higher than 2.92. The results indicated in Table 4 revealed that only the factors X_1 and X_2 were significant with 95% confidence level. X_3, the 2-way interaction (X_1X_2, X_2X_3, and X_1X_3), and the 3-way interaction ($X_1X_2X_3$) had no effect at the 95% confidence level. The importance of the data can also be judged by their *p*-value, which is the probability value that is used to determine the statistically significant effects in the model, with values closer to zero denoting greater significance. For a 95% confidence level, the *p*-value should be less than or equal to 0.05 for the effect to be considered statistically significant [25]. The final empirical model for the time of total phenol elimination therefore becomes:

$$Y = 48.1 + 12.5X_1 - 13.25X_2 \quad (3)$$

3.3. Main and interaction effects

Analyzing the coefficients of Eq. (3), it can be inferred that the amount of PAC (X_2) was an important factor which influences the phenol elimination by the composite beads since its coefficient was the higher in modulus (i.e. 13.25). The negative sign of this coefficient means that phenol elimination was better at high adsorbent values. Increasing the dose of PAC from 1 to 5 g/L decreased considerably the time of phenol elimination. This was due to the greater availability of the exchangeable sites or surface area at higher amounts of the sorbent [25]. The initial phenol concentration (X_1) also had a considerable effect on the time of phenol elimination. The positive value of its coefficient (+12.5) reveals that Y increases as this factor changes from low to high levels. An increase in the initial phenol concentration from 100 to 500 mg/L resulted in an increase in time of phenol elimination. According to *t*-value and *p*-value, X_3, the 2-way interaction (X_1X_2, X_2X_3, and X_1X_3), and the 3-way interaction ($X_1X_2X_3$) had no effect at the 95% confidence level.

The interaction effects plots are shown in Fig. 2. The parallel lines in this figure are indications of the absence of interaction between factors. In addition, the two lines corresponding to the factor X_3 (diameter of the composite beads) are superimposed, indicating that the effect of this factor is not significant at the considered level. Dursun et al. [16] have reported that

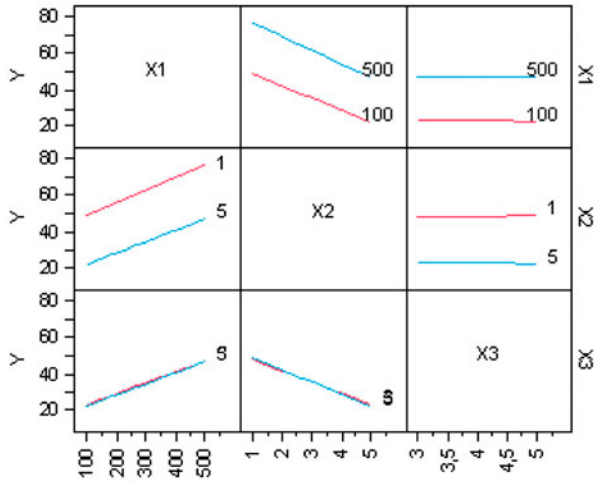

Fig. 2. Interaction effects plot for time of phenol elimination.

an increase in particle size caused an increase in diffusion limitations, although the phenol removal rates observed for the particles of diameter 3 and 4 mm were almost even.

3.4. Analysis of variance

In order to ensure that the model given in Eq. (3) is an appropriate model, the test for the significance of regression was performed by analysis of variance (ANOVA). Table 5 shows the sum of squares, mean square, *p*-value, and *F*-value. For a 95% confidence level, 2 and 7 degrees of freedom, $F_{0.05,2,7}$ is equal to 4.737, and the *F*-value obtained (51.16) is higher than $F_{0.05,2,7}$. Moreover, the *p*-value estimated (0.0193) is less than 5%. These results indicate a good adherence of the model to the experimental results at 95% confidence level.

Table 5
Analysis of variance

Source	DF	Sum of square	Mean square	*F*-value	*p*-value
Model	7	2,668.0	381.143	51.1601	0.0193
Error	2	14.9	7.450		
C. Total	9	2,682.9			

3.5. Optimization

The desirability function approach is the most current and strongly suggested method for the optimization of one or more responses [26]. Where there are multiple responses, each function (y_i) provides an individual desirability value (d_i), and the global desirability (D) is the geometric mean of these values [27] ($D = d$ when only one response is considered). The desirability function transforms the values of a response into [0,1], where 0 stands for a non-acceptable value of the response and 1 for values where higher/lower (depending on the direction of the optimization) values of the response have little merit. When a response is to be minimized, the desirability of a predicted response equal to y is defined as [28]:

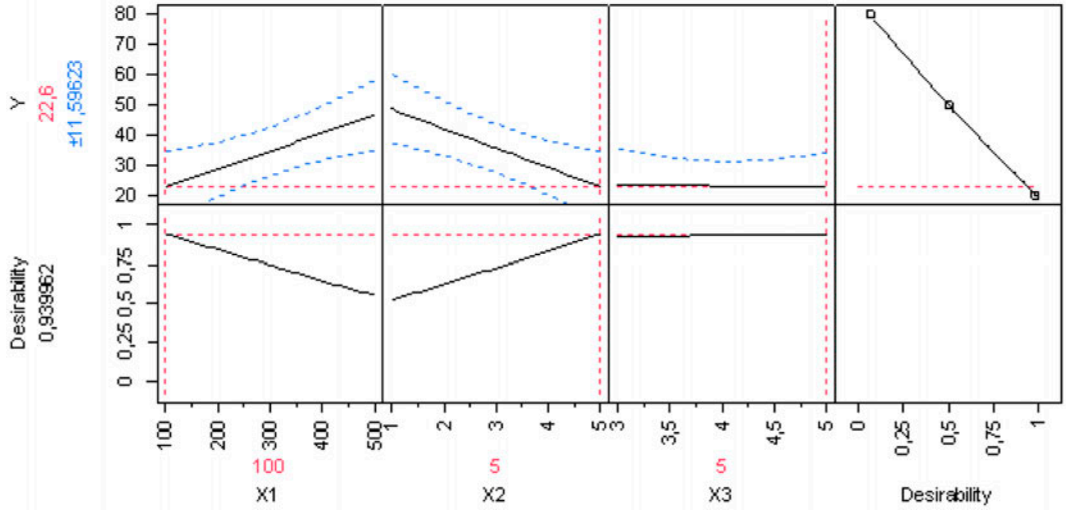

Fig. 3. Desirability functions for the optimization of the response.

$$D = \begin{cases} 1, & y < y_{\min} \\ \left|\frac{y - y_{\max}}{y_{\min} - y_{\max}}\right|, & y_{i\ \min} \le y_i \le y_{i\ \max} \\ 0, & y > y_{\max} \end{cases} \quad (4)$$

where $y_{\min}$ and $y_{\max}$ are respectively, the minimum and maximum values of the response.

As shown in Fig. 3, the desirability function decreases linearly with the increase of the phenol elimination time. The maximum desirability which corresponds to the best results is about 0.939 and was achieved at the higher level of the amount of PAC (5 g) and the lower level of initial phenol concentration (100 mg/L).

4. Conclusion

In order to determine the effect of various operating conditions (initial phenol concentration, PAC amount, and diameter of composite beads) and their possible interactions on the time of phenol elimination, a full 2^3 factorial design was performed. The statistical analysis such as *t*-test and ANOVA showed that only the initial phenol concentration and the amount of PAC were statistically significant. The adsorbent amount was found to have the most significant impact on the time of phenol elimination followed by the initial phenol concentration, while the effect of beads diameter, the 2-way and 3-way interactions were not significant. The minimum time of phenol elimination was 22.6 h, and this optimum was achieved at 5 g/L of PAC and an initial phenol concentration of 100 mg/L.

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