

Response Surface Methodology for Optimization of Operational Parameters To Remove Ciprofloxacin from Contaminated Water in the Presence of a Bacterial Consortium

Syed Wajid Ali Shah, Mujaddad ur Rehman, Muhammad Arslan, Saddam Akber Abbasi, Azam Hayat, Samina Anwar, Samina Iqbal, and Muhammad Afzal*



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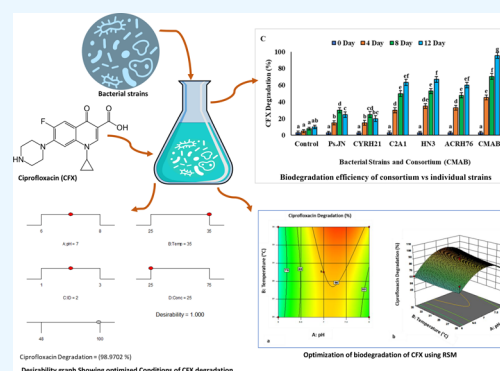
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ABSTRACT: Ciprofloxacin (CFX) is a broad-spectrum fluoroquinolone antibiotic that is widely used to treat bacterial infections in humans and other animals. However, its unwanted occurrence in any (eco)system can affect nontarget bacterial communities, which may also impair the performance of the natural or artificially established bioremediation system. The problem could be minimized by optimization of operational parameters via modeling of multifactorial tests. To this end, we used a Box–Behnken design in response surface methodology (RSM) to generate the experimental layout for testing the effect of the CFX biodegradation for four important parameters, that is, temperature ($^{\circ}\text{C}$), pH, inoculum size (v/v %), and CFX concentration (mg L^{-1}). For inoculation, a consortium of three bacterial strains, namely, *Acanitobacter lwoffi* ACRH76, *Bacillus pumilus* C2A1, and *Mesorihizobium sp.* HN3 was used to degrade 26 mg L^{-1} of CFX. We found maximum degradation of CFX (98.97%; initial concentration of 25 mg L^{-1}) at 2% inoculum size, 7 pH, and $35 \text{ }^{\circ}\text{C}$ of temperature in 16 days. However, minimum degradation of CFX (48%; initial concentration of 50 mg L^{-1}) was found at pH 6, temperature $30 \text{ }^{\circ}\text{C}$, and inoculum size 1%. Among different tested parameters, pH appears to be the main limiting factor for CFX degradation. Independent factors attributed 89.37% of variation toward CFX degradation as revealed by the value of the determination coefficient, that is, $R^2 = 0.8937$. These results were used to formulate a mathematical model in which the computational data strongly correlated with the experimental results. This study showcases the importance of parameter optimization via RSM for any bioremediation studies particularly for antibiotics in an economical, harmless, and eco-friendly manner.



INTRODUCTION

Antibiotics are administered to prevent (prophylaxis) or treat infections. Bactericidal antibiotics kill bacteria, whereas bacteriostatic antibiotics inhibit the growth and metabolism.¹ Antibiotics are generally categorized into six groups, namely, fluoroquinolones (FQs), macrolides, tetracyclines, aminoglycosides, cephalosporins, and penicillins.² FQs are a broad-spectrum class of bactericidal antibiotics, which are used to prevent or treat infections without affecting the host cells.³ As per the mode of action, FQs inhibit the synthesis of essential enzymes involved in DNA replication.⁴ FQs can only be partially metabolized within human and animal bodies and are frequently found in urban discharges and at wastewater treatment plants.⁵ FQs are recognized among other emerging environmental contaminants with great public health concern because of the ecotoxicological effects and potential to increase microbial resistance.⁶ Among several FQs, ciprofloxacin (CFX) is the most often used fluoroquinolone antibiotic.^{4,5} CFX has been found in agricultural soils ($119.8 \mu\text{g kg}^{-1}$),⁹ freshwater

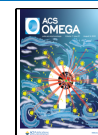
(6.5 mg L^{-1}),⁷ manure (45.59 mg kg^{-1}),⁸ and urban sewage sludge (426 mg kg^{-1}).⁹ According to Mathew and Unnikrishnan, CFX concentrations in effluents of wastewater treatment plants of pharmaceutical companies in India have reached up to 31 mg L^{-1} .¹⁰

The presence of antibiotics and/or their residues in the environment is of concern due to nontarget toxicity. Precisely, it can alter the functioning of basic nutrient cycles (e.g., carbon, nitrogen, and oxygen) after disturbing the microbial community structures in the particular (micro)ecosystem.¹ Hence, a variety of methods have been used to remove CFX from water including advanced oxidation processes,¹¹ sorption by specific materials,¹² and photodegradation.¹³ This includes treatment with ultrasonic/persulfate (US/PS), ultrasonic/hydrogen peroxide (US/ H_2O_2), US/ $\text{H}_2\text{O}_2/\text{Fe}^{2+}$, US/PS/

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Fe²⁺, and the US/PS/H₂O₂/Fe²⁺.¹⁴ Similarly, synthesized or natural absorbents such as magnetic copper ferrite/montmorillonite (CuFe₂O₄/MMT) nanocomposites,¹⁵ activated carbon magnetized with Fe₃O₄ nanoparticles,¹⁶ and activated carbon have been used for the removal of CFX from the water.¹⁷ Nevertheless, biodegradation of CFX appears to be an effective, sustainable, and environmentally friendly strategy.¹⁸ As antibiotics are designed to kill/inhibit the growth of microorganisms, their presence above minimum inhibitory concentrations could directly impair the microorganisms performing bioremediation.¹ However, the negative impact of antibiotics may be alleviated, and biodegradation potential may be enhanced by optimizing different biotic and abiotic (operational) parameters.¹⁹ The empirical identification of the optimized parameters however is crucial as it requires testing several combinations of variables individually. Such a multifactorial experiment is often not feasible.

The response surface methodology (RSM) is a multivariate statistical tool used for modeling and analyzing the interactive effects of various variables to build a mathematical model that can represent the entire process under study.²⁰ This method can optimize the response and has been successfully applied for the optimization of several other xenobiotic degradations. Among different RSM experimental designs, the Box–Behnken design (BBD) is advantageous because it considers a condition in which all parameters are at their boundary value at the same time.¹⁹ Previously, remediation potential for several other contaminants has been optimized with RSM; however, optimization of parameters for the biodegradation of CFX has never been carried out. The objective of this study was to determine the optimal parameters for the maximum biodegradation of CFX in a typical bioremediation experiment. To this end, an experimental layout was generated for multifactorial tests of CFX degradation following bioremediation assays, modeling of experimental data with RSM, and finally validating the modeled prediction for enhanced degradation. A bacterial consortium having CFX degradation potential was initially grown under the conditions directed by BBD for the maximum degradation of CFX.

MATERIALS AND METHODS

Chemicals and Media. CFX tablets (250 mg) were purchased from a local pharmacy (Sami Pharmaceuticals,

Table 1. Bacterial Strains Used to Study CFX Degradation in Liquid Minimal Salt Media

IGS type	bacterial strain	reference
PsJN	<i>Burkholderia phytofirmans</i>	24
CYRH21	<i>Acenitobacter sp.</i>	29
ACRH76	<i>Acenitobacter lwoffii</i>	29
C2A1	<i>Bacillus pumilus</i>	30
HN3	<i>Mesorhizobium sp.</i>	31

Private Limited, Karachi, Pakistan). The HPLC-grade chemicals, acetonitrile (ACN), and methanol were supplied by Sigma-Aldrich (Germany). The degradation of CFX was investigated using three different types of media [Luria Bertani (LB) medium, minimal salt medium (MSM), and Mueller–Hinton medium]. All the chemicals and media were obtained from Merck, Germany, and Sigma-Aldrich, USA.

Bacterial Strains. Five bacterial strains, *Burkholderia phytofirmans* PsJN,²⁴ *Acenitobacter sp.* CYRH21,²⁵ *Acenitobacter*

Table 2. Experimental Factors and Their Levels Used in RSM for Optimization of CFX Degradation by the Bacterial Consortium

factor	coded level of variables		
	low (−1)	center (0)	high (+1)
pH	6	7	8
temperature (°C)	25	30	35
inoculum size (v/v%)	1	2	3
concentration (mg L ^{−1})	25	50	75

Table 3. Box–Behnken Experimental Design with Coded Values of Independent Variables and the Response of Dependent Variable CFX Degradation

run	coded level of variables				response degradation (%)
	pH	temp	IS	conc	
1	6	30	2	75	52
2	8	35	2	50	80
3	6	30	1	50	48
4	7	35	1	50	76
5	7	30	2	50	91
6	7	30	2	50	90
7	7	30	2	50	92
8	8	30	3	50	77
9	7	30	2	50	88
10	7	30	2	50	88
11	7	25	1	50	65
12	8	30	1	50	58
13	6	35	2	50	64
14	6	30	2	25	66
15	7	35	3	50	99
16	7	30	1	25	63
17	7	35	2	75	94
18	8	25	2	50	76
19	6	30	3	50	60
20	7	35	2	25	98
21	7	25	3	50	87
22	7	25	2	75	86
23	6	25	2	50	74
24	8	30	2	25	97
25	7	30	1	75	73
26	7	25	2	25	88
27	7	30	3	75	96
28	8	30	2	75	75
29	7	35	2	25	98

lwoffii ACRH76,²¹ *Bacillus pumilus* C2A1,²² and *Mesorhizobium sp.* HN3,²³ were used in this study (Table 1). Each bacterium was grown in LB broth for 24 h at 37 °C and 120 rpm. The bacterium culture was centrifuged at 5000 g for 10 min. As described earlier, the pellets were washed and suspended in sterile saline (0.90% NaCl) to obtain their required numbers.²⁴

Screening of Potent Bacterial Strains for CFX Degradation. The CFX-degradation potential of the bacterial strains was determined using the method described earlier.²⁵ Briefly, liquid MSM having CFX (5 and 10 mg L^{−1}) was used as the sole source of carbon and energy. A 10 mL suspension (10⁹ cells mL) of each bacterial strain was inoculated in 200 mL MSM and was kept for 12 days in a shaker at 35 °C and 150 rpm. Samples were taken every 4 days of incubation. Using high-performance liquid chromatography (HPLC), the remaining amount of CFX in the water was determined. The

optical density of the collected samples had been recorded at 600 nm by using a spectrophotometer to evaluate bacterial growth. Furthermore, the survival of all of the bacterial strains in the media was confirmed by spreading the bacterial suspension on LB agar plates.

Development of a CFX-Degrading Bacterial Consortium. Three strains (*Acentobacter lwofii* ACRH76, *Bacillus pumilus* C2A1, and *Mesorhizobium* sp. HN3) exhibiting maximum CFX degradation potential were chosen for the development of a bacterial consortium. Their compatibility was determined, and these strains were mixed in a proportion of 1:1:1 to form a bacterial consortium.²⁴

Optimization of Conditions for Maximum CFX Degradation. Optimization of conditions for the maximum biodegradation of CFX was performed using RSM. Based on one factor at a time, pH, temperature (°C), inoculum size (%), and CFX concentration (mg L⁻¹) were chosen as the four independent variables. The biodegradation of CFX was observed in 100 mL liquid MSM on a shaking incubator at 120 rpm for 16 days. With the help of design expert software (trial version 10, Stat-Ease, Inc., MN, USA), a three-factor/five-level dominant BBD with 2³ full factorials consisting of 29 experimental runs was used. BBD is a type of second-order design which is based on three-level incomplete factorials.²⁶ The application resulted in a total of 15 coefficients by fewer runs while compared to other response surface design methods. To estimate the tuning parameters for a quadratic response surface model with *N* variables, the study must be performed at three levels.²⁶ To fit a second-order response surface equation and analyze the response, in this study, a 29-run BBD was used with four factors at three levels, as well as three replicates at the central point. These were included as a measure of data consistency and reproducibility and have proven to be useful. The *F*-test significance (0.05) and lack of it (insignificant) were used to determine whether a fit model is adequate.²⁷ As a result, the model's high accuracy, consistency, and reproducibility were reflected in its lack of it. Therefore, this model can also be used to optimize the conditions for CFX biodegradation within the limits of tested parameters. Each independent variable, that is, low, middle, and high, was assigned the values of -1, 0, and +1, respectively (Table 2).

The actual experimental setup is presented in Table 3. Here, CFX degradation was a dependent variable. The mathematical relationship between the responses of the four variables was assessed following the quadratic polynomial equation (eq 1).

$$Y = a_0 + a_1A + a_2B + a_3C + a_4D + a_{12}AB + a_{13}AC + a_{14}AD + a_{23}BC + a_{24}BD + a_{34}CD + a_{11}A^2 + a_{22}B^2 + a_{33}C^2 + a_{44}D^2 \quad (1)$$

The letter *Y* is a response value; whereas the fitting response is represented by *a*₀ at the central design point, linear coefficients are represented by *a*₁, *a*₂, *a*₃, and *a*₄; cross-product coefficients are denoted by *a*₁₂, *a*₁₃, *a*₁₄, *a*₂₃, *a*₂₄, and *a*₃₄; and *a*₁₁, *a*₂₂, *a*₃₃, and *a*₄₄ represent the quadratic coefficients. The best results of a single (one-at-a-time) method were used to determine the runs and coded levels of these four variables. The coded values were used for all variables. The degradation coefficients were calculated using regression analysis. The *F*-tests and *P*-values were used to investigate the impact of various factors on degradation. The significant factors were those with a *P*-value of less than 0.05. A typical experimental

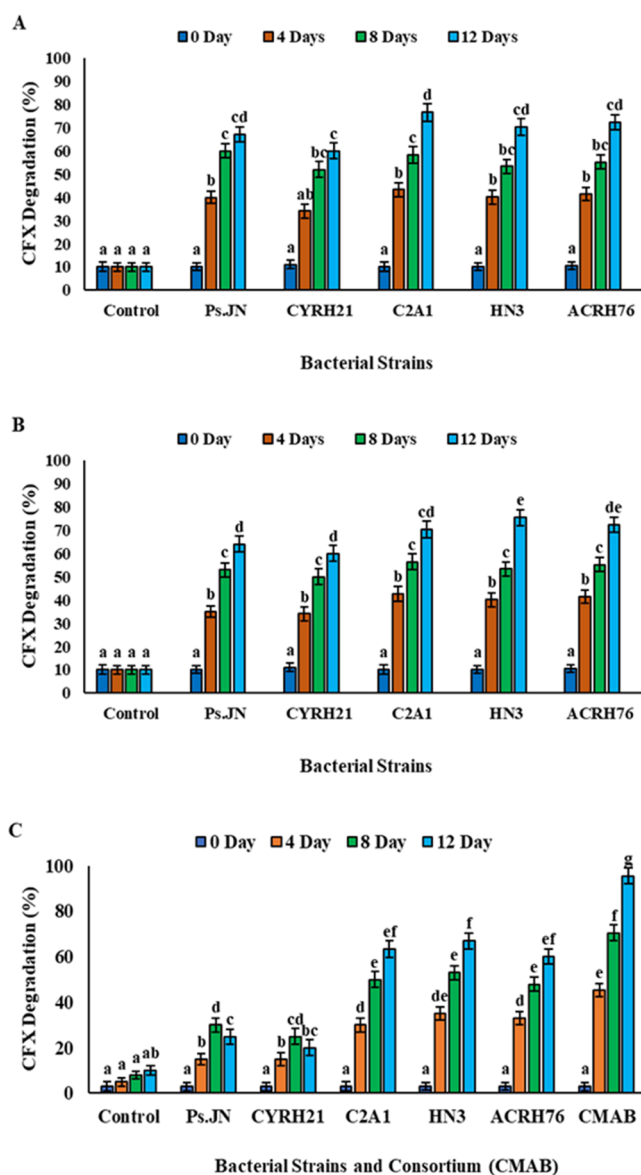


Figure 1. Degradation (%) of CFX by bacterial strains and their consortium in MSM having 5 mg L⁻¹ (A), 10 mg L⁻¹ (B), and 20 mg L⁻¹ (C) CFX after 4, 8, and 12 days of incubation. The bacterial strains, *Burkholderia phytofirmans* PsJN, *Acentobacter* sp. CYRH21, *Acentobacter lwofii* ACRH76, *Bacillus pumilus* C2A1, and *Mesorhizobium* sp. HN3, were used individually and in the consortium. Means followed by the same letters are not significantly different (*P* < 0.05), and the error bars represent the standard deviation.

design of these four variables was developed using RSM for CFX degradation to confirm the model predictions.

Analysis of Residual CFX. The remaining quantity of CFX in MSM was estimated according to a method described earlier.³⁰ Briefly, ACN was used to extract CFX from the aqueous solution. The ACN extract was analyzed by using PerkinElmer HPLC (Germany). A binary elution system consisting of water and acetonitrile, acidified with 2% phosphoric acid (H₃PO₄), had been used as the mobile phase on a reverse-phase ODS2 C18 column (Massachusetts, USA) with an isocratic flow rate of 0.8 mL min⁻¹. A diode array detector with 275 nm was used for detection, and a sample volume of 15 μL was added. The temperature of the column was set to 30 °C.

Table 4. ANOVA for the CFX Degradation Response (%)^a

source	sum of		mean	F	p-value	
	squares	df	square	value	prob > F	
model	5598.84	14	399.92	8.41	0.0001	significant
A-pH	816.75	1	816.75	17.18	0.0010	
B-Temp	102.08	1	102.08	2.15	0.1650	
C-ID	1541.33	1	1541.33	32.41	<0.0001	
D-Conc	108.00	1	108.00	2.27	0.1540	
AB	49.00	1	49.00	1.03	0.3273	
AC	12.25	1	12.25	0.26	0.6197	
AD	16.00	1	16.00	0.34	0.5711	
BC	0.25	1	0.25	5.257E-003	0.9432	
BD	1.00	1	1.00	0.021	0.8868	
CD	49.00	1	49.00	1.03	0.3273	
A ²	2266.24	1	2266.24	47.66	<0.0001	
B ²	11.10	1	11.10	0.23	0.6364	
C ²	563.03	1	563.03	11.84	0.0040	
D ²	13.33	1	13.33	0.28	0.6048	
residual	665.72	14	47.55			
lack of fit	652.92	10	65.29	20.40	0.0053	significant
pure error	12.80	4	3.20			
Cor Total	6264.55	28				

^aR² = 0.8937; Adjusted R² = 0.7875; Predicted R² = 0.3965; Adequate precision = 11.250. Significant at P < 0.05. Nonsignificant at P > 0.05.

Table 5. Regression Analysis and Model Coefficients for CFX Degradation (%) Response

source	coefficient	standard error coefficient	P-value
constant	89.80	0.18	<0.0001 ^a
A	8.25	0.11	0.0010
B	2.92	0.11	0.1650
C	11.33	0.11	<0.0001
D	-3.00	0.11	0.1540 ^b
AB	3.50	0.20	0.3273
AC	1.75	0.20	0.6197
AD	-2.00	0.20	0.5711
BC	0.25	0.20	0.9432
BD	-0.50	0.20	0.8868
CD	-3.50	0.20	0.3273
A ²	-18.69	0.15	<0.0001
B ²	1.31	0.15	0.6364
C ²	-9.32	0.15	0.0040
D ²	1.43	0.15	0.6048

^aSignificant at P < 0.05. ^bNonsignificant at P > 0.05.

Data Analysis. The quadratic models were fitted using RSM which described the mathematical relationship between each term in the model and response. Here, analysis of variance (ANOVA) was used to split the total variation into different model components, whereas, to check the significance of each component, the F-test was used.²⁸ Accordingly, for multiple comparisons of different treatments, the SPSS software package was used to analyze the data. ANOVA was used to evaluate the treatments followed by a post hoc Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Screening of CFX-Resistant Bacterial Strains. In this study, all the bacterial strains were able to degrade CFX (Figure 1A). Initially, at a low CFX concentration (5 mg L⁻¹), the strain *B. pumilus* (C2A1) displayed higher degradation (76.53%) than the other four strains (Figure 1A). However, at

a high CFX concentration (10 mg L⁻¹), the strain *Mesorhizobium* sp. (HN3) exhibited better degradation (75.32%) efficacy (Figure 1B). There was only 10% removal of CFX in the flasks without bacterial inoculation. This may be due to the natural attenuation (degradation) of CFX over time.²⁶ The degradation efficiency slightly differed among the bacterial strains at both concentrations (5 and 10 mg L⁻¹). This is likely because some bacteria degrade antibiotics more efficiently than others.²⁹ The bacterial consortium of three strains showed better CFX degradation (95.45%) in our investigation (Figure 1C), suggesting that the combined use of bacteria is more efficient for CFX degradation. Many previous investigations have previously documented that microbial cooperation increases organic pollution removal from water.³⁰ According to Liao et al.,³¹ a mixed bacterial culture exhibited higher CFX removal from the wastewater compared to the individual strains. Similarly, other studies also reported that the bacterial consortium degrades CFX more efficiently than the single strains.³² The biodegradation of CFX likely started from the cleavage of isoxazole and piperazinyl rings catalyzed by sulfite reductase and cytochrome P450 (CYP450) enzymes, respectively.³³ Analyses of degradation intermediates by HPLC and liquid chromatography/mass spectrometry suggested that 100% of CFX could be removed from water due to complete microbial degradation.³⁴

Optimization of Parameters for CFX-Biodegradation.

RSM was used to investigate the interaction and concurrent effects of four variables, namely, pH, temperature, inoculum size, and CFX concentration on the biodegradation of CFX. A BBD was used to generate an experimental design matrix which consisted of eight full factorial points, six central points, and six axial points being positioned at the center and extreme levels, resulting in 29 experimental runs/setups (Table 3).

In this study, maximum degradation (99.97%) of CFX (25 mg L⁻¹) was observed at pH 7, temperature 30 °C, and inoculum size 3%. This was followed by 97% degradation of CFX (25 mg L⁻¹) at pH 8, temperature 30 °C, and inoculum size 2% (run #24). However, minimum degradation (48%) of

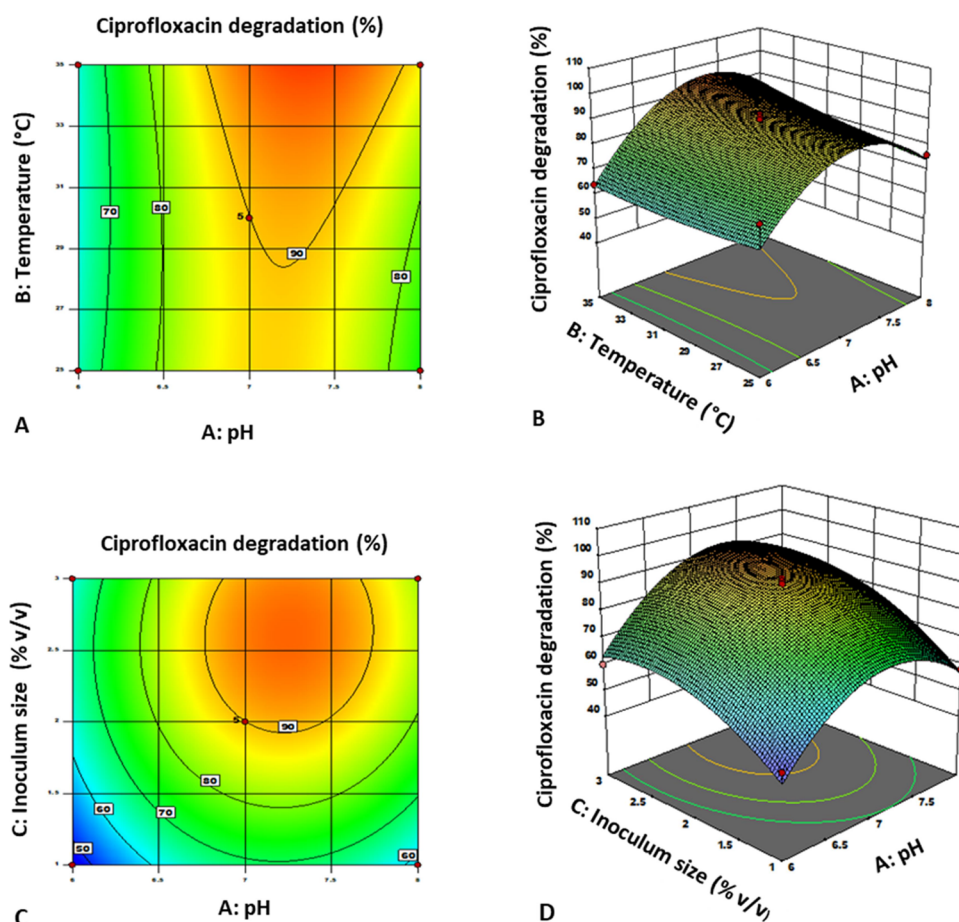


Figure 2. Contour plot (A) and 3D response surface plot (B) showing the effect of mutual interaction of temperature and pH on CFX degradation (%) at inoculum size (2% v/v) and 25 mg L⁻¹ concentration of CFX. Contour plot (C) and 3D response surface plot (D) showing the effect of mutual interaction of the inoculum size and pH on CFX degradation (%) at constant temperature (30 °C) and 50 mg L⁻¹ concentration of CFX.

CFX (50 mg L⁻¹) was found at pH 6, temperature 30 °C, and inoculum size 1% (run #3) followed by 52% degradation of CFX (75 mg L⁻¹) at pH 6, temperature 30 °C, and inoculum size 2% (run #1). These results indicate that pH is a main limiting factor for CFX degradation as compared to the temperature, inoculum size, and concentration. Independent factors attributed 89.37% of variation toward CFX degradation as revealed by the value of the determination coefficient, that is, $R^2 = 0.8937$. This also confirmed the model's efficiency as shown in Table 4. A higher adjusted determination coefficient (adj. $R^2 = 0.7875$) confirmed the model's best fit. Accordingly, the quadratic model was also significant due to its high F -value (8.41) and low P -value (0.0001). The value for lack of fit is nonsignificant showing that the level of fit is satisfactory. Individual P -values revealed that all variables had a significant effect on CFX biodegradation; however, effects of pH and inoculum size were prominent. Previously, RSM was used to optimize operational parameters for the biodegradation of cephalixin and amoxicillin.³⁵ The maximum degradation was observed in the presence of 5.57 log₁₀ CFU mL⁻¹ of bacterial cells, incubation time of 10.38 days, 36.62 °C of temperature, and 4.14 mg L⁻¹ of cephalixin/amoxicillin (R^2 : 0.99). Likewise, another study used CCD and ridge-canonical analyses and reported that 7.973 g of ceftriaxone sodium was the threshold concentration to completely remove (100%) of the antibiotic after 39 h of incubation under aerobic static conditions at 30 °C.³⁶ The optimal operational parameters

were also determined during the bioremediation of crude oil-contaminated water using RSM.²⁸ With optimized parameters, there was a 95% attenuation of the hydrocarbon concentration, which was very close to the 98% attenuation predicted by the model.

Then, RSM was applied for the mathematical model building of the experimental data obtained with the bioremediation assays. Here, multiple linear regression analysis was performed on experimental data to test for linear (A, B, C, D), quadratic (A^2 , B^2 , C^2 , D^2), and interaction effects (AB, AC, AD, BC, BD, CD) of all variables (Table 5). The following polynomial equation fitted best to the degradation (%) of CFX.

$$Y = 89.80 + 8.25A + 2.92B + 11.33C - 3.00D + 3.50AB + 1.75AC - 2.00AD + 0.25BC - 0.50BD - 3.50CD - 18.69A^2 + 1.31B^2 - 9.32C^2 + 1.43D^2 \quad (2)$$

pH, temperature, inoculum size, and CFX concentration were four independent variables represented by A, B, C, and D, respectively. The synergistic and antagonistic impacts of each variable were represented by a positive (+) and a negative (-) value of the regression coefficient. The regression equation shows that A, B, C, AB, AC, BC, B², and D² had a synergistic effect, whereas D, AD, BD, CD, and C² displayed an antagonistic effect.

Model Analysis via 2D Contour Graphs and 3D Surface Plots for CFX Degradation. When a first-order

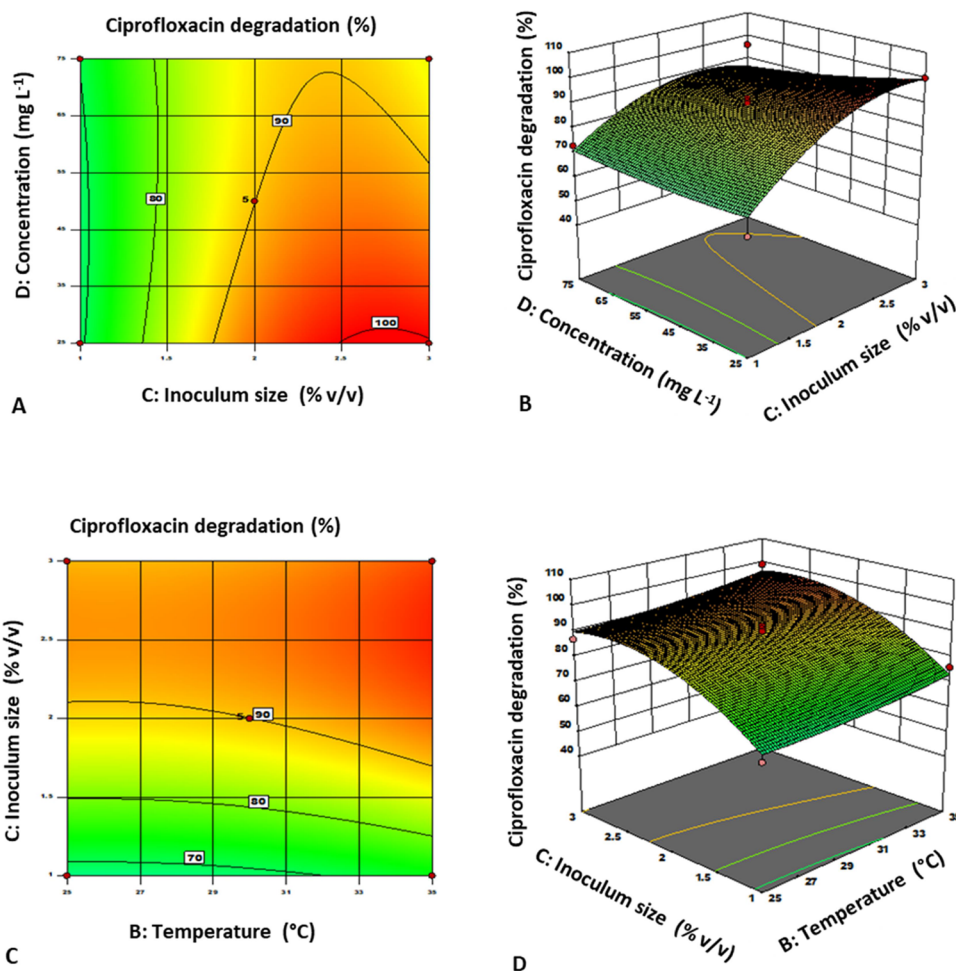
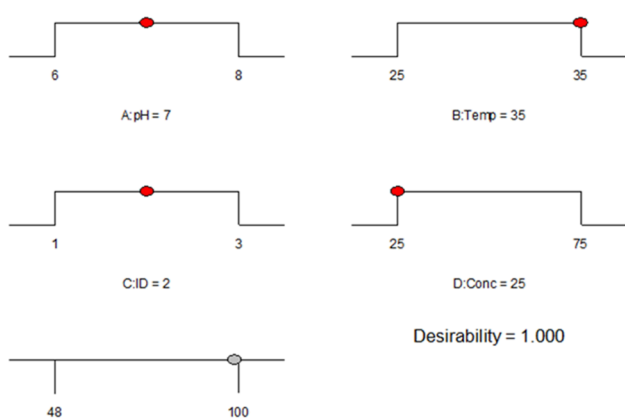


Figure 3. Contour plot (A) and 3D response surface plot (B) show the effects of mutual interaction of the concentration and inoculum size on CFX degradation while keeping pH and temperature constant. Contour plot (C) and 3D response surface plot (D) show the effects of mutual interaction of the inoculum size and temperature on CFX degradation (%) while keeping other two factors constant.



Ciprofloxacin Degradation = (98.9702 %)

Figure 4. Desirability graph of CFX degradation at 25 mg L⁻¹.

model cannot be applied due to the contact of parameters and surface curving, a second-order polynomial model can considerably improve the process of optimization.²⁶ In this study, a second-order model was used to study the relationships between experimental variables (A, B, C, and D) and the corresponding responses. The results were visualized by drawing two-dimensional contour plots and three-dimensional

response surface graphs. Here, two of the experimental variables were changed over the course of the experiment, while the third and fourth variables remained constant. The contour plot represented the relevance of the mutual effects of the response conditions (Figures 2 and 3). A circular contour plot showed no significant interaction between the variables, whereas an elliptical contour plot displayed strong mutual contact of the experimental variables.³⁷ The relationship of pH and temperature (Figure 2A,B), as well as an elliptical contour plot, suggested that these two parameters had a significant impact on CFX biodegradation. An increase in temperature and pH improved the CFX biodegradation up to a point, but a subsequent increase had a negative impact on CFX biodegradation. However, maximum biodegradation of CFX was observed at 35 °C. The relationship between the inoculum size and pH was significant (Figure 2C,D). CFX biodegradation was at its peak when the inoculum size (2%) and pH (7.0) were at their optimal levels. The relationship between the CFX concentration and inoculum size revealed that these two parameters apparently had no effect on CFX biodegradation (Figure 3A,B). The temperature and inoculum size, however, had a significant impact on CFX biodegradation (Figure 3C,D). Further, an increase in the inoculum size also increased biodegradation of CFX up to an optimum temperature.

The desirability ramp graph indicated that bacterial consortium can degrade CFX (25 mg L⁻¹) up to 98.97% under suitable conditions, that is, pH (7), inoculum size (2%), and temperature (35 °C) in 16 days of incubation (Figure 4). Previously, in a sequencing batch reactor, thermodynamics analysis revealed that CFX removal from wastewater was spontaneous (Gibbs free energy change (ΔG°) <0 kJ/mol), exothermic (enthalpy change (ΔH°) <0 kJ/mol), and the removal process involved both physisorption and chemisorption (absolute value of ΔH° = 20 to 80 kJ/mol).³³ This indicated that biodegradation of antibiotics depends on thermodynamics properties of the system, and the biodegradation rate could be enhanced with an increase in the temperature of the medium.³⁸

CONCLUSIONS

A consortium of three bacterial strains, *A. lowfi* ACRH76, *B. pumilus* C2A1, and *Mesorhizobium* sp. HN3, was found more efficient in degrading CFX than the individual strains. RSM was successfully applied to optimize the variables, namely, pH, inoculum size, and temperature for the attenuation of CFX. Maximum degradation (98.97%) of CFX was observed at pH (7), inoculum size (2%), temperature (35 °C), and low CFX concentration (25 mg L⁻¹). Further studies are needed at the pilot scale to explore the potential of the consortium under optimized conditions for the maximum remediation of CFX-contaminated water. This study shows that the use of RSM is promising to enhance the existing remediation of CFX, and likely other antibiotics, in a contaminated environment.

AUTHOR INFORMATION

Corresponding Author

Muhammad Afzal – Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad 38000, Pakistan; orcid.org/0000-0003-1511-816X; Phone: +92 41 9201316; Email: manibge@yahoo.com; Fax: +92 41 9201322

Authors

Syed Wajid Ali Shah – Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad 38000, Pakistan; Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad 22020, Pakistan
Mujaddad ur Rehman – Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad 22020, Pakistan
Muhammad Arslan – Department of Civil and Environmental Engineering, University of Alberta, Edmonton, Alberta T6G 2R3, Canada
Saddam Akber Abbasi – Department of Mathematics, Statistics and Physics, Qatar University, Doha 122104, Qatar
Azam Hayat – Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad 22020, Pakistan
Samina Anwar – Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad 38000, Pakistan
Samina Iqbal – Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic

Engineering (NIBGE), Faisalabad 38000, Pakistan; orcid.org/0000-0001-8412-6329

Complete contact information is available at: <https://pubs.acs.org/10.1021/acsomega.2c02448>

Notes

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