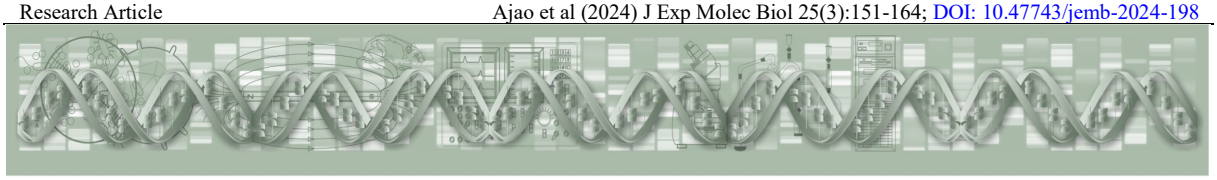

ENHANCING BIODEGRADATION EFFICIENCY OF REACTIVE BLACK-5 DYE USING *BACILLUS WIEDMANNII* STRAIN NAF4: OPTIMIZATION OF DEGRADATION PARAMETERS VIA RESPONSE SURFACE METHODOLOGY (RSM)

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Abstract

Reactive Black 5 (RB-5) dye is widely used in industries such as textile, paper, and leather, raising environmental concerns due to its persistence and adverse effects. This study aimed to develop efficient and eco-friendly strategies for RB-5 dye removal from industrial wastewater. RB-5 dye-degrading bacteria, namely NAF1, NAF2, NAF3, and NAF4, were isolated from soil contaminated with textile effluents. Evaluation of their decolorization potential revealed NAF4 as the most effective, achieving a decolorization percentage of 89%, followed by NAF3 and the co-culture at 75% and 73%, respectively. The isolate NAF4 showed significant production of tyrosinase and laccase enzymes. The 16SrRNA sequencing confirmed the identities of the isolates belonging to the following genera *Bacillus, Pseudomonas, Escherichia*, and *Citrobacter.* The biodegradation potential of B. wiedmannii strain NAF4 for RB-5 dye was assessed using Response Surface Methodology (RSM). The optimized conditions for RB-5 degradation were determined to be an agitation speed of 115.777 rpm, pH of 7.449, inoculum size of 12.255, and temperature of 29.74°C. The RSM model exhibited high statistical significance with an F-value of 53.30 and low p-values (<0.0001) as well as a correlation coefficient (R^2) value of 0.9813. Validation studies confirmed the adequacy and precision of the model. The maximum RB-5 degradation achieved was 90.2291%. This study provides insights into the potential applicability of RSM for optimizing degradation processes in various contexts and offers promising solutions for RB-5 dye removal from industrial wastewater, mitigating its environmental impact.
Keywords: *Bacillus wiedmannii*, Response Surface Methodology (RSM), Reactive Black 5, biodegradation, environmental impact, industrial wastewater

Introduction

The textile industry plays a significant role in the global economy, providing millions of jobs and meeting the growing demands for clothing and textiles (Dixit and Lal 2019). However, the production of textiles is associated with various environmental concerns, particularly the discharge of dye-containing wastewater (Al-Tohamy et al. 2022, Khan et al. 2023)
Reactive Black 5 (RB5) dye, a widely used textile dye, poses a significant threat to the environment due to its persistence, toxicity, and non-biodegradability (Elgarahy et al. 2021). The accumulation of RB5 dye in water bodies can result in severe ecological imbalances and harm aquatic life (Yusuf 2019).
Given the concerns associated with the persistence and adverse environmental effects of RB-5 dye, it is crucial to address the issue and develop effective strategies for its removal and

Received 21 May 2024; Reviewed 2 July 2024; Accepted 29 July 2024;
Published Online 29 July 2024; Published 9 October 2024

degradation. The development of sustainable and environmentally friendly methods for the treatment of RB-5 dye-contaminated wastewater is of utmost importance. Exploring and enhancing the potential of biological approaches, such as microbial degradation, it may be possible to mitigate the adverse environmental impacts associated with RB-5 dye.

Biodegradation has emerged as a promising approach for the removal of synthetic dyes from wastewater, offering a cost-effective and environmentally friendly solution (Srivastava et al. 2022). A wide range of microorganisms have been identified as potential sources of enzymes capable of degrading complex organic compounds, including synthetic dyes (Gaur et al. 2018). Consequently, there is a pressing requirement to explore and establish efficient and sustainable approaches for the removal and degradation of RB-5 dye from industrial wastewater.

This study represents a noteworthy advancement as it seeks to enhance current understanding by investigating the capabilities of biological mechanisms, specifically microbial degradation, in tackling the challenges associated with RB-5 dye contamination. The main goal of this research is to devise effective and environmentally friendly approaches for mitigating the persistent and detrimental environmental impact caused by RB-5 dye in industrial wastewater. Additionally, the efficacy of utilizing Response Surface Methodology (RSM) as an optimization tool for maximizing the degradation process by fine-tuning the degradation parameters has been demonstrated.

This study evaluates the biodegradation potential of *Bacillus wiedmannii strain NAF4* for Reactive Black 5 dye using a Surface Response Optimization (SRO) model. The SRO model will be employed to optimize the process parameters and determine the optimal conditions for maximum dye degradation efficiency. By exploring the biodegradation capabilities of *B. wiedmannii* and optimizing the process parameters using the SRO model, this study aims to contribute to the development of effective and eco-friendly solutions for the removal of RB5 dye from textile wastewater.

Materials and Methods

Chemicals. RB5 was procured from the local market in Ilorin. The structure of RB-5 is shown in Figure 1.

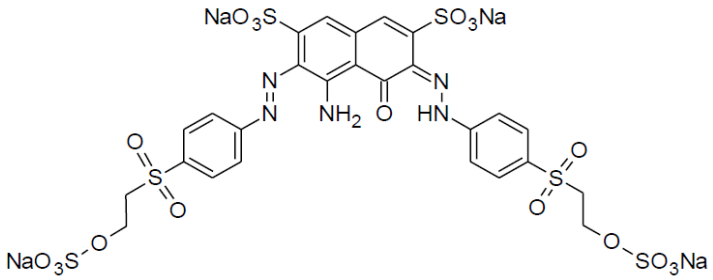

Fig. 1: Chemical structure of the dye "Reactive Black 5 (Pérez García et al. 2017)

Sample Area and sample Collection

The study was conducted at a local textile outlet situated in Adewole housing estate, Ilorin, Kwara state. This facility was chosen due to its practice of discharging effluent directly into the surrounding soil. To ensure a significant presence of dye effluent contamination, a thorough examination of the site was performed. Soil samples were collected during each phase of the study using aseptic techniques. The topsoil (0 to 5 cm) was carefully removed, and approximately 500 g of soil was obtained from various points. The collected soil samples were immediately transferred into a universal bottle using a soil auger (Chikere and Ekwuabu 2014). To maintain sample integrity, the universal bottle was securely packaged in a polythene bag and transported to the laboratory for further analysis.

Enrichment and Isolation of Dye-degrading Bacteria

The procedure described by Khan and Malik (2018) for isolating dye degrading bacteria utilized an enrichment technique based on the methodology outlined by Moosvi et al. (2005) with certain modifications. To initiate the isolation process, nutrient broth supplemented with RB5 at a concentration of 100 µg mL^{-1} was prepared. A 10% (w/v) portion of contaminated soil was inoculated into the broth, and the mixture was incubated under static conditions at 37 °C for 48 hours. This step was repeated several times using fresh dye-containing media until decolorization of the media was observed. To obtain pure cultures, a 100 µL sample of the culture suspension was plated onto nutrient agar plates containing RB5 at a concentration of 100 µg mL^{-1}. Following incubation, a bacterial colony showing the largest clear zone was selected for purification. The purified colony was then subjected to identification through 16S rDNA sequencing.

Biodegradation of RB5 by bacterial Isolates

The experimental setup involved utilizing a 250 ml conical flask containing 100 ml of nutrient broth as the growth medium. The broth was supplemented with Reactive Black 5 dye at a concentration of 10 µg/mL^{-1}. The basal conditions included a pH value of 7.0, a temperature of 37°C, and agitation at 150 rpm. For inoculation, a log growth phase culture of the target bacterium was prepared using the same nutrient medium conditions. The optical density (OD) of the inoculum was adjusted to 0.1 at a wavelength of 620 nm, corresponding to a bacterial concentration of 1×10^8 colony-forming units (cfu per ml), based on the McFarland turbidity standard of 0.5. Samples were periodically taken from the cultured broth and determined the percentage degradation.

Molecular Identification of the Dye-Degrading Bacteria

A single colony of respective dye-degrading bacteria was suspended in lysis buffer and Proteinase K for the extraction of the genomic DNA as described by Bhutia et al. (2021). The dye-degrading bacteria that were isolated had their 16S rRNA genes amplified through the use of universal primers 518F (5-CCAGCAGCCGTAATACG-3) and 800R (5-TACCAGGGTATCTAATCC-3) in a thermal cycler. The thermal cycling conditions included denaturation (92°C/min), annealing (54°C/min), and extension (72°C/min) for 25 cycles. The resulting PCR product was then assessed on 1% (w/v) agarose gels and purified using the QIA quick PCR purification kit from Qiagen, USA. The 16S rRNA genes were partially sequenced with the aid of the BIG-DYE terminator kit ABI 310 Genetic Analyzer from Applied Biosystems, USA. To align the bacterial sequences, the BLAST Search software at the National Center for Biotechnology Information was utilized to find homologous bacteria, the sequences. Neighboring Joining method was used to build the phylogenetic tree using MEGA 6. Upon submission to the National Center for Biotechnology Information (NCBI) gene bank, accessio numbers were assigned to the bacterial sequences.

Experimental Procedure

The experiments were carried out according to the Box–Behnken design and the range and level of the variables are stated in Table 1. The Box-Behnken design (BBD) of the response surface methodology (RSM) was used to optimize the experimental conditions vis-a-vis: temperature (20, 30, and 40 ^{0}C), pH (5, 7, 9), agitation (100,150, 200 rpm) and inoculum size for the degradation of reactive black-5 dye RB-5 by *B. wiedmannii*. The degradation capacity of *B. wiedmannii* was examined by inoculating the *B. wiedmannii* culture into different Erlenmeyer flasks (250 mL) containing minimal salt medium (50 mL) with above mentioned optimized conditions and incubated in an incubator shaker with the agitation speed and temperature adjusted according to the BBD After the degradation process, the samples were withdrawn and analyzed for RB-5.

The percentage degradation was calculated by

$$\text{Decolorization (\%)} = \frac{\textit{Initial OD} - \textit{Final OD}}{\textit{Initial OD}} \times 100\%$$

Experimental design by RSM

Response Surface methodology (RSM) is an empirical statistical technique employed for multiple regression analysis by using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously. Box–Behnken design was used to study the effects of the variables toward their responses and subsequently in the optimization studies. This method was suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interaction between the parameters. The coded values of the process parameters were presented in Table 1. The experimental and predicted values of percentage degradation of RB5 by dye-degrading *B. wiedmannii* NAF4 are given in Table 2.

The regression and graphical analysis with statistical significance were carried out using Design Expert software (version 7.1.5, Stat-Ease, Inc., Minneapolis, USA). In order to visualize the relationship between the experimental variables and responses, 3D plots were generated from the models. The optimum values of the process variables are obtained from the response surface.

Table 1. Level of different process variables in coded and uncoded form for degradation of RB5 by *Bacillus wiedmannii* NAF4

Variables	Code	Levels		
		-1	0	+1
pH	A	5	7	9
Temperature, ^{0}C	B	20	30	40
Innoculum concentration, %	C	5	10	15
Agitation speed, rpm	D	100	150	200

Table 2. Experimental conditions of Box Behnken design for RB5 degradation by *B. wiedmannii* NAF4

		Factor 1	Factor 2	Factor 3	Factor 4	Response 1
Std	Run	A:Temp	B:pH	C:Inoculum size	D:Agitation	Degradation
		ºC		%		%
18	1	45	7	5	150	61
23	2	35	3	15	250	0
26	3	35	7	15	150	71
9	4	25	7	15	50	57
5	5	35	7	5	50	43
24	6	35	11	15	250	51
2	7	45	3	15	150	0
22	8	35	11	15	50	37
25	9	35	7	15	150	71
27	10	35	7	15	150	71
20	11	45	7	25	150	78

21	12	35	3	15	50	0
6	13	35	7	25	50	45
28	14	35	7	15	150	71
3	15	25	11	15	150	55
17	16	25	7	5	150	61
16	17	35	11	25	150	65
1	18	25	3	15	150	0
4	19	45	11	15	150	55
13	20	35	3	5	150	0
14	21	35	11	5	150	77
15	22	35	3	25	150	0
8	23	35	7	25	250	93
10	24	45	7	15	50	62
11	25	25	7	15	250	86
7	26	35	7	5	250	66
19	27	25	7	25	150	78
12	28	45	7	15	250	86
29	29	35	7	15	150	71

Results

The laboratory evaluation of their decolorization potentials showed variations in the abilities of four isolates, namely *C. freundi* NAF1, *E. coli* strain NAF2, *P. aeruginosa* strain NAF3, and *B. wiedmannii* NAF4, to degrade RB-5 dye. Among these isolates, NAF4 exhibited the highest percentage of dye decolorization, indicating its strong potential for degrading the dye. *P. aeruginosa* strain NAF3 showed the second highest decolorization ability, more than their co-culture while NAF2 and NAF1 demonstrated the lowest potential for decolorization as shown in Figure 1. The highest percentage of decolorization for all isolates was observed at 144 hours of incubation, indicating that the degradation process reached its peak at this time point. Subsequently, the percentage of decolorization gradually decreased over time.

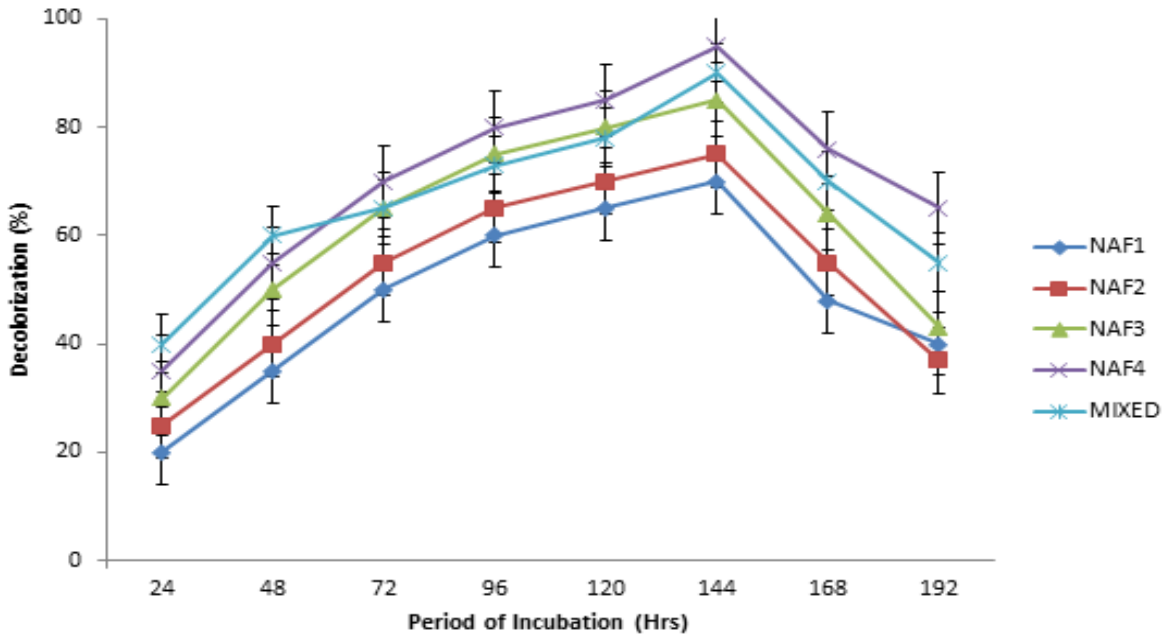

Figure 1. Decolorization of RB-5 by Bacterial Isolates from the Soil Contaminated with Textile Effluent

In this study, a total of four bacterial isolates were recovered from the soil samples contaminated with the dyes using culture-based methods. The dye-degrading bacteria belong to the following genera: *E.coli*, *Citrobacter, Pseudomonas and Bacillus.* The 16SrDNA sequencing revealed that the isolated bacterial strains exhibited homology to known species. Specifically, NAF1 to *Escherichia coli* strain 81402; NAF2, *Citrobacter freundii* strain ATCC 8090; NAF3 *Pseudomonas aeruginosa* strain DSM 50071 and NAF4, *Bacillus wiedmannii* strain BCL-Z09 was observed, as shown in Figure 2.

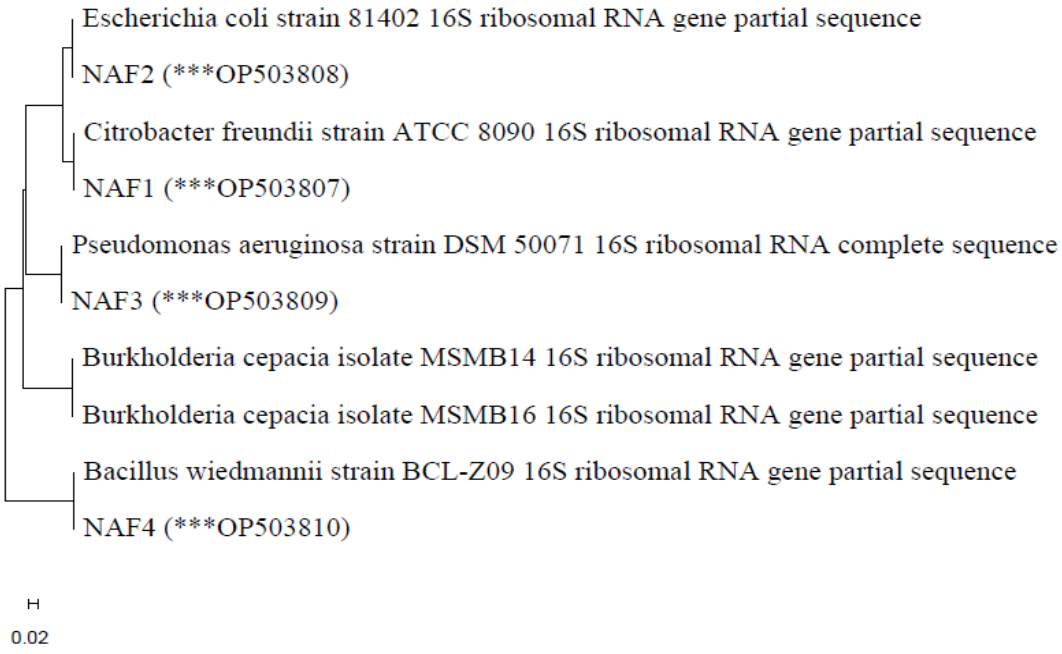

Figure 2. Phylogenetic tree constructed by Neighbor-Joining method derived from analysis of the 16S rRNA gene sequences of dye-degrading bacteria and related sequences obtained from NCBI.

The adequacy of the proposed model was evaluated by the Design expert 11.1.2.0 as depicted in Table 3. The model showed that the quadratic model possesses an *F*-value (53.30) and low p-values (<0.0001) indicating that the model is significant. The model also possesses a high correlation coefficient (R^2) value (0.9813). The adjusted (R^2) value of 0.9632 compares adequately with the predicted R^2 (0.8939) with a moderate agreement since their difference is less than 0.2. The model significance is true if its p-value < 0.05. The adequate precision value of 23.2533 is appreciably high; a value > 4.0 is desirable and reveals a signal toward the adequacy of the model. The linear terms A, B, C, D, AB, AC, CD, A^2, B^2, C^2, and D^2 are the significant terms for the degradation of RBD-5 by *B. wiedmannii* NAF4 as shown in Table 3.

Table 3. Significance of Experimental Parameters on the Degradation of RB5

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	19351.61	14	1382.26	53.30	< 0.0001	significant
A-Temp.	1260.75	1	1260.75	48.61	< 0.0001	significant
B-pH	4840.08	1	4840.08	186.63	< 0.0001	significant
C-Agitation	533.33	1	533.33	20.56	0.0005	significant
D-Inoculum size	161.33	1	161.33	6.22	0.0258	significant
AB	225.00	1	225.00	8.68	0.0106	significant
AC	306.25	1	306.25	11.81	0.0040	significant
AD	100.00	1	100.00	3.86	0.0697	
BC	0.2500	1	0.2500	0.0096	0.9232	
BD	0.0000	1	0.0000	0.0000	1.0000	
CD	441.00	1	441.00	17.00	0.0010	significant
A^2	3906.77	1	3906.77	150.64	< 0.0001	significant
B^2	9264.07	1	9264.07	357.21	< 0.0001	significant
C^2	1443.29	1	1443.29	55.65	< 0.0001	significant
D^2	921.13	1	921.13	35.52	< 0.0001	significant
Residual	363.08	14	25.93			
Residual	363.08	14	25.93			
Pure Error	0.0000	4	0.0000			
Cor Total	19714.69	28				

Mean=50.10; C.V.%=10.16; R^2=0.9813; Adjusted R^2=0.9632; Predicted R^2=0.8939; Adequate Precision=23.2533

The actual response versus predicted response plot for the quadratic model is presented in Figure 3. The data points on the plots were well dispersed along a straight line, which is an indication of strong conformity between the actual and predicted values of the response. This result indicates that the quadratic model is sufficiently adequate in predicting the response variables for the experimental data.

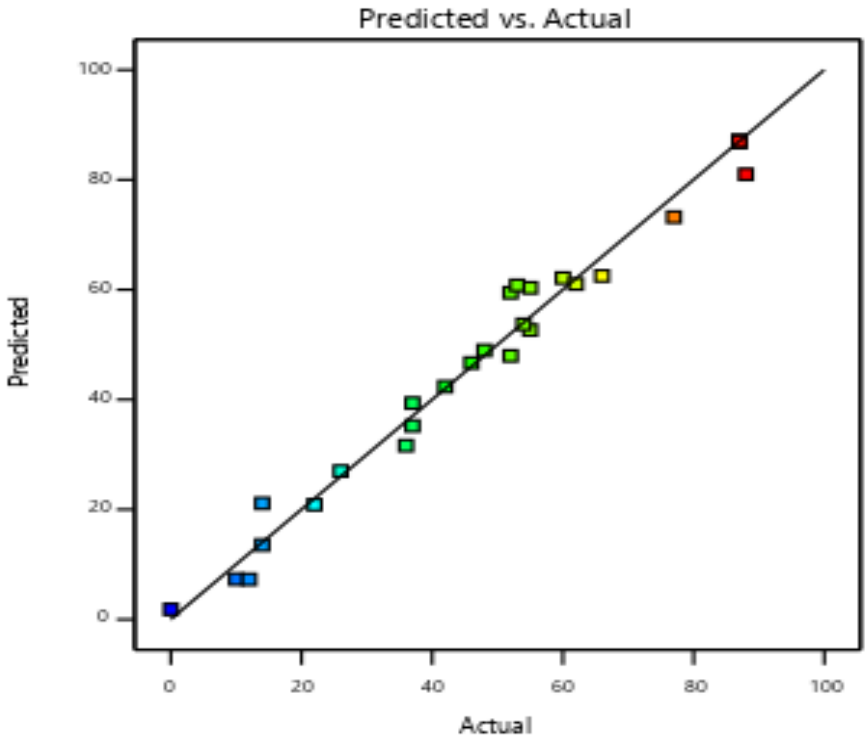

Figure 3. The actual response versus predicted response plot for the quadratic model

The size of the inoculum significantly influences the metabolic activity and microbial composition, serving as a critical determinant in identifying operational factors during the biodegradation process. In this study, the inoculum size ranged between 5 and 15. The percentage of degradation exhibits an increasing trend with an increasing inoculum size, reaching an optimal value of 12.25. However, beyond this optimum threshold, the degradation percentage declines. This decline indicates the presence of overcrowding among the *B. wiedmannii* NAF4 at sizes exceeding the optimal value, resulting in limited interaction with the RB-5 molecules and reduced efficiency of degradation.

Temperature is one of the most important factors affecting the degradation of hazardous chemicals. Bacterial activity and growth for efficient degradation were observed within a temperature range of 30 to 35°C. The optimal degradation temperature for RB-5 in this study was determined to be 29.744°C. Temperature values higher or lower than this optimum significantly impede the viability of BW cells, as depicted in the 3D response surface plots.

The agitation speed influences the degradation of RB-5, increasing it until reaching an equilibrium speed, beyond which degradation decreases. This phenomenon can be attributed to enhanced contact between *B. wiedmannii* and RB-5, facilitating the degradation process. However, further increases in agitation speed beyond the equilibrium point result in decreased degradation due to high speeds negatively affecting cell viability necessary for effective degradation. The response surface plots (Figure 4A-F) were generated to investigate the interactions among operating parameters and their impact on the degradation efficiency of RB-5 by *B. wiedmannii*. The pH of the solution significantly influences the degradation process. There is a gradual increase in the percentage of RB-5 degraded as the pH increases, reaching an equilibrium at pH 7. After pH 7, the degrading ability of *B. wiedmannii* NAF4 decreases, indicating a pH-dependent relationship between *B. wiedmannii* strain NAF4 and the nature of RB-5 in solution (Figure 4A).

The percentage of RBD-5 degraded was observed to increase with an increase in the initial inoculum size at a fixed agitation speed (115 rpm), pH (7), and temperature (30°C). The interactions among all parameters presented in Fig. 4 (A-F) demonstrate a synergistic

relationship. When one parameter increases along with another, the degradation ability of *B. wiedmannii* also increases, suggesting that each parameter plays a significant role in the degradation process.

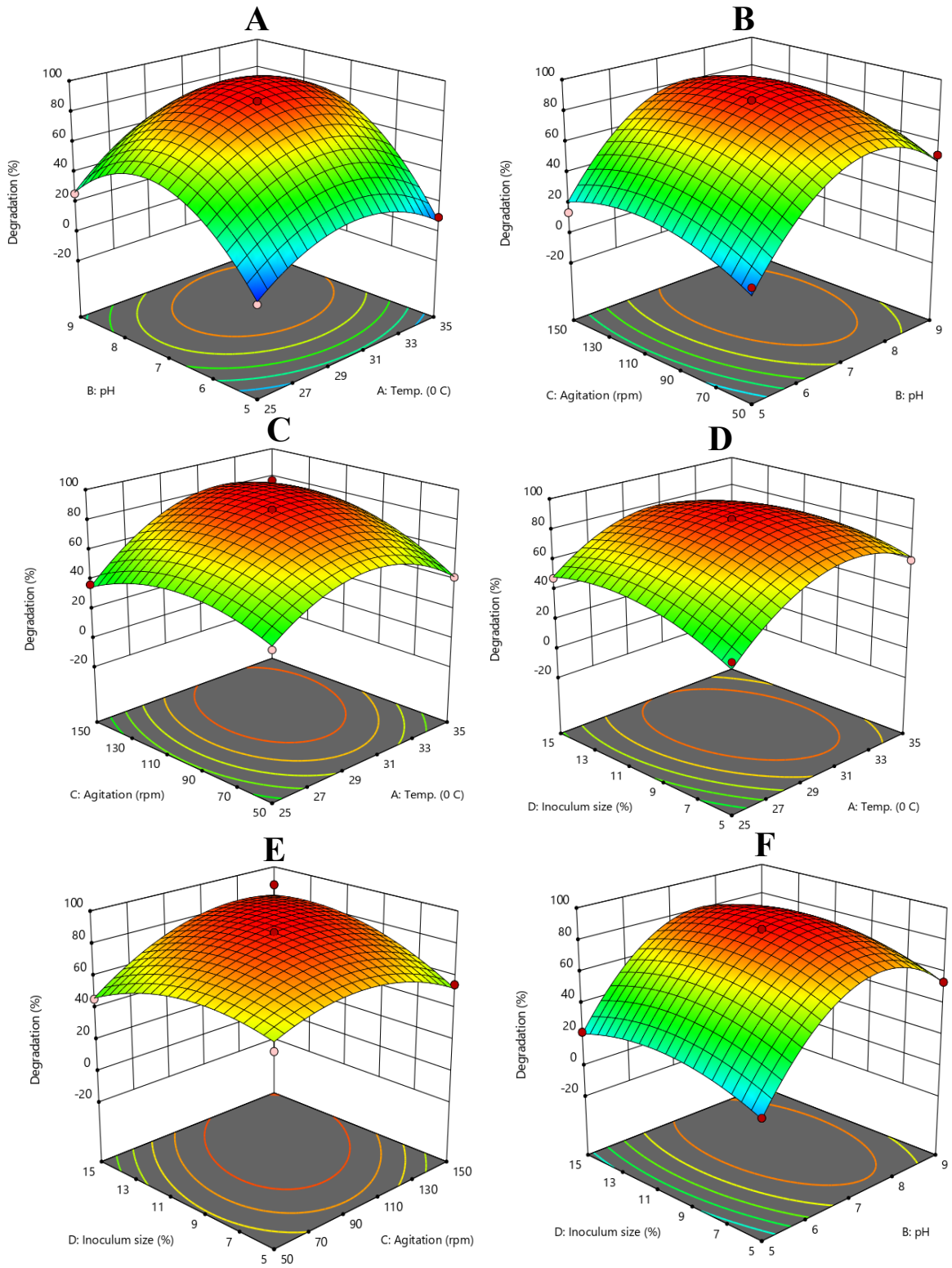

Figure 4. The 3D plots and Contour plots showing the effect of (A). pH and Temperature (B). Agitation and pH (C). Agitation and Temp. (D). Inoculum size and Temp. (E). Inoculum size and Agitation-speed (F). Inocum size and pH

However, there are optimal conditions reached during the degradation process, beyond which the degradation ability of *B. wiedmannii* NAF4 decreases. The optimum conditions identified in this study were an agitation speed of 115.777 rpm, pH of 7.449, inoculum size of 12.255, and temperature of 29.74°C, as illustrated in the Figure 5.

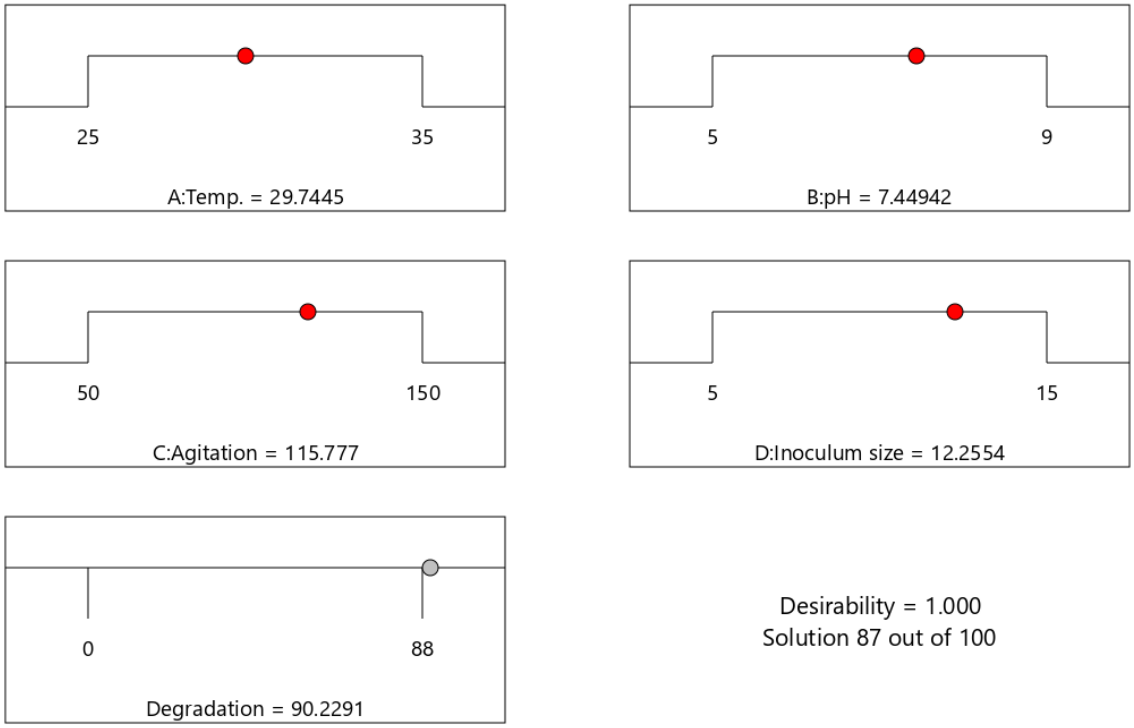

Figure 5: Optimum temp, pH, Agitation and inoculums size required to achieve 90.2291 % of the RB-5 degradation by *Bacillus wiedmannii strain NAF4*

Discussion

Reactive Black 5 (RB5) dye is a commonly used synthetic dye in various industries, including textile, paper, and leather. However, its wide-scale application has raised concerns due to its persistence and adverse environmental effects (Al Sharabati et al. 2021, Marković et al. 2023) Biodegradation, a promising approach to mitigate the environmental impact of RB5 dye, is gaining attention (Li et al. 2021) indicates their potential adaptation to the unique environmental conditions created by textile industry effluents.

The four bacterial isolates, isolated from the soil impacted by wastewater from local textiles through enrichment techniques. Enrichment techniques have been shown to be crucial in enhancing the likelihood of isolating microorganisms capable of degrading pollutants (Bokade et al. 2023). These techniques apply a selective pressure that promotes the enrichment of the desired microbial population possessing the inherent ability to utilize the pollutant as a carbon or energy source. Consequently, this selective pressure encourages the growth of pollutant-degrading microorganisms while simultaneously suppressing the growth of non-degrading or slower-degrading species (Bokade et al. 2023).

These isolates have demonstrated their ability to thrive in an environment enriched with various organic and inorganic compounds present in the wastewater. This also suggests that they possess specific physiological and metabolic characteristics that enable them to withstand the toxic effects of the textile waste contaminants. These bacteria may have developed adaptive

mechanisms to utilize the complex mixture of organic dyes, chemicals, and other pollutants present in the wastewater as a source of carbon and energy ((Giovanella et al. 2020, Bala et al. 2022).

Considering this knowledge, it is plausible that the dominant isolates identified as *C. freundi* NAF1, *E. coli* strain NAF2, *P. aeruginosa* strain NAF3, and *B. wiedmannii* NAF4 may possess similar enzymatic capabilities, facilitating the degradation and removal of textile dyes present in the contaminated soil (Saravanan et al. 2021)

These findings highlight the varying capabilities of the isolates in degrading RB-5 dye, with *B. wiedmannii* strain NAF4 showing the highest efficacy. The decrease in decolorization percentage over time suggests that the degradation process may have reached a plateau or encountered certain limitations also indicating the dynamic nature of the degradation process.

However, all the tested isolates demonstrated the capability to metabolize RB-5, with *B. wiedmannii* strain NAF4 exhibiting the highest degradation potential among them, even surpassing the degradation ability of the consortium. This finding challenges the conventional understanding that co-cultures are generally more efficient in degradation compared to pure cultures Sauer & Marx (2023). The exceptional performance of isolate *B. wiedmannii* strain NAF4 in degrading RB-5 highlights its novelty and suggests its potential as a promising candidate for further study. This study represents the first-ever investigation into the use of *B. wiedmannii* for the degradation of Reactive black dye (RB-5).

Interestingly, the consortium of isolates displayed a slightly lower performance in RB-5 degradation. This could be attributed to the antagonistic effect observed within the consortium. Previous studies have indicated that isolate *B. wiedmannii* strain NAF4 possesses cytotoxic properties, which may have contributed to the observed antagonistic effect within the consortium. This finding suggests that the interplay between different isolates within a consortium can significantly impact the overall degradation efficiency.

Understanding the varying abilities of different isolates in degrading RB-5 dye is important for the development of efficient bioremediation strategies. By identifying isolates with higher decolorization potential, such as *B. wiedmannii* strain NAF4 and *P. aeruginosa* strain NAF3, researchers can further investigate and optimize their performance for industrial applications.

Several studies have employed Response Surface Methodology (RSM) as an optimization tool in the biodegradation of various azo dyes, highlighting its effectiveness in enhancing degradation processes (Kumar et al. 2019, Khatoon and Rai 2020) RSM offers several advantages in the context of biodegradation studies. Firstly, it allows for the simultaneous investigation of multiple variables and their interactions, enabling a comprehensive analysis of the degradation process. This comprehensive approach ensures that all relevant factors are considered, leading to more accurate and reliable results.

To optimize the degradation process of RB-5, the study utilized Response Surface Methodology (RSM) in conjunction with the isolated bacterium strain. RSM is a statistical tool that enables the verification and optimization of process parameters by evaluating their effects on the degradation efficiency. In this case, the process parameters investigated were pH, temperature, inoculum size, and agitation speed (Manogaran et al. 2021).

To visualize the effects of these process parameters, 3D plots and contour plots were generated. The plots depicted the interactions between different pairs of parameters and their influence on the degradation efficiency of RB-5 by *B. wiedmannii*. Specifically, the plots illustrated the effects of pH and temperature, agitation speed and pH, agitation speed and temperature, inoculum size and temperature, inoculum size and agitation speed, and inoculum size and pH.

In this study, the application of RSM for the optimization of process parameters in the degradation of Reactive Black 5 (RB-5) dye by *Bacillus wiedmannii* strain NAF4 is affirmed. By utilizing RSM, this study showed the optimal conditions for maximum dye degradation efficiency. The identified optimal conditions included an agitation speed of 115.777 rpm, pH

of 7.449, inoculum size of 12.255, and temperature of 29.74°C. These conditions were shown to significantly enhance the degradation of RB-5, resulting in a maximum degradation efficiency of 90.2291%.

The generated response surface plots not only allow for the determination of optimum conditions but also provide valuable information regarding the interactions between the different operating parameters. The plots reveal the synergistic relationships among pH, temperature, inoculum size, and agitation speed, emphasizing the intricate interplay of these factors in the degradation process.

The identified optimum conditions hold great significance for practical applications. They serve as guidelines for optimizing RB-5 degradation processes using *B. wiedmannii* in real-world scenarios. Implementing these conditions can lead to enhanced degradation efficiency and more sustainable approaches for the remediation of RB-5-contaminated environments. Overall, this study demonstrates the immense potential of *B. wiedmannii i*n degrading RB-5 dye. The organism's enzymatic capabilities, as evidenced by the production of laccase, lignin peroxidase, manganese peroxidase, and azoreductase, indicate its suitability as a biodegradation agent. The utilization of RSM and response surface plots further enhances our understanding of the interactions between process parameters and their effects on RB-5 degradation. The identified optimum conditions provide practical insights for optimizing the degradation process and highlight the promising applications of *B. wiedmannii* in addressing RB-5 contamination challenges. Furthermore, the utilization of RSM in this study serves as an innovative approach to maximize the degradation process by optimizing degradation parameters. The successful application of RSM in this study demonstrates its effectiveness as an optimization tool and provides valuable insights for future biodegradation studies. The optimized conditions identified through RSM offer practical guidance for the implementation of efficient and environmentally friendly strategies for the degradation of RB-5 and potentially other similar azo dyes.

Conclusions

The Biodegradation study of RB-5 was carried out with the bacterium *B. wiedmannii* NAF4 strain isolated from the dye- contaminated field soil. The RSM was used with isolated bacterium strain for the verification, and optimization, of process parameters, namely pH, temperature, Inoculum size and agitation-speed for degradation of RB-5. This statistical analysis technique confirmed as useful and powerful tool, in standardizing optimum degradation conditions. This is the first research which describes the application of statistical designing tool for the degradation of RB-5 by *B. wiedmannii* NAF4 strain culture and Design expert software (new version 10.0.1). The optimum conditions identified in this study were an agitation speed of 115.777 rpm, pH of 7.449, inoculum size of 12.255, and temperature of 29.74°C. From the results, it can be concluded that RSM applied here as optimization tool can be effectively used elsewhere as well, to maximize the degradation process by optimizing degradation parameters.

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Funding: No funding was received

Authors' contributions: **AAT:** Conceived and designed the experiments, Manuscript preparation. **MN and AWT:** Data analysis and interpretation, Manuscript preparation. **JAA:**

Contributed reagents/materials/analysis tools; All authors carried out laboratory experiment. All authors read and approved the final manuscript.

Acknowledgements: The authors express profound gratitude to their Institution management for creating an enabling environment

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