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# РАВНОВЕСИЕ, КИНЕТИКА И ТЕРМОДИНАМИЧЕСКИЕ ИССЛЕДОВАНИЯ БИОСОРБЦИИ КОНГО КРАСНОГО КРАСИТЕЛЯ БИОМАССОЙ *SERRATIA MARCESCENS* MM06

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***Красители представляют собой экологические риски с канцерогенными эффектами, которые ощущаются во всем мире, в том числе в Малайзии. Загрязнение воды происходит из различных источников, таких как производственные компании, исследовательские и медицинские центры. Целью исследования является изучение биосорбции красителя Конго красный (CR) бактериальной биомассой для промышленного применения. Двенадцать различных видов бактерий были протестированы на CR. Он проверяет и оптимизирует бактериальную сорбцию с использованием однофакторной (OFAT), он исследует инфракрасную Фурье-спектроскопию (FTIR), сканирующий электронный микроскоп (SEM), кинетические и равновесные модели и термодинамику. Штамм Serratia marcescens MM06 удалил больше всего красителя среди 12 бактериальных изолятов. Биомассу инкубировали с красителем 100 ppt, затем центрифугировали и измеряли при 507 нм. Оптимальными условиями после OFAT были 25°C, pH 7,0, перемешивание 125 об/мин, 20 мин, концентрация красителя 90 мг/л и дозировка абсорбента 0,94 г. FTIR и SEM характеризовали биосорбцию. Нелинейная регрессия проанализировала изотермы и кинетику, выявив псевдовторой порядок и Ленгмюр как наилучшие соответствия. Параметры подобранных изотерм, а именно, Ленгмюра, Фрейндлиха, БЭТ, Sips, Фрица-Шлюндера IV и Фрица-Шлюндера V, составили 4,387657 мг/г, 3,750862 мг/г, 4,387657 мг/г, 2,800659 мг/г, 12,178957 мг/г, 2,557313 мг/г соответственно. Параметры термодинамической свободной энергии Гиббса (DG), энтальпии (DH) и энтропии (DS) модификации указывают на экзотермические и спонтанные процессы. Serratia marcescens MM06 оказался эффективным для удаления красителя CR из сточных вод.***

**Ключевые слова:** биосорбция, конго красный, термодинамика, изотермы

# EQUILIBRIUM, KINETICS, AND THERMODYNAMIC STUDIES OF THE BIOSORPTION OF CONGO RED DYE BY THE BIOMASS OF *SERRATIA MARCESCENS* MM06

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***Dyes pose environmental risks with carcinogenic effects, felt globally including in Malaysia. Water contamination stems from various sources like manufacturing companies, research, and medical centers. The research aims to study bacterial biomass biosorption of Congo red (CR) dye for industry applications. Twelve different bacterial species were tested against CR. The study screens and optimizes the bacterial sorption process using a one-factor-at-a-time (OFAT) approach, and characterizes the biosorbent through FTIR analysis, SEM imaging, adsorption kinetic and isotherm modeling, and thermodynamic evaluation. Serratia marcescens strain MM06 removed the most dye among 12 bacterial isolates. Biomass was incubated with 100ppm dye, then centrifuged and measured at 507nm. Optimal conditions after OFAT were 25 °C, pH 7.0, 125 rpm agitation, 20 min, 90 mg/L dye concentration, and 0.94g absorbent dosage. FTIR and SEM characterized biosorption. Nonlinear regression analyzed isotherms and kinetics, revealing pseudo-second-order and Langmuir as best fits. The parameters of the fitted isotherms, namely, Langmuir, Freundlich, BET, Sips, Fritz-Schlunder IV, and Fritz-Schlunder V were 4.387657 mg/g, 3.750862 mg/g, 4.387657 mg/g, 2.800659 mg/g, 12.178957 mg/g, 2.557313 mg/g respectively. The parameters of thermodynamic Gibbs free energy (DG), enthalpy (DH), and entropy (DS) modifications indicated exothermic and spontaneous processes. Serratia marcescens MM06 proved effective for CR dye removal from wastewater.***

**Keywords:** biosorption, congo red, thermodynamics, isotherms

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## INTRODUCTION

The treatment of wastewater is a crucial and challenging research field. Various physicochemical and biological processes are used to remove color from industrial effluents. The global production of dyes is around 900 thousand tonnes per year, with 10-15% discharged as untreated wastewater from textile, paper, chemical, and other industries that utilise colours for dyeing products [1]. Biological treatment is one of the most popular and economical dye treatment techniques; yet it is sometimes difficult and time-consuming to implement in complicated wastewater matrices with low dye concentrations [2]. These effluents often contain poisonous dyes that are harmful to living organisms, being mutagenic and carcinogenic [3]. Many developing nations with poorly enforced rules on the production and consumption of dyes are nevertheless seeing an increase in dye pollution. Examples of these countries include Bangladesh, Pakistan, India, Malaysia, and others. A significant portion of dye pollution contamination was found in Malaysia's Juru riverine area [4].

Local researchers are actively seeking ways to remove Congo red dye. One particularly effective and reasonably priced technique is bioremediation, particularly biosorption. Because of their large surface area, variety of sorption-capable functional groups, homogeneous size, and inherent availability from agricultural waste, bacteria are the preferred biosorbents [5]. AMT-Bioclaim$^{TM}$, a well-established biosorbent, is a bacterial biomass example [6]. This research presents the first report on Congo red sorption using inactivated biomass of *Serratia marcescens* strain MM06.And it is warranted as it tackles the high cost and limited availability of activated carbon for dye removal by using *Serratia marcescens* strain MM06 biomass as an affordable and sustainable alternative. This study builds on existing bioremediation techniques, with a focus on Congo red dye biosorption, delivering an eco-friendly solution for textile wastewater treatment.

## MATERIALS AND METHODS

### *Equipment, chemicals, and reagents*

The reagents consumed were of the highest rational quality. Congo red dye was brought from Sigma Aldrich Co. in the United States. Synthetic wastewater was created in the lab by mixing the dye using deionized water. A stock solution was prepared by dissolving 100 mg of dye salt in 100 mL of water. pH was altered using 0.5 N HCl or 0.1 mol NaOH. Absorbance was normalized with a spectrophotometer scanning from 400 to 800 nm, with the highest peak observed between A450 and A507 nm [7].

### *Bacterial Source*

Twelve distinct pure bacterial cultures (designated as Isolates 34XR, 34XW, 52, 2, 1, 29, 7, 8, 4, 5.2, 30, and 5.1) remained sourced from the Bioremediation, Biomonitoring, and Ecotoxicology Laboratory (BBE) within the Faculty of Biotechnology and Biomolecular Sciences at Universiti Putra Malaysia. Initially, a sample taken from the Juru riverbanks in Pulau Pinang, Malaysia, was used to identify these bacteria [8]. The river and surrounding area have been reported to have high levels of pollution due to local textile and pigment industries [9]. As a result, regular sub-culturing were carried out in aseptic laboratory settings using Nutrient Broth (NB) as a growth medium to maintain the purity of each isolate.

### *Bacterial Biomass Extraction*

Each of the twelve (12) isolates had their biomass removed and tested for the ability to absorb Congo red dyes. A 24-hour-old culture of the isolates, with an approximate absorbance of 1 OD600, Centrifuged at a speed of 10,000 rpm for 10 min [10]. The decanted liquid from each unique isolate was removed, and the pellets were rinsed two times using Tris Buffer (pH 7.0). The cells in the pellet were then rendered nonviable by heating them in a water bath for one hour at 60 degrees Celsius [11] .The adsorption of Congo red was then studied (researched) utilising pellets.

### *Bacterial biomass screening*

0.94 g of each of the 12 nonviable bacterial biomass were combined with 10 mL of Congo red dye in 50 mL flasks, labelled, and Cultivated at 25 °C at 150 rpm. A two-milliliter portion was obtained every five minutes to track the adsorption of each bacterial biomass, then centrifuged and measured at OD507 nm. Experiments were triplicated to ensure consistency, and percentage adsorption was calculated accordingly using the formula below.

$$Dye\ adsorption\ percentage\ (\%) = \\ = \frac{Y-Z}{Y}\ \chi\ 100. \qquad (2.1)$$

Where Y is the initial absorbance prior to growth and Z is the final optical density after growth. The isolate with the highest Congo red dye absorption percentage was chosen for further investigation.

### *One factor at a time (OFAT) strategy method employed to enhance Congo red dye adsorption*

Isolate 34XR (*Serratia marcescens* MM06) shows the highest adsorption of Congo red dye, as per screening results. *Serratia marcescens* MM06 was previously characterized by [8]. The adsorption of dye is influenced by various parameters. OFAT method used to optimize conditions for dye adsorption using *Serratia marcescens* mm06. Optimizing conditions such as initial dye concentration, contact time, pH, agitation speed, temperature, and adsorbent size aids industrial-scale dye removal treatment development [12]. Each parameter is checked individually while keeping previously optimized parameters constant. Experiments conducted in triplicate.

*Characterization of biosorbent (biomass)*

*Fourier Transform Infrared Spectrometer (FTIR)*

FTIR analysis of *Serratia marcescens* MM06 biomass (treated and untreated with Congo red) was performed after drying and sieving [13]. FTIR (ATR) analysis using VERTEX 80v (Bruker Optics Korea) was performed from 400-4000 $cm^{-1}$ to identify functional groups. Samples were mixed with KBr and pressed into thin pellets for spectral examination.

*Scanning electron microscopy (SEM)*

FESEM (LEO1455) operated at 20 kV and 13 mm working distance was used for morphology analysis. Gold-coated samples were imaged at 500-5000 × magnification (10-50 µm).

*Adsorption kinetics modeling*

Batch kinetics experiments were conducted across varying amounts of dye (10, 20, 30, 40, 50, 70, and 90 ppm) based on OFAT and RSM results. Supernatant analysis occurred via a spectrophotometer at 507 nm, with absorbance measurements plotted on a graph. The best kinetics model was selected from previously assessed options, involving pseudo-first-order (PFO) and pseudo-second-order (PSO) kinetics [14, 15]. PFO, proposed by Lagergren, assumes an adsorption rate proportional to the difference between adsorbed and available concentrations [16]. PSO suggests a second-order relationship between rate and saturation concentrations, indicating chemisorption as the rate-limiting step [17]. Equations for PFO and PSO models were expressed in Equations 2.2 and 2.3 accordingly.

$$q_t = q_e(1 - e^{-K_1 t}), \quad (2.2)$$

$$q_t = \frac{K_2 {q_e}^2 t}{(1 + K_2 q_e t)}. \quad (2.3)$$

Where $q_e$ and $q_t$ stand for the quantity of dye adsorbed (*mg/g*) at equilibrium and time *t* (min), respectively. $K_1$ and $K_2$ are the rate coefficients of pseudo-first-order ($min^{-1}$) and pseudo-second-order sorption (*g mg/min*), respectively.

*Adsorption isotherm model*

Equilibrium data underwent analysis via various isotherm models after batch experiments across variations in dye concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 70 ppm, and 90 ppm. Eight isotherm models, ranging from one to five parameters, were employed to determine the best-fit model for Congo red dye adsorption, employing nonlinear regression instead of linear regression [13, 18].

*Data Fitting*

Nonlinear regression was used to fit the adsorption kinetics and isotherms nonlinear pattern in data using the Marquardt algorithm in CurveExpert Professional software, Version 2.6.5.

*Thermodynamics study*

The dimensionless equilibrium constant (KL) between phases was determined using the Langmuir equation (Equation 2.4).

$$q_e = \frac{q_{mL} K_L C_e}{1 + K_L C_e}. \quad (2.4)$$

Equation 2.5 was then used to transform the Langmuir $K_L$ value into a dimensionless form $K_C$.

$$K_C = 696.6 \cdot 55.5 \cdot 1000 \cdot K_L. \quad (2.5)$$

Congo red dye has a molecular weight of 696.6 $gmol^{-1}$, its molarity is 55.5, and the term 696.6 · 55.5 · 1000 · $K_L$ will be dimensionless.

The exact evaluation of the two-phase equilibrium constant, known as $K_C$, is directly related to the accuracy of the thermodynamic parameter estimates that are produced.

Equation 2.6 describes the dimensional endless $K_L$'s relationship to the dimensionless equilibrium constant $K_C$, C° represents the specified standard of adsorbate (C = 1mol/L), $K_L$ (L/mol) signifies Langmuir constant, and γ (dimensionless) symbolises activity coefficient of adsorbent in solution.

$$K_C \approx \frac{K_L\left(\frac{L}{mol}\right) \cdot C^{\circ}\left(\frac{mol}{L}\right)}{\gamma}. \quad (2.6)$$

The thermodynamics parameters, namely Gibbs free energy Change (*ΔG*), Enthalpy change (*ΔH*), and Entropy change (*ΔS*) were computed through the van't Hoff equation and applying principles of thermodynamics [13, 19] as shown in the following equations:

$$\Delta G^{\circ} = -RT \ln KL, \quad (2.7)$$

$$\Delta G^{\circ} = \Delta H - T\Delta S^{\circ}, \quad (2.8)$$

$$lnK_C = \frac{-\Delta H^{\circ}}{R} \cdot \frac{1}{T} + \frac{\Delta S^{\circ}}{R}. \quad (2.9)$$

*R* stands for universal gas constant, 0.00831 kJ/mol·K.

## RESULTS WITH DISCUSSION

*Examination and screening of bacterial biomass*

Fig. 1 displays twelve bacterial isolates' screening results against Congo red dye. Isolate 34XR absorbed 82% of the dye, while Isolate 34XW absorbed 64%, Isolate 52 absorbed 51%, Isolate 2 absorbed 71%, Isolate 1 absorbed 61%, Isolate 29 absorbed 50%, Isolate 7 absorbed 69%, Isolate 8 absorbed 19%, Isolate 4 absorbed 46%, Isolate 5.2 absorbed 68%, Isolate 30 absorbed 49%, and Isolate 5.1 absorbed 81%. Isolate 34XR showed the highest adsorption percentage among the eleven isolates, reaching 82%. Each isolate displayed the capability to adsorb Congo red dye in wastewater. Previous studies revealed most of the isolates can decolorize or proliferate in environments containing Reactive Red 120 dye [8]. Additionally, past research has highlighted the potential of bacterial biomass in dye adsorption [20]. Isolate 34XR was recently identified and characterised as *Serratia marcescens* strain MM06 [8]. It belongs to the Gram-negative bacterium *Serratia marcescens* of the Yersiniaceae lineage. It is characterised as a facultative anaerobe and an opportunistic pathogen, initially discovered in 1819 by Bartolomeo Bizio in Padua, Italy. Various commercial biosorbents and their manufacturers were stated in previous work [21, 5]. Bacterial biosorption, particularly for Congo red dye, is a promising technique for pollutant reduction.

All *Serratia* strains have been shown to grow most effectively at pH 9 and temperatures between 20 and 37 °C. The isolate, *Serratia marcescens* strain MM06 16S ribosomal RNA gene, the partial sequence has 1,442 bp linear DNA and Accession number MW031902.1 GI: 1908125079 [8].

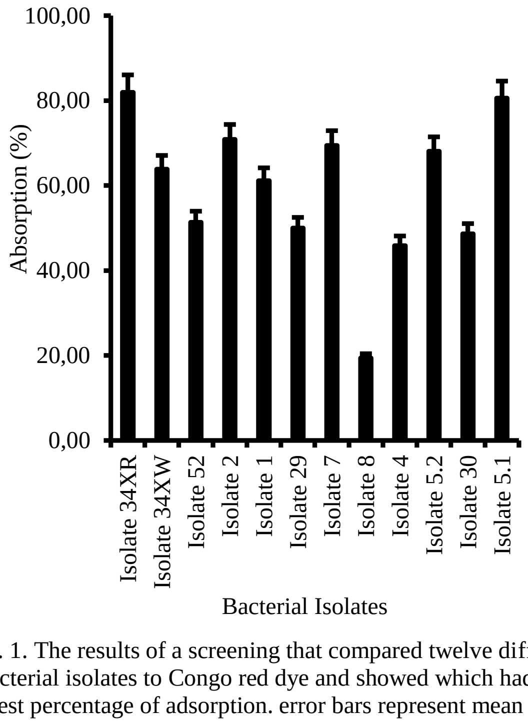


Fig. 1. The results of a screening that compared twelve different bacterial isolates to Congo red dye and showed which had the highest percentage of adsorption. error bars represent mean +/- std deviation (n=3)

Рис. 1. Результаты скрининга, в ходе которого сравнивались двенадцать различных бактериальных изолятов с красителем Конго красным и был выявлен тот, который имел самый высокий процент адсорбции. Погрешности представляют собой среднее значение +/- стандартное отклонение (n=3)

*Enhancement of the adsorption of congo red dye employing the OFAT approach*

The OFAT method was applied to explore how a variety of environmental conditions had an impact on Congo red dye's adsorption Fig. 2.

*Influence of the Initial Dye Concentration*

Despite the decrease in adsorption proportion, the amount of dye adsorbed increases as solution concentration rises at constant adsorbent dosage [22]. *Serratia marcescens* strain MM06's resilience to higher dye concentrations was studied in a batch experiment spanning 10 to 100 ppm dye concentrations, as depicted in Fig. 2a. As dye concentration rises, the proportion of adsorption diminishes. Calibration of Congo red dye at various concentrations, including 10, 20, 30, 40, 50, 90, and 100 ppm, facilitated absorbance identification. Verma and Madamwar found that *Serratia marcescens* effectively decolorized Ranocid Fast Blue (RFB) and Procion Brilliant Blue-H-GR (PBB-HGR) at day 8 and day 5, respectively, achieving over 90% decolorization at 26 °C, pH 7.0, and 100 mg/L concentration in stagnant circumstances [23]. Decolorization depended on initial dye concentration, pH, and temperature, despite a final concentration of 100 mg/L. The bacterium showed resistance to dyes at 100 mg/L, but decolorization time increased with dye concentration.

*Effect of Contact Duration*

The capacity to adsorb typically increases over the period until equilibrium, where no more dye can be adsorbed. Equilibrium time represents the duration to achieve this state [22]. To assess the impact of contact duration on Congo red dye adsorption, % dye adsorption was calculated at 5 to 100-min intervals**.** Fig. 2b indicated suboptimal adsorption between 5 and 15 min, However, adsorption was at its peak at 20 min and thereafter dropped from 40 to 100 min, suggesting that binding site saturation caused the biosorbent to reach its contact time limit.

A different research assessed the influence of contact time on the Methylene Blue and Congo Red biosorption using processed waste *Streptomyces fradiae* biomass [24]. They investigated a contact time range of 5 to 180 min, finding rapid dye uptake initially, slowing until equilibrium at 70 and 80 min for CR and MB respectively. Similar findings were noted in CR dye removal using *Eichhornia crassipes* roots [25]. The dye removal rate was initially rapid but slowed as vacant adsorbent sites diminished over time, reaching equilibrium when all sites were occupied.

*The pH's effect*

The dye solution's pH and the activity of functional groupings on the surface are crucial parameters affecting biosorption removal. Elevated pH enhances the elimination of basic dyes nevertheless decreases the elimination of acidic dyes [24]. In Fig. 2c, the bacterial biomass achieved the highest Congo red dye removal rate at pH 7. The percentage of dye removal increases from 50 to 61% as pH rises from 5.0 to 7 but drops at pH 8.5. However, increasing pH leads to a negatively charged biosorbent surface, facilitating electrostatic interactions with oppositely charged sorbate. At low pH, the biosorbent surface is positively charged, resisting the biosorption of cationic species [24, 26].

*Agitation rate's effect*

The shaking rate significantly impacts the adsorption process by influencing solute distribution and boundary film formation. Higher agitation rates generally increase dye adsorption rates [22]. The agitation rate enhances system mobility while reducing boundary layer resistance. Effects of agitation rate on Congo red dye adsorption by *Serratia marcescens* strain MM06 were examined, as depicted in Fig. 2d across various agitation speeds (50, 75, 100, 125, 150, 175, 200, and 250 rpm). Studies suggest that using raw adsorbent with an unaltered structure may damage it, as observed in lead biosorption by *Bacillus cereus*, where high shear rates at 200 rpm caused biomass damage [27].

*Temperature Effect*

Temperature greatly influences the adsorption reaction, revealing modifications in enthalpy and entropy during the process [28]. Previous studies found temperature impacts sorption processes in two main ways: reducing solution viscosity, accelerating adsorbate diffusion into pores, and altering adsorbent capacity for equilibrium with a specific adsorbate [29]. The Congo red dye's absorption was examined at various temperatures from 25 to 60 °C, as shown in Fig. 2e. Optimal adsorption occurred at 25 °C, with decreasing adsorption as temperature increased, suggesting a lower energy requirement for the process. Another study found that biomass had reduced biosorption capacity with higher temperatures [30]. Some research noted an increase in adsorption capacity from 30 to 50 °C, indicating an endothermic adsorption process [31]. Similar research revealed that absorption increases with decreasing temperature, indicating temperature's influence on biosorption is similar to physical adsorption [32]. Consequently, absorption may rise or fall with temperature changes, contingent upon the sorption type, whether physisorption or chemisorption.

*Adsorbent dosage's effect*

Fig. 2f illustrates the impact of the amount of adsorbent on Congo red eliminations by *Serratia marcescens* strain MM06. Increasing the amount of bacterial biomass, by raising the adsorbent dose from 0.5 to 1.5 g, enhances Congo red dye removal. Dye uptake rises from 55% to 74%, likely as a result of a rise in adsorbent adsorptive surface and accessible binding sites. This aligns with previous findings that indicate a decline in adsorption capacity with increased adsorbent dosage but a notable rise in adsorption percentage [19]. Additionally, studies found that a greater adsorptive surface correlates to a greater number of available adsorption sites [33]. Similar research demonstrated that raising the bulk of the biosorbent improved the elimination of colour percentages for AO7 and RB5 between 41 and 51%, and 41 and 95%, respectively [34].

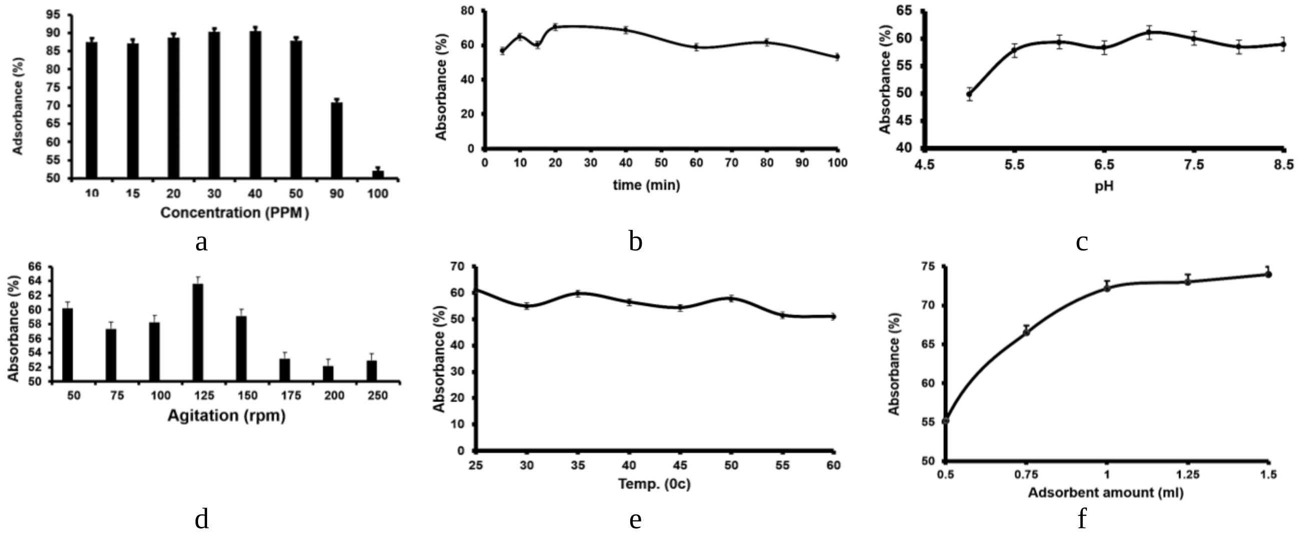


Fig. 2. OFAT-based optimization of Congo red dye absorption with *Serratia marcescens* strain MM06; a. An influence of initial dye concentration, b. Effect of contact duration, c. The pH's effect, d. Agitation rate's effect, e. Temperature Effect and f. Adsorbent dosage's effect

Рис. 2. Оптимизация абсорбции красителя Конго красный на основе OFAT штаммом *Serratia marcescens* MM06; a. Влияние начальной концентрации красителя, b. Влияние продолжительности контакта, c. Влияние pH, d. Влияние скорости перемешивания, д. Влияние температуры и e. Влияние дозировки адсорбента

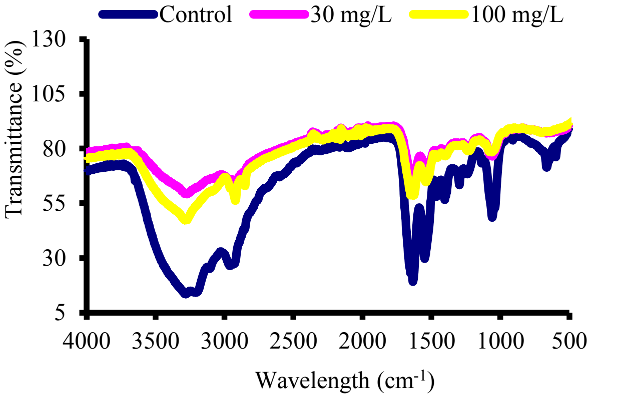


Fig. 3. Assessment in the FTIR spectrum of *Serratia marcescens* strain MM06 biomass ahead of and after being treated using 30 and 100 mg/L

Рис. 3. Оценка в спектре ИК-Фурье биомассы штамма *Serratia marcescens* MM06 до и после обработки с использованием 30 и 100 мг/л

Optimal conditions for Congo red dye adsorption using *Serratia marcescens* strain MM06 biomass were stated through OFAT optimization. Optimized Parameters included initial dye concentration, contact duration, pH, agitation rate, temperature, and absorbent dosage. Effective adsorption occurred at 90 ppm concentration of dye, 20 min contact time, pH 7, 125 rpm agitation rate, 25 °C temperature, and 0.94 g adsorbent amount. Numerous ranges of optimization conditions for dye uptake parameters were documented in the literary works [8]. Successful research removed malachite green using dry *Bacillus cereus* M116 cells as a biosorbent. Environmental factors, such as pH 5.0, 0.5 g/L biomass amount, 400 mg/L dye content at the start, and 360 min contact time, yielded optimal conditions at the highest dye absorption potential of 485 mg/g [35]. Similarly, conducted research determined that for Azo blue dye breakdown by *Streptomyces* DJ15, ideal conditions included 40 °C temperature, pH 7.0, 3% (v/v) inoculum size, 50 mg/L initial dye content, and 0 rpm agitation speed [36]. Optimizing environmental parameters significantly enhances the adsorption process, as noted in this study and others.

*Characterization of bacterial biosorbent using FTIR and SEM techniques*

*FTIR analysis*

Prominent peaks are found in the 4000-400 cm$^{-1}$ wavenumber region, as depicted in Fig. 3. Peaks surrounding the current peak are divided into two groups: one for molecular fingerprinting, below 1500 cm$^{-1}$, and the other (functional region) for detecting active groups (1500 cm$^{-1}$ to 4000 cm$^{-1}$), where most stretching frequencies occur. The fingerprint region uniquely identifies molecules [31]. Fig. 3 depict the differences in FTIR spectra of *Serratia marcescens* strain Mm06 biomass prior to and after being treated using 100 mg/L and 30 mg/L. This analysis helped identify major functional groups involved in biosorption within bacterial biomass. Adsorption peaks in FTIR spectra suggested straightforward biosorption aided by various groups and substances in Congo red dye, including $-NH_2$, $-SO_3$, C-H, and C-O, as indicated in conducted studies [37].

*Scanning Electron Microscopy (SEM) Examination*

SEM analysis of the biosorbent before and after adsorption was conducted using a JEOL JSM-7600F (×5000 magnification), with cleaned, dried, and ground samples examined and micrographs recorded [38]. Samples were dried at 65 °C before SEM analysis, revealing smooth, dense, non-porous surfaces with clear dye–biomass adhesion [39]. SEM analysis showed irregular macro-pores enhancing CR dye adsorption, with micrographs of *Serratia marcescens* MM06 revealing pore presence and internal surface structure [40]. Fig. 4, showing asymmetrical pores capable of pore diffusion.

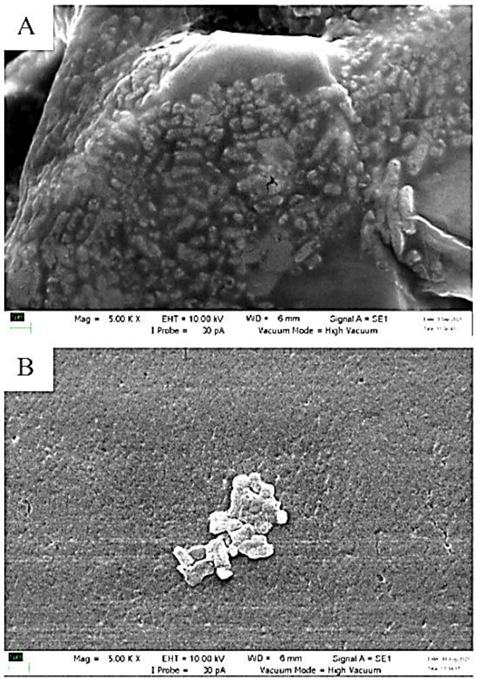


Fig. 4. Scanning electron micrographs of *Serratia marcescens* strain MM06 (A) ahead of dye adsorption and (B) after dye being adsorbed (Magnification: x5,000)

Рис. 4. Сканирующие электронные микрофотографии штамма *Serratia marcescens* MM06 (A) до адсорбции красителя и (B) после адсорбции красителя (Увеличение: x5000)

*Determining the appropriate kinetic and best isotherm model for batch adsorption processes*

In this study, a batch kinetic experiment investigated Congo red dye adsorption at diverse concentrations varying between 10 to 90 mg/L for 30 min. Both low and high dye concentrations were explored over time, with all other parameters held constant based on OFAT results. Pseudo-first-order and pseudo-second-order equations were utilized to analyze and fit adsorption data using nonlinear kinetic regression and curve-fitting software [41]. The fitting of pseudo-first- and pseudo-second-order kinetics for Congo red dye is depicted in Fig. 5A and B, respectively.

Adsorption kinetics, specifically pseudo-second-order, govern the rate of reaching equilibrium. This implies chemisorption, supported by valence forces, but kinetics alone can't fully explain adsorption mechanisms [42]. Multiple studies have identified pseudo-first and pseudo-second-order kinetics as the best models for adsorption, as demonstrated by previous researchers [14, 42, 43]. A particular study found excellent agreement between experimental and theoretical data using the second-order model [44] while concluding that the pseudo-second-order model was appropriate for Brilliant Green dye sorption in a different study [45].

Various adsorption isotherms for instance Henry, Langmuir, Freundlich, BET, Toth, Sips, Fritz-Schlunder IV, and Fritz-Schlunder V were examined and then characterized via theoretical and empirical models. Table 1 display the different isotherm models and error function analysis for fitting dye adsorption isotherms.

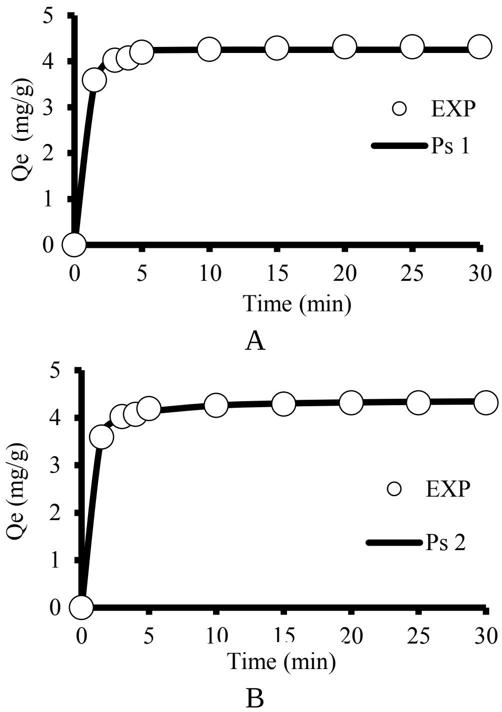


Fig. 5. Adsorption profile of *Serratia marcescens* strain MM06 biomass and Congo red dye fitted to (A) 1st pseudo-order kinetic model and (B) pseudo-second-order kinetic model

Рис. 5. Профиль адсорбции биомассы штамма *Serratia marcescens* MM06 и красителя Конго красный, подобранный по (A) кинетической модели первого псевдопорядка и (B) кинетической модели псевдовторого порядка

***Table 1***

**Evaluation of Error function assessment for the adsorption model applied to the 8 chosen isotherms**

***Таблица 1*. Оценка функции ошибки для адсорбционной модели, примененной к 8 выбранным изотермам**

| Model | *p* | RMSE | *adR2* | AICc | BIC | HQC | BF | AF |
|---|---|---|---|---|---|---|---|---|
| Henry | 1 | 2.44 | -0.33 | 24.47 | 19.06 | 18.42 | 0.47 | 2.44 |
| Langmuir | 2 | 0.02 | 1.00 | -67.74 | -77.13 | -78.40 | 0.80 | 1.25 |
| Freundlich | 2 | 0.11 | 0.99 | -31.75 | -41.14 | -42.41 | 1.29 | 1.31 |
| BET | 3 | 0.04 | 1.00 | -47.31 | -62.40 | -64.30 | 1.28 | 1.29 |
| Toth | 3 | 1.73 | -0.92 | 29.39 | 14.30 | 12.40 | 0.54 | 1.86 |
| Sips | 3 | 0.02 | 1.00 | -60.85 | -75.94 | -77.85 | 0.79 | 1.26 |
| F4 | 4 | 0.02 | 1.00 | -49.86 | -73.65 | -76.19 | 0.80 | 1.25 |
| F5 | 5 | 0.02 | 1.00 | -31.74 | -70.22 | -73.39 | 0.79 | 1.26 |

Примечание: RMSE – Среднеквадратичная ошибка (Root Mean Square Error)
p – Количество параметров
$adR^2$ – Скорректированный коэффициент детерминации
BF – Коэффициент смещения (Bias Factor)
AF – Коэффициент точности (Accuracy Factor)
AICc – Скорректированный информационный критерий Акаике (Adjusted Akaike Information Criterion)
BIC – Байесовский информационный критерий (Bayesian Information Criterion)
HQC – Информационный критерий Ханнана-Куинна (Hannan–Quinn Information Criterion)
Note: RMSE Root mean Square Error
p no of parameters
$adR^2$ Adjusted Coefficient of determination
BF Bias factor
AF Accuracy factor
AICc Adjusted Akaike Information Criterion
BIC Bayesian Information Criterion
HQC Hannan–Quinn information criterion

*Thermodynamic studies*

Thermodynamic parameters were calculated using nonlinear regression of the van't Hoff plot and the Langmuir constant, $K_C$, from previous studies [13, 46]. The study employed five temperature data points (25, 30, 35, 40, and 45 °C) to enhance fitting through non-linear regression. More data points favor the use of non-linear equations. Kinetic and Langmuir equilibrium isotherms were investigated at various concentrations (10, 20, 50, 70, and 90 mg/L) and temperatures (25, 30, 35, 40, and 45 °C). Pseudo-second-order (PSO) kinetics were observed, and qe coefficients from the PSO model were used to calculate the Langmuir constant, KL transformed into a dimensionless form ($K_C$) for thermodynamic calculations. The thermodynamic determinants for the entire adsorption procedure are briefed in Table 2. $\Delta H°$ and $\Delta S°$ values are computed based on the van't Hoff plot in Fig. 6, where the slope determines if the system is exothermic (negative slope) or endothermic (positive slope) [47].

***Table 2***

**The thermodynamic values (ΔG, ΔH, and ΔS) evaluated based on Vant-Hoff graph and the dimensionless Langmuir constant for dye adsorption**

***Таблица 2.*** **Термодинамические значения (ΔG, ΔH и ΔS), оцененные на основе графика Вант-Гоффа и безразмерной константы Ленгмюра для адсорбции красителя**

| Temp (K) | $K_{C(\times 10^6)}$ | ΔG° (kJ/mol) | 95% CI (Lower) | 95% CI (Upper) | ΔH° (kJ/mol) | ΔS° (kJ/(mol·K) |
|---|---|---|---|---|---|---|
| 298.15 | 1.44 | -34.15 | -39.52 | -28.79 | | |
| 303.15 | 1.91 | -36.24 | -37.55 | -34.92 | | |
| 308.15 | 2.84 | -37.68 | -40.00 | -35.35 | 65.46 | 336.1 |
| 313.15 | 4.08 | -39.47 | -40.37 | -38.57 | | |
| 318.15 | 6.55 | -41.25 | -42.82 | -39.69 | | |

Примечание: CI – доверительгый интервал
Note: CI =Confidence interval

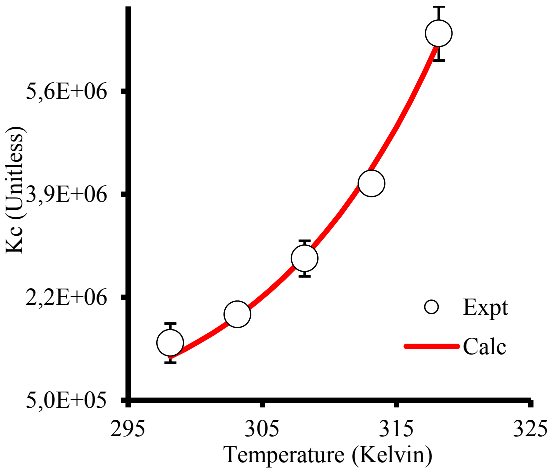


Fig. 6. A non-linear van't Hoff plot of the dimensionless constant, $K_C$ against temperature, K of the dye adsorption process. The error bars reflect the mean ± standard error (n = 3)

Рис. 6. Нелинейный график Вант-Гоффа безразмерной константы $K_C$ в зависимости от температуры K процесса адсорбции красителя. Погрешности отражают среднее значение ± стандартная ошибка (n = 3)

With -ΔG° values starting from -400 to -80 kJ/mol, the study's findings show chemisorption as a result of adsorption. Heat is absorbed from the environment during the adsorption of Congo red dye onto *Serratia marcescens* strain MM06 biomass, as indicated by the positive standard enthalpy change (ΔH°). However, the Gibbs free energy change (ΔG°) is used to establish feasibility and spontaneity, ΔH° alone does not [13, 47].

The study findings align with the results of the work, demonstrating endothermic (+ΔH), spontaneous (-ΔG°), and increased randomness (+ΔS°) adsorption processes [48]. Similarly, different researches indicate spontaneous, endothermic, and increased randomness adsorption processes [26, 13]. Positive entropy (+$\Delta S°$) suggests a structural exchange between adsorbent active sites and dye molecules.

## CONCLUSION

*Serratia marcescens* strain MM06 demonstrated superior dye removal (81.9%) compared to other bacterial isolates tested against Congo red dye. Adsorption was influenced by factors like initial dye concentration, contact time, pH, temperature, and adsorbent dosage which were Optimized using One-Factor-at-a-Time (OFAT). FT-IR analysis confirmed the involvement of surface functional groups in dye sorption, indicating the occurrence of biosorption. SEM examination revealed macro-pores on the biomass surface, enhancing Congo red dye adsorption onto *Serratia marcescens* strain MM06. Bacterial biomass adsorbed dye, following pseudo-second-order kinetics. Langmuir model best-described dye adsorption. Thermodynamic parameters ($\Delta G°$, $\Delta H°$, and $\Delta S°$) assessed adsorption feasibility.

AUTHORS’ DECLARATIONS

Data and material availability.

The appropriate author will provide any models, data, or programs developed or utilised during the study upon request for data availability.

COMPETING INTERESTS

A conflict of interest does not exist. According to COPE criteria, each author is responsible for all facets of the manuscript's accuracy and integrity after having read and approved its content.

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AUTHORS' ROLES AND PARTICIPATIONS

The first author, Ibrahim Alhaji Sabo, conducted the experiment and penned the paper's first draft. The statistics for isothermal and kinetics modeling were carried out by Motharasan Manogaran. The article was evaluated and edited by Yunus Shukor, Nur Adeela Yasid, and Ahmad Razi Othman, who also offered recommendations for improvement.

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

*Авторы заявляют об отсутствии конфликта интересов, требующего раскрытия в данной статье.*

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