Biological Decolorization of C.I. Basic Green 4 Solution by Microalga Chlorella sp.: Effect of Operational Parameters

Alireza Khataee
M. Pourhassan
M. Ayazloo

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Biological Decolorization of C.I. Basic Green 4 Solution by Chlorella sp.: Effect of Operational Parameters

Khataee A. R.1*, Pourhassan M.2 & Ayazloo M.2

(1Department of Applied Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran) (2Laboratory of Ecological Research, Department of Biology, Faculty of Science, University of Tabriz, Tabriz, Iran)

Abstract In recent years, the ability of microorganisms to decolorize textile wastewater has received great attention due to the environmental persistence and toxicity of these pollutants. In this paper biological decolorization of triphenylmethane dye, C.I. Basic Green 4 (BG 4), by Chlorella species was investigated. The effect of operational parameters (temperature, pH, initial dye concentration and algal concentration) on decolorization efficiency was examined. Results indicated that the desired initial pH was 9. The stability and efficiency of the algae in long-term repetitive operations were also examined. Michaelis–Menten kinetics was employed to describe the apparent correlation between the decolorization rate and dye concentration. The optimal kinetic parameters, \( v_{max} \) (specific decolorization rate) and \( K_m \) (maximum specific decolorization rate) were 4.6 mg dye g cell\(^{-1}\) h\(^{-1}\) and 151.0 mg L\(^{-1}\), respectively. Fig 10, Tab 2, Ref 24

Keywords biological treatment; triphenylmethane; C.I. Basic Green 4; microalgae; Chlorella; decolorization.

CLC X172

Pollution control is one of the prime concerns of society in recent years. Delivering untreated or partially treated wastewater and industrial effluents into natural ecosystems poses a serious problem to the environment \([1]\). Especially organic, inorganic and dye pollutions from industrial effluents disturb human health \([2]\). Among various industries, the textile industry ranks first in the usage of dye for colorizing fibers. The textile sector alone consumes about 60% of the total dye production for coloration of various fabrics \([3]\). The dye based effluents induce persistent color coupled with organic load and affect the total ecological symbiotic balance of water and receiving water stream \([4, 5]\).

Color affects the nature of water and inhibits the sunlight penetration into stream, thus reducing the photosynthetic activity \([6, 7]\). Furthermore, the effluents from dyeing industry constitute one of the most problematic wastewaters to be treated not only for high chemical and biological oxygen demands, suspended solids, contents of toxic and carcinogenic, mutagenic or teratogenic compounds, but also for color, which is the first contaminant to be recognized by human eyes \([8, 9]\). So the removal of dyes from water body has received considerable attention within environmental research \([10]\).

There are several methods currently used to decolorize textile wastewater but they are not universally applicable and are not cost effective for all dyes \([10]\).

Dye wastewater is usually treated by physical or chemical treatment processes. These include chemical coagulation/flocculation, ozonation, photooxidation, ion exchange, irradiation, precipitation and adsorption. Some of these techniques have been shown to be effective, although they have limitations \([11–19]\). Currently, research community is focused on techniques for the treatment of polluted environments that are less costly and ecofriendly.

In recent years, a number of studies have focused on some microorganisms which are able to biodegrade or bioaccumulate the dyes in wastewaters. A wide variety of microorganisms including bacteria, fungi and algae are capable of decolorizing a wide range of dyes via anaerobic, aerobic and sequential anaerobic-aerobic treatment processes \([14, 15, 16–20]\). Although bacteria play a key role in the biodegradation of organic pollutants, recent studies indicate that in addition to providing oxygen for aerobic bacterial biodegradation, microalgae can also biodegrade organic pollutants directly \([21, 22]\).

Algae are photosynthetic organisms and distribute in nearly all parts of the world in all kinds of habitats. Algae can degrade large number of dyes, postulating that the reduction appears to be related to the molecular structure of dyes and the species of algae used.

This study aims to investigate the potential of microalga Chlorella sp. for decolorizing of the wastewater containing a cationic textile dye, C.I. Basic Green 4. The correlation of the kinetic properties with dye concentration and other rate dependent environmental parameters (temperature, pH, dye concentration and algal concentration) was characterized. In addition, the stability and reusability of algae during repetitive decolorization operations were examined.

1 Material & Methods
1.1 Algal biomass
An alga was collected from a natural lake and used
immediately. According to its morphology and microscopic observations, it is identified as _Chlorella_ sp. belonging to green algae.

### 1.2 Growth medium

The algae was grown in a medium composed of Ca(NO₃)₂ (1.0 g L⁻¹), KH₂PO₄ (0.2 g L⁻¹), KCl (0.1 g L⁻¹), FeSO₄·7H₂O (0.01 g L⁻¹) and Agar (10 g L⁻¹). The pH of the medium was adjusted to 7.5 with diluted H₂SO₄ and NaOH solutions and then the pH values were measured with pH meter (654 pH-meter, Metrohm, Switzerland) before sterilization. Cells were cultivated in petri dishes containing of sterile medium. After cultivation for 7 days, the cells were transferred to the liquid medium composed of Ca(NO₃)₂ (6.5 g L⁻¹), K₂HPO₄ (1.5 g L⁻¹), KNO₃ (1.5 g L⁻¹) and FeCl₃ (0.01 g L⁻¹). The composition of the media was obtained from Merck, Germany.

### 1.3 Dye analysis

The triphenylmethane dye used in this study was C.I. Basic Green 4 (C.I. 42000, Merck), and its chemical structure and characteristics are given in Table 1. The absorbance was measured with spectrophotometer (UV/V is spectrophotometer, WPA light wave S2000, England) at maximum absorption wavelengths, λ_max=619 nm. Decolorization was determined from absorbance calibration curve of standard solutions (Fig. 1). The removal efficiency of color was expressed as the percentage ratio of the decolorized dye concentration to that of the initial one (Eq. 1).

\[
\text{Dye removal (\%) = } \frac{C_o - C}{C_o} \times 100
\]

where \(C_o\) and \(C\) are the initial concentration of BG 4 (mg L⁻¹) and the concentration of BG 4 during decolorization process, respectively.

### Table 1 Characteristics of C.I. Basic Green 4

<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>C.I. number</th>
<th>C.I. name</th>
<th>Class</th>
<th>Ionization</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td>42000</td>
<td>C.I. Basic Green 4</td>
<td>Triaryl methane</td>
<td>Basic</td>
</tr>
</tbody>
</table>

### 1.4 Batch decolorization operation

The experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of the synthetic dye solution and algal biomass. To evaluate the effects of operation and environmental factors on the efficiency of color removal, the batch decolorization experiments were carried out at different initial dye concentrations (0~15 mg L⁻¹), algal concentrations (0~9 \times 10⁶ cells mL⁻¹), temperatures (5~45 °C) and pH values (2.0~11.0). The pH was adjusted using diluted NaOH and HCl solutions. The batch decolorization experiments were performed under a static-incubation condition.

### 1.5 Kinetic model

The kinetic trends were interpreted according to conventional Michaelis-Menten model[24], presented by Eq. 2:

\[
v_{\text{dye}} = \frac{v_{\text{dye,max}} C_{\text{dye}}}{K_C + C_{\text{dye}}}
\]

where \(v_{\text{dye}}\) is specific decolorization rate (mg dye g cell⁻¹ h⁻¹), \(C_{\text{dye}}\) is the dye concentration (mg L⁻¹), \(v_{\text{dye,max}}\) and \(K_C\) are the maximum specific decolorization rate (mg dye g cell⁻¹ h⁻¹) and dissociation constant (mg L⁻¹), respectively. The constant \(v_{\text{dye,max}}\) and \(K_C\) were calculated by using the Lineweaver-Burk Equation expressed in Eq. 3[11]

\[
\frac{1}{v_{\text{dye}}} = \frac{1}{v_{\text{dye,max}}} + \frac{1}{v_{\text{dye,max}}} \frac{1}{C_{\text{dye}}}
\]

### 2 Results & Discussion

#### 2.1 UV-Vis spectra changes

Fig. 2 shows a typical time-dependent UV-Vis spectrum of BG 4 solution during biodegradation. The absorbance peaks corresponding to the dye diminished indicated that the dye had been removed. The spectrum of BG 4 in visible region exhibits a main peak with a maximum at 619 nm. The decrease in absorbance peak of BG 4 at λ=619 nm in this figure indicates a rapid degradation of the dye. According to the pervious literature[19], biodecolorization of dyes can be due to adsorption onto biomass or biodegradation. In the adsorption process, all peaks of the dye, in UV-Vis region, decreased approximately in proportion to each other. However, if the dye removal was attributed to biodegradation, either the major visible light absorbance peak would disappear or a new peak would appear. As seen in Fig. 2, the main absorbance peak of the dye approximately disappeared within 7 h. Therefore this process seems to be the biodegradation.

#### 2.2 Effect of different algal biomasses

The results obtained from the present investigation revealed the ability of _Chlorella_ sp. in biodegradation of BG 4. To estimate the desired biomass needed by _Chlorella_ sp., removal of 10 mg L⁻¹...
of initial dye concentration. Initial concentration provided an important driving force to overcome all mass transfer resistances of the dye between the aqueous and solid phases. Hence, a higher initial concentration of dye may enhance the process.

2.4 Kinetic model

Fig. 6 shows the dependence of the dye concentration on the biological decolorization rate. When the initial dye concentration was increased from 2.5 to 400 mg L⁻¹, the decolorization rate also increased. The optimal kinetic parameters, \( v_{\text{dy}, \text{max}} \) and \( K_a \), were 4.6 mg dye g cell⁻¹ h⁻¹ and 151.0 mg L⁻¹, respectively (Fig. 7).

2.5 Effect of temperature and pH

The effect of temperature on decolorization rate was significant (Fig. 8). In the experiments carried out at different temperatures, the initial dye concentration and pH were fixed at 10 mg L⁻¹ and 9.0, respectively. The temperature effect was
investigated at the temperature range from 5 to 45 °C. Over the examined range decolorization rate increased as the temperature rose. The results showed essentially no thermal deactivation of decolorization activity under operational temperatures. Therefore, the used species could acclimatize in a broad range of temperature. The standard deviation values for dye removal (%) under different temperatures after three times repeated at the reaction time of 7 h have are shown in Table 2.

<table>
<thead>
<tr>
<th>θ/°C</th>
<th>Minimum dye removal (r/%)</th>
<th>Maximum dye removal (r/%)</th>
<th>Mean value of dye removal (r/%)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>59.9</td>
<td>60.6</td>
<td>60.4</td>
<td>0.40</td>
</tr>
<tr>
<td>10</td>
<td>63.3</td>
<td>64.7</td>
<td>64.2</td>
<td>0.81</td>
</tr>
<tr>
<td>15</td>
<td>76.2</td>
<td>76.3</td>
<td>76.3</td>
<td>0.06</td>
</tr>
<tr>
<td>20</td>
<td>78.9</td>
<td>78.0</td>
<td>77.2</td>
<td>0.85</td>
</tr>
<tr>
<td>25</td>
<td>81.6</td>
<td>79.6</td>
<td>79.2</td>
<td>0.35</td>
</tr>
<tr>
<td>30</td>
<td>86.0</td>
<td>83.1</td>
<td>82.2</td>
<td>0.81</td>
</tr>
<tr>
<td>35</td>
<td>90.8</td>
<td>86.6</td>
<td>86.4</td>
<td>0.32</td>
</tr>
<tr>
<td>40</td>
<td>95.2</td>
<td>91.2</td>
<td>91</td>
<td>0.26</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Previously, several studies proved that biosorption processes using algae were highly pH dependent [11]. Fig. 9 shows the color removal efficiency of BG 4 solution as a function of pH. The effect of initial pH of the solution on the biodegradation efficiency was analyzed over a pH range from 2.0 to 11.0. It was observed that the amount of removed dye varied with pH. The results showed that it increased with the increase of pH till pH reached 9, and then decreased at higher pH values. The isoelectric point of *Chlorella* sp. has been reported at a pH of 3.0 [10]. At lower pH below this point, the H⁺ ions competed effectively with dye cations, causing a decrease in color removal efficiency. At higher pH above this point, the surface of biomass was negatively charged, which enhanced the positively charged dye cations through electrostatic force of attraction. This could be observed from the considerable increase in color removal efficiency from 21.1 to 68.0% when pH was increased from 3.0 to 4.0.

### 2.6 Effect of repeated uses

Repeated-batch operations were performed to examine the reusability of *Chlorella* sp. in BG 4 decolorization process (Fig. 10). During five repeated runs, *Chlorella* sp. showed the same decolorization rate that obtained from the first run. This might be attributed to an adaptation effect, since *Chlorella* sp. was repeatedly exposed to the dye. The results indicated that *Chlorella* sp. possessed reasonable reusability in repetitive decolorization operations.

### 3 Conclusion

The present study revealed that *Chlorella* sp. possessed the ability to decolorize C.I. Basic Green 4. The results obtained from this work indicated that this algal species had high decolorization efficiency, reusability and stability at the process of decolorization, which was dependent on dye concentration, algal concentration, pH and temperature. The dependence of the specific decolorization rate on dye concentration could be described by Michaelis-Menten model. Over the temperature range between 5~45 °C the decolorization rate increased with the temperature rising. The pH value of 9.0 was determined as the optimal decolorization pH.
References