KINETIC AND THERMODYNAMIC STUDY ON DECOLORIZATION OF REACTIVE RED M5B USING *Fusarium oxysporum*

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ABSTRACT

The present investigation focused on the decolorization of Reactive Red M5B using *Fusarium oxysporum*. Decolorization capability of this fungal species against Reactive red M5B was carried out under static in vitro condition. With an aim of determining the optimal conditions required for maximum decolorization, the effect of pH, temperature and initial concentration on decolorization of Reactive red M5B was studied. Highest percentage and rate of decolorization was obtained at, temperature of 313K and initial dye concentration of 5 ppm after five days of incubation. Further, the kinetics of decolorization was compared using Michaelis-Menten plot and Lineweaver plot. From these plots, the maximum substrate consumption rate (V\(_{\text{max}}\)) and decolorization rate constant (K\(_{m}\)) were determined. Thermodynamic study was done to determine the activation energy, Gibb's free energy, enthalpy and entropy of the decolorization. The negative values of ΔG at 313K indicates the reaction is spontaneous. The ΔH value implies an exothermic decolorization reaction. ΔS value predicts the randomness of the process. Current study has revealed that the potential of *Fusarium oxysporum* towards the decolorization of reactive red extends the scope for the future analysis in the treatment of textile effluent.

Keywords: Decolorization, Optimal condition, Thermodynamics, Kinetics, Rate constant.

1. INTRODUCTION

A high volume of colored waste water is generated by textile industries. Textile, printing and dyeing industry effluent possess reactive dyes which induce a strong color to the effluent and are then discharged to many water resources. Hence it becomes a major contributor for water pollution causing toxic and carcinogenic effects including, cut-off of sunlight passage into water
underneath blocking photosynthesis of aquatic system, reduction in dissolved oxygen content, etc [7]. Usually reactive dyes which have complex aromatic molecular structure are mainly used dyes in these industries and so its removal prior to effluent discharge to environment is necessary [10].

Favourable characteristics of reactive dyes like bright colour, water-fastness, and simple application techniques with low energy consumption enhanced the common usage of these dyes in textile industries [8]. Azo-based chromophores with different types of reactive groups such as vinyl sulfone, chlorotriazine, trichloropyrimidine and difluorochloropyrimidine forms the reactive dyes [14]. Hydrolyzed reactive dyes are present in effluent as some amount of those dyes does not get fixed on the substrate, representing 20-30% of the reactive dyes applied (on average 2 gL-1). Coloration of the effluents due to residual amount of these dyes causes the effluent not to be recycled. These reactive dyes are difficult to get removed from effluent due to their ability of easy passage through conventional treatment systems [2].

For decolorization of dyes general physical–chemical methods namely, chemical coagulation/flocculation, ozonation, oxidation, ion exchange, irradiation, precipitation and adsorption are used [4]. Since they are very expensive and experience operational problems, they are not feasible techniques [1]. An economical and eco-friendly alternative is Biological wastewater treatment [5]. Therefore microbial decolorization methods are applied to the treatment of textile industry effluents because various microorganisms, such as bacteria, yeasts, algae and fungi removes different classes of dyes [13,9]

This study aims at investigating the potential of Fusarium oxysporum in decolorizing a reactive dye ie, for present study reactive red M5B is taken as an example. Optimum parameters such as pH, temperature and initial dye concentration are to be found by studying their effects on dye decolorization.

2.1 MATERIALS AND METHODS
2.1 MICROORGANISM AND GROWTH MEDIUM
The filamentous fungus Fusarium oxysporum was maintained on Potato Dextrose Agar (PDA) at 4°C. The fungus was sub-cultured on PDA plates. Fusarium oxysporum is a saprotrophic organism which has been reported in the degradation of Reactive Red HE7B [12].

1.2 REACTIVE RED MB SOLUTIONS
From Erode Scientific Chemicals, Reactive Red M5B was obtained. Stock solutions (50mg/L) of RR was prepared by dissolving accurate quantity of dye in distilled water. Using spectrophotometer, absorbance of 50mg/L of dye stock solution was measured at 200nm-800nm. Depending on measured absorbance values, stock solution was subjected to wavelength scanning in the range from 400nm to 600nm. Working standards varying between 1000, 2000, 3000, 4000 and 5000 mg/L (1, 2, 3, 4 and 5ppm respectively) were obtained by diluting the stock solution.

1.3 OPTIMIZATION OF DECOLORIZATION CONDITIONS
In this study, decolorization experiments were run under various experimental conditions. Optimization of Reactive Red M5B concentration (1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L and 5 mg/L), then the temperature (298K, 303K, 308K and 313K) were done. Along with corresponding experiment condition, controls were also maintained. Culture flasks and control flasks were kept at respective culture conditions for 5 days. Absorbance of both were measured before treatment and after 5days of treatment using spectrophotometer at 537.5nm.
1.4 PERCENTAGE DECOLORIZATION
Percentage of Reactive Red M5B decolorized by *Fusarium oxysporum* was calculated for each condition using the following formula,

\[
\% \text{ Decolorization} = \frac{\text{final conc.} - \text{initial conc.}}{\text{final conc.}} \times 100
\]

1.5 RATE OF DYE DECOLORIZATION
Determination of volumetric rate of dye decolorization (r\text{vol}:mg/L/h) was carried out using the given formula,

\[
r_{\text{vol}} = \frac{\Delta C(t)}{\Delta t}
\]

where, \(\Delta C(t) \text{ (mg/L)}\) is the change in dye concentration over the time interval \(\Delta t \text{ (h)}\) [6].

1.6 MAXIMUM SUBSTRATE CONSUMPTION RATE (VMAX) AND DECOLORIZATION RATE CONSTANT (Km)
Kinetic study on Reactive Red M5B decolorization by *Fusarium oxysporum* was carried out through two kinetic approaches namely, Michaelis Menten and Lineweaver-Burk. Interpreting from these two approaches the maximum substrate consumption rate (Vmax) and decolorization rate constant (Km) were determined.

1.7 ACTIVATION ENERGY DETERMINATION
Apparent activation energy for Reactive Red M5B decolorization at different temperatures (298K, 303K, 308K and 313K) was calculated from the plot of lnK against reciprocal of temperature according to the linearized Arrhenius equation.

\[
\ln K' = \ln A - \frac{(E_a/RT)}{\text{--(3)}}
\]

where A is the frequency factor, \(R\) is the gas constant (8.3145 J mol\(^{-1}\) K\(^{-1}\)), and \(T\) is the absolute temperature (K) [6].

1.8 THERMODYNAMIC PARAMETERS
At standard conditions Gibbs free energy was calculated using the following equation,

\[
\Delta G = -RT \ln k_{eq} \text{--(4)}
\]

where \(k_{eq}\) is the apparent equilibrium constant at standard conditions, \(R\) is the gas constant (8.3145 J mol\(^{-1}\) K\(^{-1}\)), and \(T\) is the absolute temperature (K).

Also,

\[
k_{eq} = \frac{[P]_{eq}}{[Q]_{eq}} \text{--(5)}
\]

where \([P]_{eq}\) is the initial dye concentration - dye concentration remaining at equilibrium, and \([S]_{eq}\) is the dye concentration remaining at equilibrium [6]. Van’t Hoff analysis was used to determine the enthalpy and entropy of dye decolorization. According to Van’t Hoff equation the temperature dependence of Keq is expressed as follows:

\[
\ln K_{eq} = (-\Delta H/RT) + (\Delta S/R) \text{--(6)}
\]

where, \(\Delta H\) is van’t Hoff enthalpy (Jmol\(^{-1}\)) and \(\Delta S\) is the entropy (Jmol\(^{-1}\)K\(^{-1}\)) [6].

2. RESULTS AND DISCUSSIONS
3.1 CHARACTERISTICS OF REACTIVE RED M5B SOLUTIONS
At 537.5nm, maximum value of optical density was obtained. Characteristics of Reactive Red M5B solutions were 303K and pH8. The pH of the working solutions was adjusted to the required values by 0.1 M HCl or 0.1 M NaOH.
3.2 EFFECT OF DIFFERENT TEMPERATURES AND INITIAL DYE CONCENTRATION.

Maximum percentage of Reactive Red M5B decolorization was found at 313K for 5ppm solution. It’s found that at 303K, Reactive Red decolorization percentage was maximum when treated with Rhizopus arrhizus [11]. Here, the graph plotted with decolorization rate against different temperatures (Fig. 1) gives a clear indication of increase in dye decolorization rate at temperatures 303K and 313K for all the five different dye concentrations. It shows that F. oxysporum enhances its ability to decolorize the dye upon increase in the temperature. Also, the rate of decolorization increases linearly with the initial dye concentration.

![Graph showing decolorization rate at different temperatures](image)

**Figure 1:** Decolorization rate at different temperature.

3.3 DETERMINATION OF MAXIMUM SUBSTRATE CONSUMPTION RATE (VMAX) AND DECOLORIZATION RATE CONSTANT (KM)

Microbial decolorization of dyes is mainly a process due to action of extracellular enzymes [3]. To determine the values of Km and Vmax for reactive red dye decolorization, the kinetic study based on Michaelis - Menten and Lineweaver – Burk models were performed.

\[ V = \frac{V_{\text{max}} \times S}{(K_m + S)} \] \hspace{1cm} (7)

Equation 7 represents Michaelis-Menten equation, where Vmax is the maximum substrate consumption rate in mg L\(^{-1}\) h\(^{-1}\); \( V \) is the substrate consumption rate in mg L\(^{-1}\) h\(^{-1}\); S is the substrate concentration in mg L\(^{-1}\); Km is the Michaelis-Menten constant in mg L\(^{-1}\) [8]. Concentration against rate of decolorization was plotted for each temperature (Fig.2). The slope from each plot was estimated to be the first order rate constants k’, and noted in Table1.
Figure 2: Michaelis-Menten plots.

Table 1: First order rate constants obtained from M.M plots.

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Rate constants, k’ (h⁻¹) ×10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>298K</td>
<td>0.409</td>
</tr>
<tr>
<td>303K</td>
<td>0.987</td>
</tr>
<tr>
<td>308K</td>
<td>0.5</td>
</tr>
<tr>
<td>313K</td>
<td>0.875</td>
</tr>
</tbody>
</table>

Lineweaver-Burk equation as follows:

\[
\frac{1}{V} = \frac{K_{m}}{(V_{\text{max}} S)} + \frac{1}{V_{\text{max}}} \tag{8}
\]

The reciprocal of reaction rate was plotted against reciprocal of concentration. From the slope and intercept values, corresponding Km and Vmax of concentration (Fig.3) values were calculated [8] (Table.2). Higher value of Km represents a feeble binding between the dye and the cells of organism.
3.4 ACTIVATION ENERGY DETERMINATION
Estimation of apparent activation energy (Ea) for the reactive red dye decolorization was done using a linearised Arrhenius plot (Fig. 4). Activation energy was calculated for temperatures 298K, 303K, 308K and 313K. The Ea value calculated from the slope of linearized Arrhenius plot was equal to 25.001KJ mol$^{-1}$ and was consistent with the Ea values for enzyme-catalyzed reactions that generally range from 16 to 84 kJ mol$^{-1}$ [6].

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>$V_{\text{max}}$ (mg L$^{-1}$ h$^{-1}$)</th>
<th>$K_m$ (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>2.873</td>
<td>2.407</td>
</tr>
<tr>
<td>303</td>
<td>6.505</td>
<td>0.0117</td>
</tr>
<tr>
<td>308</td>
<td>2.35×10$^{-3}$</td>
<td>0.7028</td>
</tr>
<tr>
<td>313</td>
<td>5.6242</td>
<td>0.01036</td>
</tr>
</tbody>
</table>
Figure 4: Linearized Arrhenius plot to calculate the activation energy for Reactive Red M5B decolorization.

3.5 DETERMINATION OF THERMODYNAMIC PARAMETERS

Apparent equilibrium constant $K_{eq}$ was calculated for each experimental condition using (5). These $K_{eq}$ values were then used in (4) to obtain Gibb’s free energy ($\Delta G$). The calculated $\Delta G$ values are listed in Table 4 respective to 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm, at various temperatures. The negative value of $\Delta G$ at 313K indicates the reaction is spontaneous and the reaction is feasible at this temperature. According to (6), $\ln K_{eq}$ was plotted against $1/T$ (Fig. 5), the slope of which is $\Delta H/R$ and intercept is $\Delta S/R$. Enthalpy $\Delta H$, entropy $\Delta S$ of reactive red dye decolorization at each experimental condition was therefore calculated from respective plots and listed in Table 3. The $\Delta H$ value was negative which implies an exothermic reaction. Positive $\Delta S$ indicate increased randomness during the decolorization process due to the decomposition of dye molecules.

Table 3: $\Delta G$, $\Delta H$ and $\Delta S$ values calculated for Reactive Red M5B decolorization.

<table>
<thead>
<tr>
<th>T (K)</th>
<th>$K_{eq}$</th>
<th>$\Delta G$ (J/mol)</th>
<th>$\Delta H$ (J/mol K)</th>
<th>$\Delta S$ (J/mol K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>-3.178</td>
<td>7874.3307</td>
<td></td>
<td></td>
</tr>
<tr>
<td>303</td>
<td>-1.9928</td>
<td>5018.3709</td>
<td>-96.281</td>
<td>297.16</td>
</tr>
<tr>
<td>308</td>
<td>-2.7515</td>
<td>7046.3677</td>
<td></td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>2.33</td>
<td>-2201.3201</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4. CONCLUSION
The current research work on reactive red dye decolorization by *F. oxysporum* enabled the optimization of the dye’s decolorization parameters like initial dye concentration and temperature. Also, this work afford a proof on the ability of *F. oxysporum* in decolorization of reactive red dye. Hence it can be applied in biological treatment of textile industrial effluent. From the kinetic evaluation of the dye decolorization, the reaction was found to follow first order reaction kinetics. The activation energy value calculated as 25.001 KJ mol\(^{-1}\) and the \(V_{\text{max}}\) and \(K_m\) values indicates the activity of enzyme on decolorization of Reactive Red M5B. Thermodynamic study reveals the decolorization reaction as spontaneous and exothermic.

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REFERENCES


