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ОПТИМИЗАЦИЯ БИОСОРБЦИИ КРАСИТЕЛЯ КОНГО КРАСНОГО БИОМАССОЙ *SERRATIA MARCESCENS* ММ06 С ИСПОЛЬЗОВАНИЕМ МЕТОДА ПОВЕРХНОСТИ ОТКЛИКА

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Сточные воды, загрязненные красителями, представляют собой серьезную экологическую проблему, особенно в районах с ограниченной инфраструктурой очистки. В то время как биологические методы продолжают развиваться, адсорбция становится эффективным и широко распространенным методом очистки. Хотя активированный уголь остается наиболее эффективным адсорбентом, его высокая стоимость вызвала новый интерес к недорогим альтернативам. В таких странах, как Малайзия, неадекватные системы очистки усугубляют проблему, подчеркивая необходимость экономически эффективных и экологически безопасных решений. Бактериальные биосорбенты обеспечивают устойчивое решение. В данной работе для оценки шести важных переменных, влияющих на биосорбцию красителя Конго красного бактериальной биомассой, использовался план Плакетта-Бермана. Было проведено двенадцать испытаний с высокими (+1) и низкими (-1) значениями параметров. Дисперсионный анализ (ANOVA) выявил статистически значимые факторы ($p < 0,05$), которые затем были оптимизированы с помощью трехуровневого полного факторного плана. Максимальная скорость удаления красителя на этой фазе составила 85,08% (запуск 12). Последующие тринадцать экспериментов, основанные на полном факторном планировании, дали максимальную адсорбционную емкость 91,75%. Регрессионная модель продемонстрировала хорошее соответствие данным ((коэффициент детерминации (R^2) = 0,9935, скорректированный R^2 (R^2-adj) = 0,9889, F-тест для значимости модели (F) = 214,89, точность адекватности = 36,685)), и не было обнаружено существенной разницы между прогнозируемыми и экспериментальными значениями ($p > 0,05$), что подтверждает надежность модели.

Ключевые слова: краситель, сточные воды, методология поверхности отклика, адсорбция

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OPTIMIZATION OF CONGO RED DYE BIOSORPTION BY *SERRATIA MARCESCENS* MM06 BIOMASS USING RESPONSE SURFACE METHODOLOGY

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Dye-contaminated wastewater poses a significant environmental challenge, particularly in areas with limited remediation infrastructure. While biological methods continue to evolve, adsorption is emerging as an effective and widely accepted treatment option. Although activated carbon remains the most effective adsorbent, its high cost has sparked renewed interest in low-cost alternatives. In countries such as Malaysia, inadequate treatment systems exacerbate the problem, emphasising the need for cost-effective, environmentally friendly solutions. Bacterial biosorbents provide a sustainable solution. In this work, the Plackett-Burman design was utilised to test six important variables that influence Congo red dye biosorption by bacterial biomass. Twelve trials were conducted with parameters set at high (+1) and low (-1) values. Analysis of Variance (ANOVA) revealed statistically significant factors ($p < 0.05$), which were then optimised with a 3-level complete factorial design. The highest dye removal rate during this phase was 85.08% (run 12). Subsequently, thirteen experiments based on the full factorial design yielded a maximum adsorption capacity of 91.75%. The regression model demonstrated a strong fit to the data ((Coefficient of Determination (R^2) = 0.9935, Adjusted R^2 (R^2-adj) = 0.9889, F-test for model significance (F) = 214.89, adequacy precision = 36.685)), and no significant difference was found between predicted and experimental values ($p > 0.05$), confirming the model's reliability.

Keywords: dye, wastewater, response surface methodology, adsorption

INTRODUCTION

Environmental challenges arising from growing human-induced pollution have become increasingly significant in the current phase of societal development. Notably, the volume of untreated wastewater released into surface water bodies continues to rise each year [1]. Biological methods for treating dye-contaminated wastewater are still under development. Adsorption, however, is quickly becoming a widely accepted technique for treating aqueous wastewater in various treatment technologies. Activated carbon is widely recognised as the predominant sorbent and also is regarded as a highly effective adsorbent. However, its high cost, particularly in developed countries, has limited its use, prompting researchers to explore alternative methods. This includes the use of bacteria that can utilize agricultural and food waste as a substrate, facilitating the sustainable production of biosorbents [2]. Nevertheless, these dyes represent a serious risk to aquatic ecosystems by interfering with photosynthetic processes. Moreover, some dyes and their breakdown products have been found to be toxic, carcinogenic, and mutagenic, and may also pose neurotoxic risks to humans [3]. Many industries utilise dyes or pigments for colouring, primarily in pulp and paper, food colouring, textile finishing, dye production, and cosmetics industries. Many of these chemicals can have an adverse effect on the environment if they are released into wastewater [4]. Worryingly, dye pollution in Malaysia and many other parts of the world is compounded by a lack of remediation [5, 6].

Bacterial biomass is employed as biosorbents for their potential application as biosorbents, and has been studied in the biosorption of dyes, including mixed organism/biomass systems [7]. Biomasses from plants, animals, and microorganisms (algae, bacteria, and fungi) as well as different byproducts have been used in several biosorption experiments to eliminate colours, metals, and other toxins. However, there is still a lack of research on the large-scale industrial use of biosorption [8]. Bacteria are preferred biosorbents due to their high surface area, a diversity of functional groups for sorption, uniform size, and natural availability from agricultural byproducts [9]. The goal of this study is to use the biomass of *Serratia marcescens* MM06 to maximise the removal (biosorption) of Congo Red dye from polluted solutions. Response Surface Methodology (RSM) will be used to find the optimal operating settings. Due to its toxicity, colour intensity, and resistance to degradation, Congo Red, a synthetic azo dye that is frequently used in textile and industrial operations, offers significant environmental dangers when found in wastewater.

The study intends to determine the most efficient environmental factors, including pH, dye concentration, biomass dose, agitation rate, temperature, and contact time, that maximise dye removal using *Serratia marcescens* MM06 biomass as an inexpensive, environmentally friendly biosorbent. The work helps to the development of an effective and sustainable treatment strategy for dye-polluted settings by optimising these parameters through RSM.

MATERIALS AND METHODS

Optimization of Congo red (CR) dye adsorption by response surface methodology (RSM)

The traditional single-factor optimization method is time-consuming and insufficient. Response surface methodology (RSM) optimizes and examines correlations between bioprocess parameters efficiently [10]. Placket-Burman design and 3-level full factorial were employed in Design-Expert 6.0.8 software to identify significant factors in Congo red dye adsorption [10 ,11]. Design-Expert 6.0.8 software was employed in RSM's statistical analysis and experimental design.

Optimization With PBD

The Plackett-Burman design identified key process parameters affecting Congo red dye biosorption by bacterial biomass. Variables were assigned high (+1) and low (-1) levels based on exploratory trials and literature review [5, 12]. Twelve experiments followed software instructions to screen six factors affecting Congo red dye adsorption, predicted using OFAT parameters. ANOVA identified statistically significant parameters ($p < 0.05$). After that, a 3-level full factorial was employed for further optimization [5, 11, 13].

Optimization using 3-Level full factorial

Following PBD optimization, significant parameters such as pH and adsorbent size were identified. These parameters were examined in more detail through a 3-level full factorial optimization, with pH ranging from 4.0 to 8.0 and adsorbent sizes ranging from 0.5 to 1.5 g. A 3-level full factorial [10, 11] was utilized to examine both significant parameters identified by PBD and their interactions, comprising a total of 13 trial runs conducted in triplicate. The adsorption percentage for each run was determined and recorded. After that, experimental validation was conducted on the software's final projected RSM response [14].

The experimental model's validation

In triplicate, the response surface model was verified under the anticipated circumstances, utilizing results from a statistical RSM study (3-level full factorial). The setup of the experiment chosen from the RSM model aimed to validate the established three-level full factorial model [14]. Most significant parameters validated experimental results. Actual % adsorption of Congo red dye using *Serratia Marcescens* MM06 biomass compared to the predicted value from three-level full factorial analysis [10].

RESULTS WITH DISCUSSION

Optimization of CR Compound adsorption by Response Surface Methodology (RSM)

After identifying the best condition for each factor using OFAT, the optimization process proceeded with the multivariate statistical method of Response Surface Methodology (RSM). The ideal state of Congo red dye uptake was furthermore determined via statistical methods, including the Plackett-Burman approach and a 3-level full factorial design.

Plackett-Burman design (PBD) technique

The Plackett-Burman Design technique analyzed Congo red dye adsorption by screening and selecting crucial variables affecting the process. Six independent parameters were screened through 12 experiments, revealing significant elements. Each parameter had two levels: low level (-1) and high level (+1), matching the lowest and highest OFAT values. Regression analysis of experimental findings evaluated with Design Expert (version 6.0.8) mapped the response surface and optimized key variables. The six variables affecting dye removal percentage range as follows: Initial dye content (50-100 mg/L), contact duration (5-20 min), pH (4-8), agitation rate (100-150 rpm), temperature (25-35 °C), and adsorbent dosage (0.5-1.5 g) (Table 1). With these settings, 12 successful Plackett-Burman tests were conducted. The dye adsorption was highest in run 12 (85.08%) and lowest in run 5 (7.96%). Dye removal percentage was selected as the response for statistical analysis, and important factors underwent more optimization using the 3-level full factorial model.

Table 1

Experimental setup and the proportion of Congo red dye that was adsorbed in 12 Plackett-Burman design (PBD) experimental runs

Таблица 1. **Экспериментальная установка и доля красителя Конго красного, который был адсорбирован в 12 экспериментальных прогонах по схеме Плакетта-Бермана (PBD)**

Run	A: Dye Conc. (PPM)	B: Contact Time. (min)	C: pH	D: Agitation (rpm)	E: Temp. (°C)	F: Adsorbent quantity (g)	Removal %
1	50	20	8	100	35	0.5	55.24
2	100	5	4	100	35	1.5	39.80
3	50	5	4	150	35	1.5	22.39
4	100	5	8	150	25	1.5	65.35
5	100	20	4	150	25	0.5	7.96
6	100	20	4	150	35	0.5	12.94
7	100	20	8	100	35	1.5	79.26
8	100	5	8	100	25	0.5	48.68
9	50	20	4	100	25	1.5	69.65
10	50	5	8	150	35	0.5	55.01
11	50	5	4	100	25	0.5	13.93
12	50	20	8	150	25	1.5	85.08

Table 2 presents the analysis of variance (ANOVA) and regression analysis for the first-order model. The models exhibited poor significance and linearity with minor curvature. Dye concentration, contact time, agitation speed, and temperature were all insignificant. Therefore, pH and adsorbent amounts were utilized in designing a three-level full factorial design. The model recorded an F-value of 10.52, showing noteworthy differences based on ANOVA analysis (Table 2). A p-value of 0.05 indicates significant model terms, with just a 1.03% possibility of such a large "model F-value" arising due to noise. According to ANOVA, the coefficient of determination (R^2) was 0.9266, indicating a poorly fitted regression line. The modified and anticipated R^2 values for dye adsorption were 0.8385 and 0.5771 respectively, showing disagreement. The sufficient precision value for signal-to-noise measurement was determined to be 9.379, indicating a satisfactory signal. Fig. 1 shows the plot of similarities between the real and expected values obtained by the PBD. The last equation with respect to real factors is shown in Equation 1:

$$Y = 2.74036 - 0.15775A + 0.72189B + 9.2482C - 0.1928D - 0.43359E + 27.96056F. \quad (1)$$

Adsorption optimization of dye using three-level full factorial design

The significant parameters from the design of Plackett-Burman were further improved via a three-level full factorial design. Thirteen experimental setups, including 5 center points, were performed as illustrated in Table 3. The R^2 value assesses the goodness of fit between predicted and experimental values, with higher values indicating better fit [15]. The highest adsorption capacity was observed in run 13 (91.75%). Regression analysis demonstrated a good fit of experimental data to the third-order polynomial model [15, 16]. The R^2 value in this study was 0.9935, indicating that the model accounts for over 99.35% of the variation in the response. The modified R-squared (R^2-adj) values were also great at 0.9889, confirming the model's significance. The model's F-value of 214.89 indicates high significance. Moreover, insignificant parameters are absent, as shown by the proximity of R-squared (R^2) and modified R-squared (R^2-adj) values. The "predict R-squared" (0.9462) and "adjust R-squared" (0.9889) values agree reasonably. The ade quacy precision ratio, measuring the signal-to-noise ratio, ideally exceeds 4. Within this study, the sufficient precision is 36.685, which denotes a good signal and prediction performance [17]. The analysis of Variance (ANOVA) is given in Table 4.

Table 2

Analysis of Variance (ANOVA) from PBD for Congo Red Dye Adsorption

***Таблица 2*. Дисперсионный анализ (ANOVA) по данным PBD для адсорбции красителя Конго красный**

Source	Sum of Squares	*df*	Mean Square	*F*-Value	Prob > F
Model	7324.38	6	1220.73	10.52	0.0103 Significant
Dye concentration (PPM)	186.65	1	186.65	1.61	0.2606
Contact time (min)	351.76	1	351.76	3.03	0.1422
pH	4105.4	1	4105.4	35.37	0.0019
Agitation speed (rpm)	278.8	1	278.8	2.4	0.1819
Temperature (ºC)	56.4	1	56.4	0.49	0.5168
Adsorbent quantity (g)	2345.38	1	2345.38	20.2	0.0064
Residual	580.4	5	116.08		
Cor Total	7904.79	11			
Std. Dev.	10.77		R^2	0.9266	
Mean	46.27		Adj R^2	0.8385	
C.V.	23.28		Pred R^2	0.5771	
PRESS	3343.11		Adeq Precision	9.379	

Key:
Sum of Squares: A measure of variation.
Degrees of Freedom (*df*): Represent the number of independent pieces of information available to estimate a parameter.
Mean Square: A measure of the average variation.
F-value: The F-value checks if the model is significant.
Prob > F (p-value): Probability that the model is significant by chance.
Residual: Numerical difference between the actual experimental value and the value predicted by a statistical or mathematical model.
Cor Total (Corrected Total Sum of Squares): The total variation in the response data after removing the effect of the mean.
Std. Dev. (Standard Deviation of the Model Residuals): The square root of mean square error (MSE).
C.V. (Coefficient of Variation): % measure of precision of the model.
PRESS (Predicted Residual Sum of Squares): Measures the predictive ability of the model.
Условные обозначения:
Sum of Squares (сумма квадратов) - мера вариации.
Степени свободы (df) представляют собой количество независимых фрагментов информации, доступных для оценки параметра.
Mean Square (средний квадрат) - мера средней вариации.
F-Value - F-значение проверяет, является ли модель значимой.
Prob > F (p-значение) - вероятность того, что модель случайно значима.
Residual (остаток) - численная разница между фактическим экспериментальным значением и значением, предсказанным статистической или математической моделью.
Cor Total (скорректированная общая сумма квадратов) - общая вариация данных отклика после удаления эффекта среднего.
Std. Dev. (стандартное отклонение остатков модели) - квадратный корень из средней квадратической ошибки (MSE).
Коэффициент вариации (C.V.) - процентная мера точности модели.
PRESS (предсказанная сумма квадратов остатков) - измеряет предсказательную способность модели.

Table 3

Experimental layout and result for the optimization of dye using a three-level full factorial design

***Таблица 3.* Экспериментальная схема и результат оптимизации красителя с использованием трехуровневого полного факторного дизайна**

Run	A: pH	B: Adsorbent quantity (g)	Actual value (%)	Predicted value (%)
1	6	1	89.29	88.25
2	8	0.5	57.67	58.56
3	4	1	85.93	85.02
4	8	1.5	90.77	89.93
5	6	1	87.23	88.25
6	4	0.5	56.27	57.50
7	8	1	86.33	86.28
8	6	0.5	62.90	60.69
9	6	1	87.83	88.25
10	6	1	87.96	88.25
11	4	1.5	90.98	90.11
12	6	1	87.96	88.25
13	6	1.5	90.51	91.75

Table 4

Analysis of Variance (ANOVA) for a three-level full factorial model

***Таблица 4.* Дисперсионный анализ (ANOVA) для трехуровневой полной факторной модели**

Source	Sum of Squares	*df*	Mean Square	*F*-Value	Prob > F
Model	2016.45	5	403.29	214.89	< 0.0001
A	2.37	1	2.37	1.26	0.2983
B	1447.67	1	1447.67	771.39	< 0.0001
A2	18.67	1	18.67	9.95	0.0161
B2	399.63	1	399.63	212.95	< 0.0001
AB	0.093	1	0.093	0.05	0.8298
Residual	13.14	7	1.88		
Lack of Fit	10.85	3	3.62	6.31	0.0536
Pure Error	2.29	4	0.57		Significant
Cor Total	2029.59	12			
Std. Dev.	1.37	R-Squared	0.9935		
Mean	81.5	Adj R-Squared	0.9889		
C.V.	1.68	Pred R-Squared	0.9462		
PRESS	109.19	Adeq Precision	36.685		

Note: A: pH, B: biosorbent dosage (g)

Примечание: A: pH, B: дозировка биосорбента (g)

The findings align with previous work, where the model's R-squared (R^2) value was close to 1 (0.9994), and the modified R-squared (R^2-adj) was elevated as well (0.9990). The "predict R-squared" and "adjust R-squared" values were in agreement at 0.9978 and 0.9984, respectively. Adequate precision was 152.669, indicating sufficient signal for accurate prediction [16]. The study highlighted the influence of pH, adsorbent size, and their interaction on dye biosorption. The relationship between real and anticipated values in the three-level full factorial design is depicted in Fig. 1, showing a significant correlation. Equations 2 and 3 represent these relationships based on coded and real factors.

$$Y = -15.30+7.96A+126.38B-0.65A^2-48.12B^2+0.15AB \quad (2)$$

$$Y = 88.25+0.63A+15.53B-2.6A^2-12.03B^2+0.15AB \quad (3)$$

3D response visualisation plot of Congo red dye sorption

3D response surface graphs illustrate relationships between dependent and independent factors, aiding in understanding their main and interaction effects [18]. Design-Expert software was utilized to generate graphs and contours for the 3D reaction surface model, depicting mutual interactions between independent parameters and their impact on the dependent parameter, such as adsorption [19]. Elliptical contours signify significant interactions between independent parameters. Fig. 2 displays 3D reaction surfaces and contour graphs depicting interactions between pH (A) and adsorbent dosage (B) concerning Congo red dye removal. At a constant concentration of 90 PPM, contact time of 20 min, agitation rate of 125 rpm, and temperature of 25 °C, the relationships with pH and adsorbent dose were significant. Within the experimental ranges, dye adsorption increases with larger adsorbent sizes but decreases with lower pH levels. According to past conducted studies, 3D response surface and contour plots in a two-level factorial design illustrate interactions among temperature, pH, and ionic strength, aiding in parameter optimization [20]. Similarly, past research utilized 3D response surface and contour plots in a three-level factorial design to depict interactions among volume fraction, temperature, and ionic nanoparticle size, assisting in understanding variable interactions and optimal parameter selection [16].

Validation of RSM experiment

To experimentally verify the predicted Congo red dye adsorption, the two significant variables were set at optimal values of pH 7.18 and absorbent dose of 1.24 g, as determined by a three-level full factorial. Other factors (concentration, contact time, agitation, and temperature) remained constant based on OFAT due to their insignificance in the PBD analysis, and the experiment was conducted in triplicate. The experimental value of 90.37% dye absorption closely matched the model's predicted value of 88.24%, indicating no significant difference between them (t-test, $p > 0.05$) and supporting the model's validity.

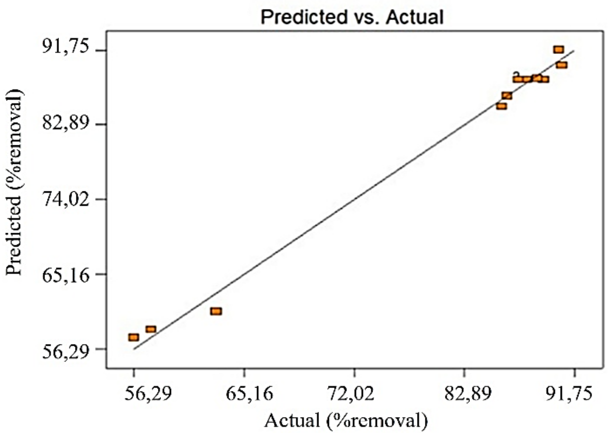

Fig. 1. Experimental and predicted factors for optimal dye adsorption responses
Рис. 1. Экспериментальные и прогнозируемые факторы для оптимальной реакции адсорбции красителя

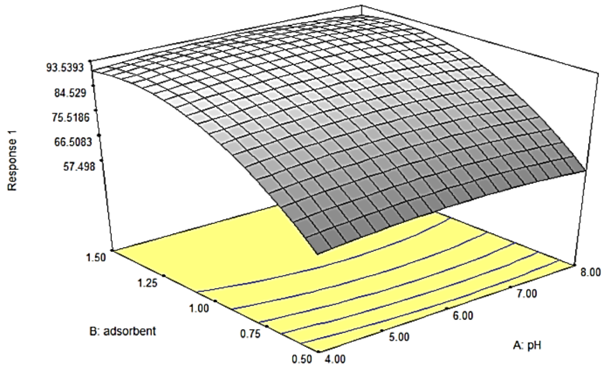

Fig. 2. Three-dimensional (3D) reaction surface graphs of dye adsorption illustrating the relationship between adsorbent size and pH
Рис. 2. Трехмерные (3D) графики реакционной поверхности адсорбции красителя, иллюстрирующие связь между размером адсорбента и pH

CONCLUSION

The potential of bacterial biosorbents as an affordable and environmentally friendly substitute for wastewater colour removal is highlighted by this study. Response Surface Methodology (RSM) statistical optimisation greatly improved adsorption efficiency, raising removal rates from 85.08% to 91.75%. The model's practical application in wastewater treatment was supported by its great predicted accuracy and capacity to identify the most favourable conditions.

DECLARATIONS BY THE AUTHORS

Information and Data availability

Any models, data, or programs created or used during the study will be made available by the relevant author upon request.

CONFLICTING INTERESTS

There is no conflict of interest. After reading and approving the manuscript’s content, each author bears responsibility for all aspects of its accuracy and integrity in accordance with COPE standards.

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THE ROLE AND INVOLVEMENT OF AUTHORS

Ibrahim Alhaji Sabo, the first author, wrote the first draft of the article and carried out the experiment.Motharasan Manogaran performed the statistics.

Yunus Shukor, Nur Adeela Yasid, and Ahmad Razi Othman reviewed and revised the article and provided suggestions for enhancements.

The authors declare the absence of conflict of interest warranting disclosure in this article.

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