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Biosorption of Methylene Blue from Aqueous Solution using Dried *Rhodotorula glutinis* Biomass

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Abstract – The biosorption of methylene blue (MB) from aqueous solution was investigated using dried *Rhodotorula glutinis* as the biosorbent. The effects of pH, initial dye concentration, biosorbent dosage, and kinetic studies were determined to obtain valuable information for biosorption. Results indicated that most of the adsorbed MB bound within 30 minutes of contact and the MB adsorption capacity increased from 21.1 to 101.8 mg/g with the initial MB concentration increased from 50 to 300 mg/L. Additionally, the MB adsorption capacity gradually increased from pH 4.0 to 9.0, reaching its peak at an initial pH of 9.0. As the biomass load was increased from 0.25 to 4.0 g/L, the MB removal efficiency increased from 14.1 to 84.5%. The Langmuir model provided the best fit throughout the concentration range, and the maximum adsorption capacity (q_{max}) and Langmuir constant (b) were determined to be 135.14 mg/g and 0.026 l/mg, respectively. Furthermore, the biosorbent process of *R. glutinis* was found to follow pseudo-second-order kinetics and the calculated $q_{eq,cal}$ value showed good agreement with the experimental q_{eq} value. Overall, the biosorption of MB by *R. glutinis* can be characterized as a monolayer, single site type phenomenon, and the rate-limiting step was determined to be the chemical reaction between the adsorbent and the adsorbate.

Key words: *Rhodotorula glutinis*, Methylene blue, Biosorption, Langmuir isotherm, Pseudo-second-order kinetics

1. Introduction

The environmental pollution caused by industrial dyes has become a major concern in recent years. Dyes are used in a wide range of industries, including textiles, paper, food, and cosmetics, among others. According to some reports, the global dye market was valued at around US$24 billion in 2020, and it is projected to grow at a Compounded Annual Growth Rate (CAGR) of about 4% during the forecast period of 2021 to 2028 [1]. Dyes can be released into the environment through discharge of wastewater from dye factories and textile mills. Water pollution caused by dyes can have a significant impact on aquatic life and the overall health of the ecosystem. Dyes can change the color of the water and make it difficult for aquatic plants and animals to survive. Dyes can also be toxic to aquatic life and can cause a range of health problems, such as reproductive failure, cancer, and organ damage [2]. The presence of dyes in water can also make it unsafe for human use, as they can cause skin irritation, allergic reactions and other health problems, if ingested [3]. Proper treatment and disposal of dye waste is important to prevent water pollution and protect the health of aquatic life, human and the environment.

Methylene blue (MB), a widely used dye in the textile and paper industries, is used in dyeing, printing, and as an oxidizing agent in many chemical and electrochemical processes. It can be used in the dyeing of silk, wool, and other natural fibers, as well as for printing and dyeing of paper [4]. Research has shown that some of the hazards associated with MB include skin and eye irritation, toxicity to aquatic life, and causing neurological effects such as confusion, disorientation, and hallucinations [5]. It is crucial to handle MB in a safe and responsible manner, following proper safety protocols and guidelines, and to properly dispose of any waste solutions to prevent environmental pollution.

Biosorption of dyes from aqueous solutions using microorganisms has gained significant attention as a sustainable, environmentally friendly, and cost-effective method for treating dye-contaminated wastewater. The use of microorganisms such as bacteria, fungi, and algae has been extensively studied for the removal of different types of dyes, including methylene blue [6-8]. Studies have shown that microorganisms can effectively remove methylene blue dye from aqueous solutions through various mechanisms such as adsorption, precipitation, and biosorption. The biosorption process involves the binding of the dye molecules to the cell surface or extracellular polymeric substances (EPS) of the microorganisms.

Rhodotorula glutinis (*R. glutinis)* has been extensively studied for its ability to remove heavy metals from aqueous solutions through the process of biosorption. This microorganism has been found to have several characteristics that make it a suitable biosorbent for the

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removal of contaminants, such as high biomass production, low cost, and easy cultivation [9,10]. The literature on biosorption by *R. glutinis* has mainly focused on the removal of heavy metals and has been limited in its examination of the biosorption of dyes. Despite the potential of this microorganism as a biosorbent, further research is needed to investigate the biosorption of dyes by *R. glutinis* and to determine its effectiveness as a treatment option for dye-contaminated wastewater [11].

The objective of this research is to investigate the biosorption of methylene blue dye from aqueous solution using dried *R. glutinis* biomass. The effects of pH, initial dye concentration, and biosorbent dosage were studied to optimize the biosorption process. In addition, kinetic studies were conducted to obtain valuable information for the design and optimization of treatment systems using biosorption. The results of this study will provide valuable insights into the potential of biosorption as a sustainable and cost-effective method for treating dye-contaminated wastewater.

2. Materials and Methods

2-1. Microorganism and Biomass Preparation

The biosorption of MB dye involved using *Rhodotorula glutinis* KCTC (Korean Collection for Type Cultures) 7989. The medium in which the cells were cultured contained 5.0 g/L of peptone, 3.0 g/L of yeast extract, 3.0 g/L of malt extract, and 10 g/L of glucose. On an orbital shaker operating at 150 rpm, culture was incubated at 25 ℃. After a 30-hour incubation, the biomass was extracted using centrifugation at 10,000 × g for five minutes. After being harvested, the biomass underwent two rounds of deionized distilled water washing. The biomass was washed, dried at 70 ℃ for 24 hours, and then ground with a mill and pestle. Studies on MB biosorption used the resulting powdered *R. glutinis* biomass.

2-2. MB Biosorption Experiments

The desired concentrations of MB were prepared by dilution of stock solution (500 mg/L) with deionized distilled water. For MB binding experiments, 2.0 g of biomass was added to 50 ml of MB solutions in 250 ml Erlenmeyer flasks shaken at 150 rpm in an orbital shaker at 25 ℃ for 3 h unless stated otherwise. Before adding biomass, the pH of the solution was adjusted to the desired values using 0.1N NaOH and 0.1N HNO_3. After sorption, the biomass was then separated by centrifugation at 10,000 × g for 5 min and the concentration of MB in the supernatant was determined at 600 nm by using a spectrophotometer (Varian Cary 50, USA).

2-3. Biosorption Isotherm

The adsorption capacity was calculated using the following equation:

$$q_{eq} = V(C_i - C_{eq})/1000\mathrm{M}$$

where q_{eq} is the adsorption capacity (mg-MB/g-biomass), V is the volume of MB solution (mL), C_i and C_{eq} are the initial and equilibrium concentration of MB (mg-MB/L) respectively, M is the dry weight of the biomass (g).

The biosorption equilibrium-isotherm was obtained using the Langmuir isotherm and Freundlich isotherm model [12,13]. The mathematical description of the Langmuir model for a single component adsorption is

$$q_{eq} = q_{max} b C_{eq}/(1 + bC_{eq})$$

where q_{max} is the maximum MB sorption (mg-MB/g-biomass) and b is the Langmuir isotherm constant (l/mg-MB). The Freundlich model takes the following form for a single component adsorption:

$$q_{eq} = K_F C_{eq}^{1/n}$$

where K_F and n are the Freundlich constants related to the adsorption capacity and adsorption intensity of the sorbent, respectively.

2-4. Biosorption Kinetics

Different kinetic models, such as pseudo-first-order and pseudo-second-order, were assessed in order to study the rate of MB biosorption over *R. glutinis*.

Lagergren suggested a pseudo-first-order equation for the sorption of liquid/solid system based on solid capacity [14].

$$dq/dt = k_1(q_{eq} - q_t)$$

where q_{eq} (mg/g) and q_t (mg/g) are the amounts of adsorbed MB ions on the biosorbent at equilibrium and at time t, respectively, and k_1 (l/min) is the rate constant of pseudo-first-order sorption.

The pseudo-second-order kinetic model based on the sorption capacity of the solid phase can be used in this case assuming that measured concentrations are equal to cell surface concentrations [15]. If the rate of sorption is a pseudo-second-order mechanism, it may be represented as follows:

$$dq_t/dt = k_2(q_{eq} - q_t)^2$$

where, k_2 (g/mg min) is the pseudo-second-order biosorption rate constant.

The adjustable parameters of isotherms and kinetics were obtained by adopting the least-squares method to converge the experimental data using Excel software (Microsoft Corp.). The goodness of fit of linear regression was described by using the square of correlation coefficients, R^2.

3. Results and Discussion

3-1. Effect of Initial MB Concentration

As shown in Fig. 1, the impact of initial methylene blue (MB) concentration on the binding capacity of *R. glutinis* biomass (2.0 g/L) was investigated. It was observed that *R. glutinis* adsorbed MB very rapidly, with most of the adsorbed MB bound within 30 minutes of

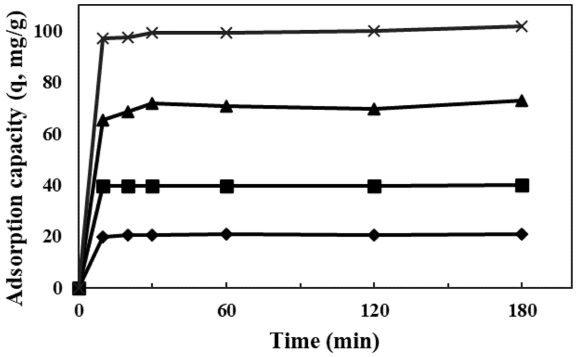

Fig. 1. Effect of initial MB concentration on MB biosorption (2.0 g/L biomass, pH 9.0, ◆; 50 mg/L, ■; 100 mg/L, ▲; 200 mg/L, X; 300 mg/L).

contact. The adsorption capacity of the biomass increased with an increase in the initial concentration of MB and reached a saturation point. This result suggests that the adsorption of MB by *R. glutinis* is a chemically equilibrated and saturable process, where the capacity to adsorb increases as long as binding sites are available.

When the initial MB concentration increased from 50 to 300 mg/L, the MB adsorption capacity increased from 21.1 to 101.8 mg/g. The amount of adsorbed MB did not change significantly over time after very fast adsorption. This can be attributed to a higher mass transfer driving force resulting from a higher initial adsorbate concentration, leading to a higher adsorbate adsorption capacity [16].

3-2. Effect of pH

The pH of the solution plays a significant role in the biosorption capacity of the biomass. *R. glutinis* was used to adsorb methylene blue (MB) in a pH range of 4.0 to 10.0 in a solution containing 300 mg/L of MB, as shown in Fig. 2. The results indicate that the MB adsorption capacity gradually increased from pH 4.0 to 9.0, reaching its peak at an initial pH of 9.0. The pH of the solution affects the availability of ligands required for the adsorbate to bind to the adsorbent. Additionally, the protonation or deprotonation of the functional groups in the cell wall also affects the MB biosorption [17]. The high proton concentration at low pH can hinder the biosorption of MB. This is because the cationic form of MB and protons compete for binding sites, reducing the amount of MB that can be adsorbed. However, as the pH increases, deprotonation of the MB binding sites results in an increase in the number of functional groups on the cell wall with a negative charge, which can boost MB sorption. Additionally, the pH of the solution greatly influences the speciation of MB. According to the literature, the cationic form of MB is mainly found at pH values higher than 6.0. This means that at higher pH values, there are more positive charges on the MB molecules, which will be attracted to the negatively charged functional groups on the *R. glutinis* biomass, increasing the biosorption capacity [18]. According to the report, the cell surface of *R. glutinis* was found to have multiple binding sites containing carboxyl and phosphoryl groups. The total concentration of these binding sites was quantified to be 20.63 mmol/g [19]. The abundance of negatively charged cell surfaces of dried *R. glutinis* biomass is believed to be the major contributing factor for the observed high MB adsorption capacity.

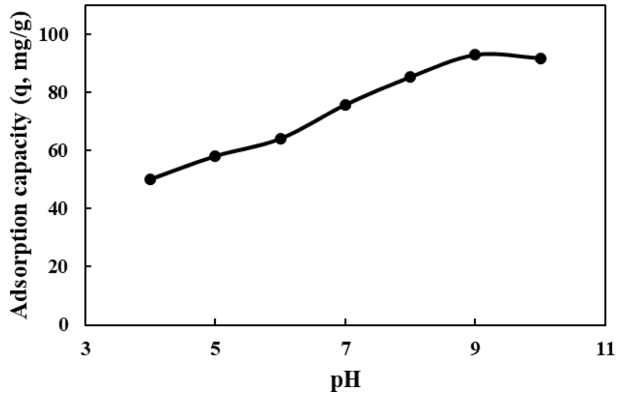

Fig. 2. Effect of pH on MB biosorption (2.0 g/L biomass, 300 mg/L MB).

3-3. Effect of Biomass Dosage

Fig. 3 illustrates the percentage of removal efficiency and methylene blue (MB) adsorption capacity at different biomass dosages in a solution containing 300 mg/L of MB. It is observed that as the biomass concentration increases, the MB removal efficiency improves. As the biomass load was increased from 0.25 to 4.0 g/L, the MB removal efficiency increased from 14.1 to 84.5%. This is attributed to the increase in biomass surface area and adsorption active sites with increased biomass dose, leading to greater removal effectiveness. On the other hand, the adsorption capacity was found to decrease as biomass concentration increased. This can be explained by the partial saturation of biomass active sites [20]. Furthermore, as biomass concentration increased, the intensity of electrostatic interactions between cells also increased, resulting in cell aggregation and a decrease in the number of accessible binding sites [21].

3-4. Biosorption Isotherm

The Langmuir and Freundlich isotherms are two commonly employed empirical adsorption models that can be utilized to model equilibrium biosorption data. The ability of these isotherms to predict

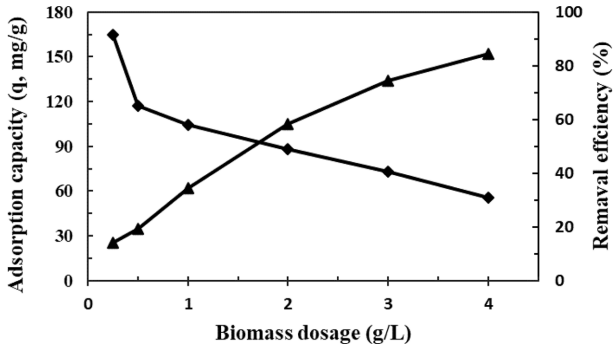

Fig. 3. Effect of biomass dosage on MB biosorption (300 mg/L MB, pH 9.0, ◆; adsorption capacity▲; removal efficiency).

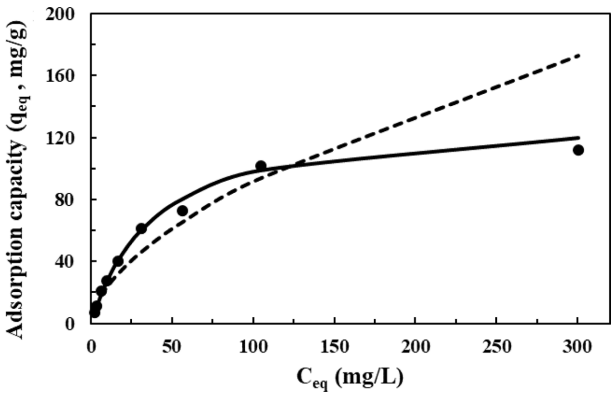

Fig. 4. Correlations between experimental data and Langmuir and Freundlich isotherm models for MB biosorption (2.0 g/L biomass, pH 9.0, ●; experimental data, …; Freundlich isotherm, —; Langmuir isotherm).

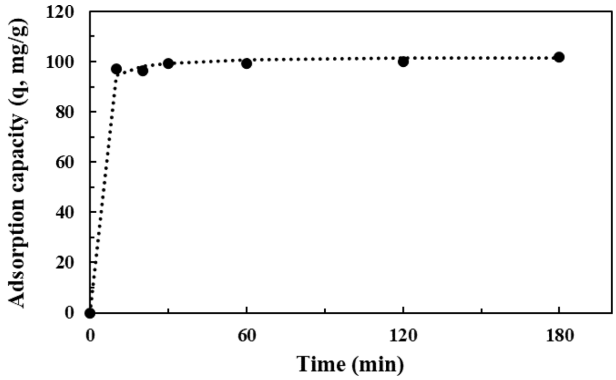

Fig. 5. Correlations between experimental data and pseudo-second-order kinetic model for MB biosorption (2.0 g/L biomass, 300 mg/L MB, pH 9.0, ●; experimental data, …; pseudo-second-order kinetic model).

experimental results was evaluated by comparing theoretical plots from each isotherm to the experimental data for the adsorption of MB by *R. glutinis*. The adsorption isotherm is characterized by specific constants that can be used to determine the surface properties and affinity of the sorbent, and to calculate the biosorption capacity of the biomass.

As depicted in Fig. 4, the Langmuir model provided the best fit throughout the concentration range, indicating that the biosorption of MB by *R. glutinis* can be characterized as a monolayer, single site type phenomenon with no interaction between ions adsorbed in neighboring sites. The results of the Langmuir isotherm and Freundlich isotherm modeling for the adsorption of MB by *R. glutinis* are presented in Table 1. The maximum adsorption capacity (q_{max}) and Langmuir constant (b) were determined to be 135.14 mg/g and 0.026 l/mg, respectively. The high q_{max} and low b value in the adsorption isotherm suggest that *R. glutinis* biomass has a strong affinity for and can efficiently absorb large amounts of MB. The maximum adsorption capacity (q_{max}) of 135.14 mg/g for this study was found to be higher than that of MB biosorption by immobilized *Pseudomonas aeruginosa* (75.7 mg/g) but lower than that of MB biosorption by *Rhizopus arrhizus* (370.3 mg/g) [6,22].

3-5. Biosorption Kinetics

The understanding of the underlying mechanisms of sorption, as well as the design and optimization of treatment systems, is greatly enhanced by conducting kinetic studies of the process. Such studies can provide insight into the time required to reach equilibrium, the rate of adsorption, and the variables that affect the sorption of solutes. To validate the experimental data, pseudo-first-order and pseudo-second-order kinetic models were utilized.

Table 1. Isotherm and kinetic parameters for biosorption of MB by *R. glutinis*

Isotherm & Kinetic model	Parameters	Value	R^2
Langmuir	q_{max} (mg/g)	135.14	0.999
	b (l/mg)	0.026	
Freundlich	K_F	6.39	0.936
	n	1.73	
Pseudo-first-order	$q_{eq,cal}$ (mg/g)	24.94	0.774
	K_1 (l/min)	0.043	
Pseudo- second-order	$q_{eq,cal}$ (mg/g)	102.04	0.999
	K_2 (g/mg min)	0.0121	

The biosorption parameters of methylene blue (MB) for an initial concentration of 300 mg/L are presented in Table 1. Analysis of the data revealed that the pseudo first-order model was not an appropriate fit, as evidenced by the low correlation coefficients obtained and the theoretical $q_{eq,cal}$ values calculated, which were found to be unreasonable. However, the pseudo-second-order kinetic model exhibits a relatively high coefficient of determination (R^2 value), indicating that the biosorption process follows pseudo-second-order kinetics. Additionally, as can be seen in Table 1, the calculated $q_{eq,cal}$ value shows good agreement with the experimental q_{eq} value. The correlation between the experimental data and the pseudo-second-order kinetic model is illustrated in Fig. 5, which clearly shows the strong agreement between the two sets of data, further supporting the conclusion that the biosorbent process of *R. glutinis* follows pseudo-second-order kinetics. In the pseudo-second-order kinetic model, it is assumed that the rate-limiting step is the chemical reaction between the adsorbent and the adsorbate, and that the rate of this reaction is dependent on the concentration of both the adsorbent and the adsorbate. Similar phenomena have been observed for the biosorption of MB by feathers and maize silk powder [16,23].

4. Conclusion

Microorganisms have been found that have significant potential for the biosorption of dyes. Studies have shown that various microorganisms have high adsorption capacity and rapid adsorption rates for dyes, such as methylene blue. *R. glutinis* has been identified as a promising biosorbent due to its high biomass production, low cost, and ease of cultivation for the removal of contaminants. Despite its potential as a biosorbent, only one study has been conducted on the biosorption of

acid green 1 dye by *R. glutinis*. Therefore, further research is necessary to evaluate the effectiveness of this strain in the biosorption and treatment of dye-contaminated wastewater. In this study, the potential of *R. glutinis* biomass as an effective adsorbent for methylene blue (MB) was evaluated. Results indicated that *R. glutinis* has a high adsorption capacity and rapid adsorption rate. The Langmuir model provides the best fit for the adsorption data, indicating that adsorption occurs on a homogeneous surface with a limited number of adsorption sites. The maximum adsorption capacity (q_{max}) was determined to be 135.14 mg/g, which is higher than the maximum adsorption capacity of methylene blue biosorption reported for other biosorbents. Overall, the results of this study suggest that *R. glutinis* biomass has potential for use in the treatment of MB-contaminated water.

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