Chapter 11
Impact of Acinetobacter Baumannii on Dye Degradation and a Molecular Analysis Study

V. Nivetha, S. Harini, J. Maria Shyla, and G. Sophia Reena

Abstract *Acinetobacter baumanni* was isolated from polluted soil. An attempt to study the *A. baumannii* to degrade dye was explored. It was found effective against azo dye and was able to completely degrade the dye under 48 h in a shake flask. Molecular analysis on the isolate *A. baumanni* was performed together with *Hedychium flavum*, and the sequence was submitted to the NCBI database to procure accession number MT192652.1. Response surface Methodology-Box-Behnken design (RSM-BBD) was used to optimize the condition and achieve 98–99% dye decolorization.

Keywords *Acinetobacter baumanni* · Dye degradation · Optimization · Molecular analysis

11.1 Introduction

The demand on every industry to provide enough consumer goods for the world's now-staggering 8 billion people was brought on by the Industrial Revolution, which aided in the population expansion that led to that number. The textile sector is vital to meeting the needs of a growing global population. An estimated one million tonnes of dyes and pigments are produced and consumed each year, with a sizable amount of these synthetic colors ultimately finding their way into the environment via wastewater discharges. The environment and human health are in grave danger due to this. Therefore, much water is consumed in the textile industry during the dying process, and the resulting effluent contains a lot of organics and hazardous chemicals. Furthermore, the dissolved oxygen levels of aquatic life are negatively impacted when this wastewater is dumped into water bodies without treatment. Thus, even at 1 ppm, dyes can contribute to diminished oxygen solubility and sunlight dispersion,

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A. Jeyaseelan et al. (eds.), *Sustainable and Cleaner Technologies for Environmental Remediation*, Environmental Science and Engineering,
https://doi.org/10.1007/978-3-031-29597-3_11

which are essential for aquatic life (Arora 2014; Barikbin et al. 2017). As a result, these effluents must be treated to remove the colors before being released into waterways. Recent reports have highlighted the public health risk posed by wastewater discharges containing the dye, which can cause eye burns, nausea, vomiting, and diarrhea. Numerous treatment technologies have been developed for decolorizing and degrading dyes in wastewater; hence, it is crucial to find an effective treatment method for wastewater containing dyes. Adsorption, reverse osmosis, ultrafiltration, chlorination, and biological approaches, including aerobic and anaerobic treatments, are also examples of such processes. However, most of these conventional pollution control methods only relocate the pollutants from one phase to another rather than transforming them into a less harmful product (Unnikrishnan et al. 2018).

Compared to conventional approaches, bioremediation is a safer, cheaper, greener, and more practical option for dealing with textile waste (Fletcher et al. 2021). Using microbes in the form of bacteria, fungi, algae, yeast, and mixed culture for bioremediation is an exciting new field (Li et al. 2019). The bacteria have an advantage in treating textile effluents due to their capacity to adapt and break down textile effluents at high concentrations. Bacterial methods for degrading synthetic dyes, especially azo dyes, may be helpful (Mishra et al. 2020). Research and development cannot proceed without isolating, identifying, and preserving various dye-degrading bacteria. To decolorize and break down azo dyes, scientists have experimented with a wide range of oxidoreductive enzymes (Srinivasan et al. 2017). Thus, optimizing the significance and interaction among protein ligands can be done cheaply through molecular docking and simulation. Dye decolorizing bacterial systems for real-time wastewater treatment in the textile industries can be effectively screened using this combinatorial approach by first employing bioinformatics tools, then conducting analysis in a wet lab (Gao et al. 2018).

The application of response surface methodology (RSM) has been validated as an efficient way to optimize processes involving several interrelated variables. When designing, modeling, and optimizing a process, the RSM, known as a Box Behnken design (BBD), has proven to be effective in estimating the correlation coefficient for a specific model, resulting in lower experimental errors and better data fitness.

This study focuses on the dye degradation capability of the isolated bacteria, enhancing its potential via optimizing process parameters like the inoculum load, temperature, and dye concentration.

11.2 Materials and Methods

11.2.1 Material

Coimbatore, Tamil Nadu, India, is home to a sizable textile sector, where we sourced our azo dye. The dye has a molecular weight of 967.5 g/mol and an absorption wavelength of 530 nm.

11.2.2 Sample Collection and Isolation of Dye-Degrading Bacteria

Under aerobic circumstances, samples of textile effluent were gathered in Coimbatore, Tamil Nadu, India. Samples that had been adequately diluted were plated on Luria–Bertani (LB) agar that contained azo dye. For additional research, the bacterial strain that could stand up to 10 g/L of the dye was chosen. The chosen bacterial isolates' capacity to discolor was confirmed (Shah 2016).

11.2.3 16S rRNA Sequencing and Submission

Using a kit, genomic DNA was isolated from the bacterium (Gislin et al. 2018). In order to find closely identical sequences, the 16S rRNA sequence was then entered into a National Center for Biotechnology Information (NCBI) blast similarity search engine.

11.2.4 Decolorization Studies

One milliliter of the isolated bacterial strain was added to a flask containing 50 mL 500 mg/L of azo dye amended in LB broth and incubated at 37 °C for 3 days. With the aid of an ultraviolet–visible spectrophotometer, the degree to which a dye lost its color was determined by observing changes in the dye's absorbance at 530 nm (Satar and Husain 2011). The percentage rate of dye decolorization, E, was calculated using the formula:

$$E = [(A_0 - A_t)/ A_0] * 100\% \quad (11.1)$$

where A_0 is the preliminary absorbance and A_t is the ultimate absorbance.

11.2.5 Immobilization of Potential Isolate

Calcium alginate beads were used to immobilize the potential isolate. Beads made of calcium alginate are durable and may be re-used after being immobilized by encapsulation. Hot water was added until wholly dissolved, followed by adding 30 g/L of sodium alginate and 5% of the bacterial culture while stirring frequently. The cell/sodium alginate combination was extruded drop by drop into a chilled, sterile calcium chloride solution using a syringe to produce calcium alginate beads with a diameter of 2 mm. $CaCl_2$ and sodium alginate are combined to create calcium

Table 11.1 Variables chosen for RSM optimization

Factor	Name	Units	Type	Minimum	Maximum
A	Temperature	Celsius	Numeric	25	35
B	Immobilized inoculum	g/L	Numeric	0.1	0.5
C	Incubation time	hours	Numeric	12	36

alginate beads. For increased hardness and durability, calcium alginate beads were resuspended in a calcium chloride solution and chilled in the refrigerator for 24 h.

11.2.6 Optimization of Process Variables

Using the Response Surface Methodology (RSM), statisticians and mathematicians can forecast ideal circumstances with the fewest runs. We studied the connections between temperature, incubation period, and inoculum volume using a three-level factorial Box-Behnken design based on the one factor at a time (OFAT) technique (BBD). The experimental intervals used in the BBD are shown in Table 11.1.

11.3 Results and Discussion

11.3.1 Isolation of Potential Bacteria and Identification

Decolorization effectiveness was evaluated after isolating a bacterial strain that can survive in solutions containing up to 10 g/L of azo dye. It was observed that the bacteria entirely removed maximal color up to 98% (Prasad and Aikat 2014; Permpornsakul et al. 2016). 16S rRNA sequencing helped identify the isolate as *A. baumannii*. *A. baumannii* is a gram-negative bacterium that can be detected in soil and water samples on rare occasions. Few studies have explored the potential of this bacteria for decolorizing dyes. GenBank now contains the sequence in the entry as Accession no. MT192652.1. Results from a phylogenetic study are shown in Fig. 11.1 (seq 1) (Unnikrishnan et al. 2018).

1 cgagcggggg tagggagctt gctactggtc ctagcggcgg acgggtgagt aatgcttatg.
61 aatctgccta ttagtggggg acaacatctc gaaagggatg ctaataccgc atacgtccta.
121 cgggagaaag caggggatct tcggaccttg cgctaataga tgagcctaag tcggattagc.
181 tagttggtgg ggtaaaggcc taccaaggcg acgatctgta gcgggtctga gaggatgatc.
241 cgccacactg ggactgagac acggcccaga ctcctacggg aggcagcagt ggggaatatt.
301 ggacaatggg gggaaccctg atccagccat gccgcgtgtg tgaagaaggc cttatggttg.
361 taaagcactt taagcgagga ggaggctact gtaattaata cctatggata gtggacgtta.
421 ctcgcagaat aagcaccggc taactctgtg ccagcagccg cggtaataca gagggtgcga.
481 gcgttaatcg gatttactgg gcgtaaagcg tgcgtaggcg gctttttaag tcggatgtga.

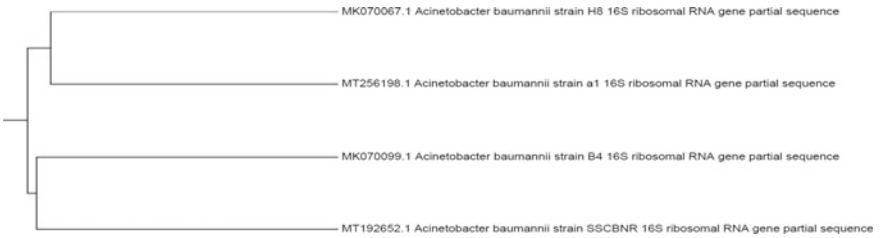

Fig. 11.1 Phylogenetic tree for *Acinetobacter baumanni* (Acc.No. MT192652)

541 aatccccgag cttaacttgg gaattgcatt cgatactggt gagctagagt atgggagagg.
601 atggtagaat tccaggtgta gcggtgaaat gcgtagagat ctggaggaat accgatggcg.
661 aaggcagcca tctggcctaa tactgacgct gga.

Sequence 1: 16 s RNA partial Sequence of Acinetobacter.

11.3.2 Optimization Using Box-Behnken Design

Optimizing decolorization processes with BBD is standard practice. For example, in 2013, Papadopoulou et al. explored the potential of *Pleurotus pulmonaris* AMRL 177 with BBD for decolorizing Remazol brilliant blue, Fazli et al. (2010) employed BBD to enhance Ganoderma species-based reactive blue 19 decolorization. Time, temperature, and inoculum volume were used in the BBD experiment. Table 11.2 details the design, while Table 11.3 provides an ANOVA for the response surface model.

$$\%removal = 82.4 + 0.625 * A + 18 * B + 10.875 * C + 0.25 * AB + -1.5 * AC + 0.25 * BC + -1.95 * A^2 + -7.7 * B^2 + -3.45 * C^2$$

When R1 represents the response, A, B, and C represent temperature, immobilized inoculum amount, and incubation period, respectively, while the other variables represent the interaction of one variable against another. Design expert software was utilized to simulate the design and analyze the results of the testing runs. The model's F-value of 58.26 suggests that it is significant at p0.0001 (Table 11.3). The most critical model terms in the current model are B, C, B2, and C2. Based on the normal-plot, actual vs. predicted plots, the actual and expected outcomes (response- dye decoloration) were good (Figs. 11.2 and 11.3). The outcome was equivalent to that of Unnikrishnan et al. (2018).

It is also possible to predict and compare the interaction of the design parameter on each other and their effect on the dye decolorization evident from the 3-D contour plots (Figs. 11.4a, b, and c) (Papadopoulou et al. 2013).

Table 11.2 Experimental runs with dye decolorization outcome

	Factor 1	Factor 2	Factor 3	Response 1
Run	A: Temperature	B: Immobilized Inoculum	C: Incubation time	% Removal
	Celsius	g/L	Hours	
1	25	0.3	12	61
2	30	0.1	12	45
3	25	0.3	36	88
4	30	0.3	24	82
5	35	0.5	24	91
6	25	0.1	24	55
7	25	0.5	24	93
8	30	0.1	36	64
9	35	0.1	24	52
10	30	0.3	24	83
11	35	0.3	12	69
12	30	0.3	24	83
13	30	0.5	12	78
14	30	0.3	24	82
15	30	0.3	24	82
16	30	0.5	36	98
17	35	0.3	36	90

Table 11.3 ANOVA analysis information

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3891.11	9	432.35	58.26	<0.0001	Significant
A-Temperature	3.13	1	3.13	0.4211	0.5371	
B-Immobilized inoculum	2592.00	1	2592.00	349.26	<0.0001	
C-Incubation time	946.13	1	946.13	127.49	<0.0001	
AB	0.2500	1	0.2500	0.0337	0.8596	
AC	9.00	1	9.00	1.21	0.3072	
BC	0.2500	1	0.2500	0.0337	0.8596	
A^2	16.01	1	16.01	2.16	0.1853	
B^2	249.64	1	249.64	33.64	0.0007	
C^2	50.12	1	50.12	6.75	0.0355	

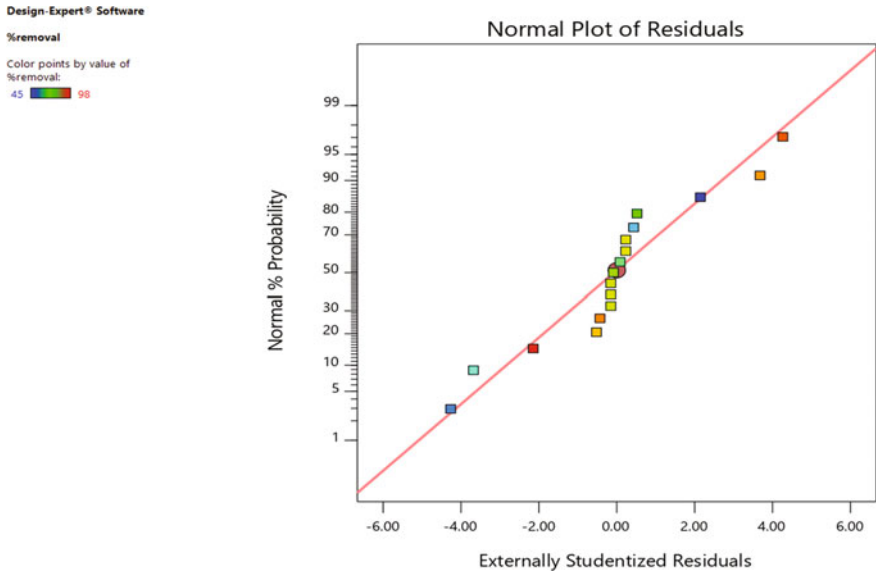

Fig. 11.2 Normal plot

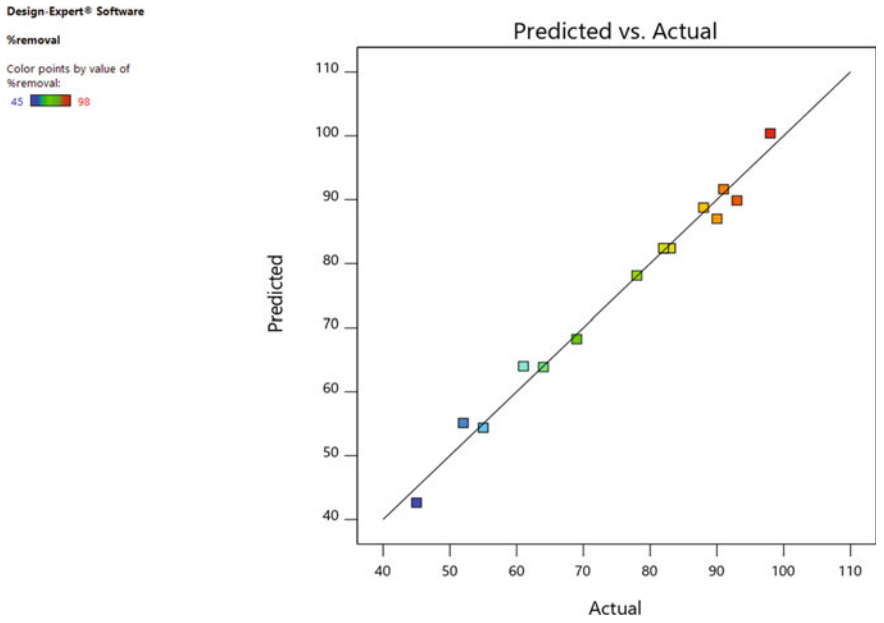

Fig. 11.3 Predicted versus actual plot

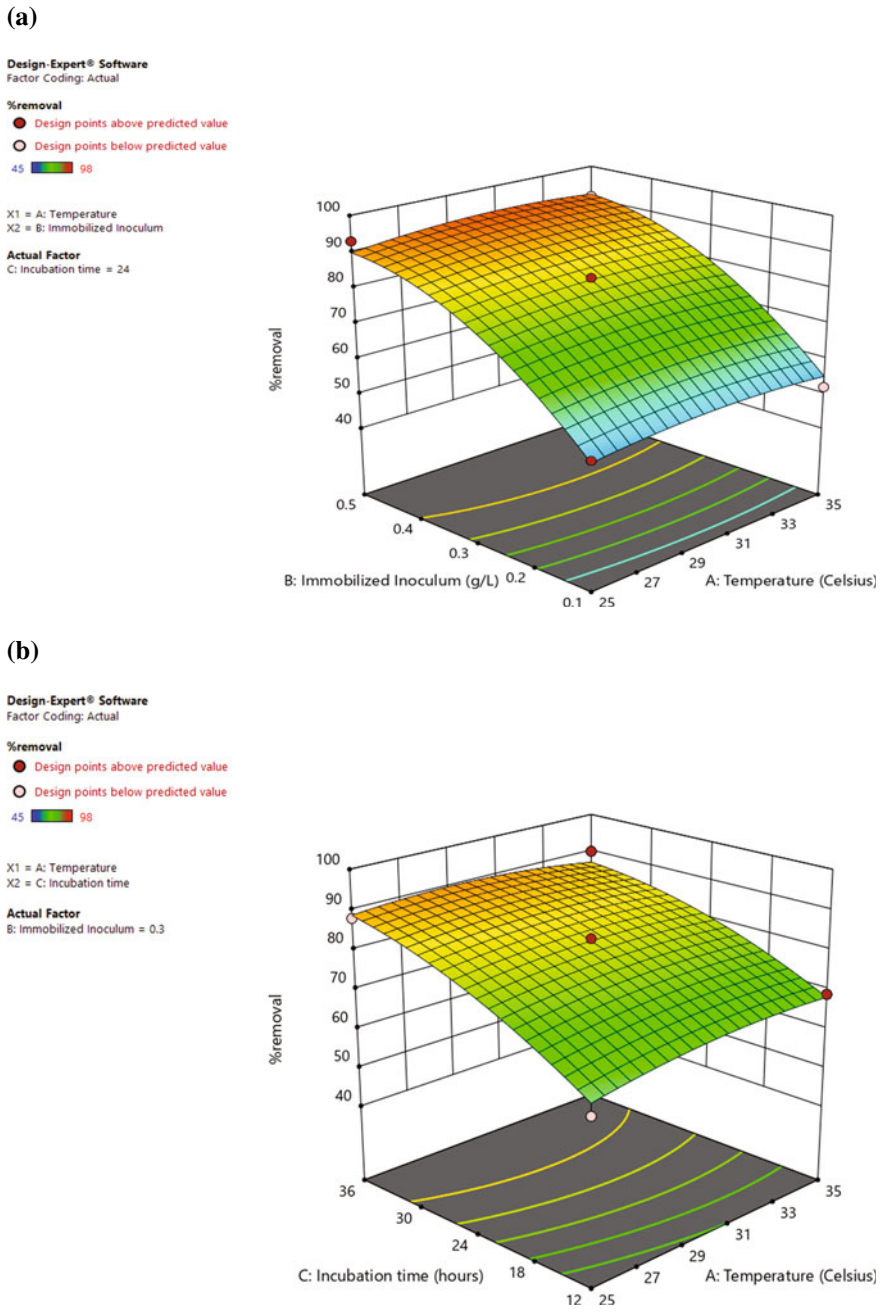

Fig. 11.4 **a** 3D contour plot of temperature versus immobilized inoculum on dye decolorization **b** 3D contour plot of temperature vs. incubation time on dye decolorization **c** 3D contour plot of incubation time vs. immobilized inoculum on dye decolorization

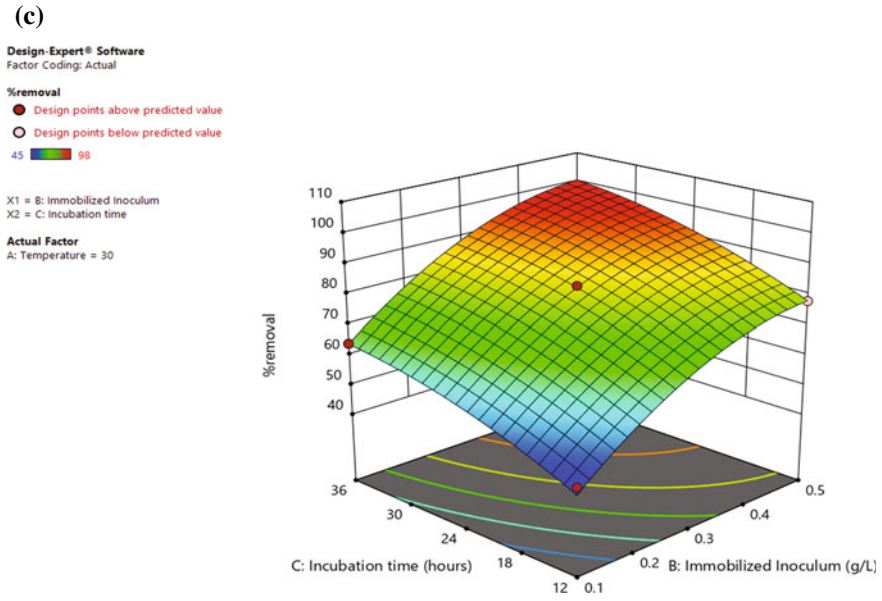

Fig. 11.4 (continued)

11.3.3 Model Validation and Experimental Confirmation

The RSM BBD output was validated with an independent run to achieve peak dye decolorization. For this dye decolorization, the experimental decolorization rate (99.15%) was achieved for an optimized condition of 28 °C with an inoculum load of 450 mg/L and an incubation period of 34 h.

11.4 Conclusion

Acinetobacter baumannii dye decolorization capabilities were evaluated and optimized to the experimental condition via RSM-BBD. As a result, 99% of dye discoloration was achieved for optimized conditions of 28 °C temperature, inoculum load of 450 mg/l, and the maximal dye discoloration was achieved in 35 h. Hence this isolate can be used for larger-scale exploration of dye degradation under optimal conditions.

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