Bacterial Degradation of Azo Dye Congo Red by *Bacillus sp.*

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Abstract. In recent decades, azo dyes are more popularly used as synthetic dye for colorants compared to natural dye. The demand for dye colorants by industries is getting higher and this really gives negative effects on human health and ecology. Textile dyes are a major source of environmental pollution, aesthetic pollution, eutrophication, and problems in aquatic ecosystems. Biological treatment has come to be considered as cheaper than the physiochemical method and it is also environmentally eco-friendly. The broadly used synthetic azo dye is Congo red, as the target of textile effluent in wastewater. This study is to investigate the efficient degradation of bacteria against azo dyes by using biological treatment under aerobic condition by *Bacillus sp.* bacteria. The highest percentage of efficiency of degradation at different conditions was 83.12% at 25 ppm within 5 days at pH 7.55 and 30°C, whereas the lowest percentage of efficiency of degradation at two different conditions is 9.00% at 5 ppm within 1 day at pH 9.40 and 26°C. As the conclusion, the effectiveness by degradation of Congo red dye *Bacillus* bacteria was within 5 days in 25 ppm. Thus by this present study, *Bacillus* could be used as a good microbial source for wastewater treatment, specifically in biological degradation of textile dye effluent in aerobic condition.

1. Introduction

It has been estimated that around 10-15% of the dyes are discharged as coloured effluent to the water bodies from dyeing process and finishing treatment [1]. India is the second largest dyes producer because its dye industry produces every type of dyes and pigments which is about 80,000 tones [2]. According to [3], textile, tannery, food, paper and pulp, printing, carpet and mineral processing industries are the examples of industries that use dyes and pigments in dyeing their output. Nearly 80% of dyestuff is used in textile industries. Day by day, the demand for dye colorants by industries has become higher and it really gives negative effects on human health and ecology [4]. Textile dyes are a major source of environmental pollution, aesthetic pollution, eutrophication, and problems in aquatic ecosystems. [1]. Even a small amount of dye colors, around 10-50 mgL-1 concentration, could contribute to aesthetic pollution. It is because azo dyes are reactive dyes which consist of highly water-soluble polyaromatic molecules. Furthermore, it can cause problems to photosynthetic aquatic plants due to the reducing light penetration in water bodies since the dye in the effluent is highly visible [4].
Azo dyes are the major classes of synthetic dye which have complex aromatic structure. Generally, these dyes are very stable because the compound does not easily degrade [5]. Azo dyes are the biggest problem to the environment because having a nitro group which is toxic in nature [6]. These azo dyes are carcinogenic and mutagenic due to the process of mineralisation which can break down into aromatic amine (arylamine) [5]. Mostly in the dyeing process of the textile industry, it has been estimated that 10-15% of the initial dye does not bind to fabric [7]. Thus, without any treatment, these dyes that are directly discharged into water bodies can lead to severe environmental pollution. The high concentration of synthetic dyes released into surface water obstruct the light from the sunlight to penetrate through the water. This causes the inhibition of photosynthesis process and lack of oxygen content in water, hence affecting photosynthetic aquatic life [8]. Unfortunately, continuous release of wastewater containing azo dyes which are very mutagenic and carcinogenic will affect the environment and human life [9]. According to [2], azo dyes as the major of textile dyes has aromatic amines which are a carcinogen to nature. In mammals, azo dyes are directly connected to bladder cancer due to a metabolic reduction of azo dye by anaerobic bacterial activity.

There are three types of treatment methods that can be applied to treat the effluent azo dyes in wastewater, which are physical, chemical and biological [4]. Physical and chemical treatments include adsorption, flocculation, electrocoagulation, precipitation, ozonation, and irradiation. Although these methods have the ability in colours removal from the wastewater, it can produce sludge which can create the second pollution. Besides, these methods are also very complex and expensive, and discharge of residues which lead to secondary pollution. Physical and chemical methods are only minimizing the hazardous level without neutralising it [3]. Therefore, a biological method is another alternative to overcome the limitation of using physical and chemical treatment [10]. This is because the biological method is cheaper than the physiochemical method and it is more environmentally eco-friendly. Moreover, this method is often used in the removal of textile dyes because of the better effectiveness of organic pollutant mineralisation [1]. Currently, it is now known that fungi, bacteria, yeast, and algae are microorganisms that have potential to decolourise and mineralise the textile dye under different environmental conditions [11].

In this research, the biological method has been used as an alternative method because the physical and chemical method have more disadvantages compared to the little benefits promised. However, in the biological method, bacteria cannot decolourise and mineralise azo dyes solely under anaerobic degradation because it will produce aromatic amines which is mutagenic [2]. Therefore, we need to focus on how aerobic bacteria decolourise azo dyes. In this study, bacteria named Bacillus were used in the biological treatment. Generally, the growth of bacteria is faster than fungi. They are easy to culture [12].

The physicochemical operational parameter affects the ability of bacteria degradation to decolourise azo dyes like a concentration of salts, concentration of dyes, pH, temperature and presence or absence of oxygen [13]. The significance study of factors affecting bacterial degradation is to make the process of bacterial degradation faster and more efficient [14]. In this study, the abiotic conditions focus on pH, temperature and dye concentration.

The target pollutant is Congo Red (CR), a synthetic azo dye which is used widely in textile dyeing process. This study focused on the efficiency of degradation through biological treatment by bacteria on stimulated industrial wastewater as a culture medium. This study also to investigate the degradation under aerobic conditions. The main objective of this study is to investigate the degradation of azo dyes by bacteria by aerobic conditions. The influence of the different experimental conditions that affect the degradation of azo dyes, which is pH and temperature were investigated.

## 2. Materials and Methods

### 2.1 Materials

All materials in this experiment were prepared in the laboratory and of analytical grade. Congo red as the model dye was purchased from a company. The stock solution of the Congo red dye was prepared...
in distilled water because dye can be soluble in water. The Congo red dye was used for all experiments. The instrument UV-Vis Spectrophotometer- UV 1800 Shimadzu was used to measure the efficiency of dye degradation.

2.2 Methods
Bacteria named Bacillus were used in this research to study the percentage of degradation of azo dyes, Congo red in certain environmental conditions. Firstly, 250 ml of the conical flask containing 0.4g of nutrient broth and 10 ml of 5 ppm from Congo Red dye solution were autoclaved under 121°C within 15 minutes. Then, 1 ml of bacteria was added to 250 ml of the conical flask containing the mixture of the solution. The pH meter was dipped into the solution and pH of 7 was adjusted by addition of either 1N NaOH or 1N. After that, the solutions were incubated for 1 day using the incubate shaker. The reading of pH and the temperature was recorded. After 1 day, the suspension was centrifuged under 4000 rpm for 30 minutes. Then, the supernatant liquid was filtered using a filter funnel with filter paper. 3 ml of the sample liquid was obtained from the conical flask and monitored by UV/Vis spectrophotometer instrument. By using a UV/Vis spectrophotometer instrument, the absorbance reading of the degradation of the samples was obtained. All of the methods were repeated with the presence of bacteria in a concentration of 10 ppm, 15 ppm, 20 ppm, and 25 ppm respectively in 2 days, 3 days, 4 days and 5 days.

3. Result and Discussion
3.1 Effect of pH of Congo Red Dye in Different Concentration
Based on the experiment, it was proven that Bacillus bacteria have the ability to degrade the Congo red dye in several days. Figure 1 shows the graph of pH with different concentration within days interval. The readings were recorded by pH meter. From the data collected, it shows that the effectiveness of the degradation increases with the decrease of pH.

Figure 1. The graph of pH with different concentrations within days interval

Figure 1 shows the pH for day 0, day 1, day 2, day 3, day 4 and day 5 and the readings are pH 7, pH 9.42, pH 9.06, pH 8.20, pH 8.80 and pH 7.55 at the concentration of 25 ppm. The pH medium plays a major role in the efficiency of dye decolourization as it was associated with the overall biochemical processes and the growth of microorganisms. It was considered as another limiting factor for microbial activities and azo dye decolourisation. In the present study, the optimum pH for bacteria, Bacillus to degrade the colour of Congo red dye is around 7 within 5 days. Generally, the pH for colour removal by bacteria often exists between 6.0 and 10.0 with a good efficiency [3]. Majority of the azo dye
reducing bacterial species were able to reduce the dye at pH near 7. This may be related to the transport of dye molecules across the cell membrane [1]. However, beyond pH 7, a decrease in decolourisation by bacteria was observed, due to reduction of the azo bond to form aromatic amine metabolites, which are more alkaline than the parent azo dye [14].

3.2 Effect of Temperature of Congo red Dye in Different Concentration

Based on the experiment, it was proven that Bacillus bacteria have the ability to degrade the Congo red dye in several days. The effectiveness of the degradation also increases with the increase of concentration. Figure 2 shows the graph of temperature with different concentrations within days interval. The reading is recorded by thermometer. From the data collected, it is shown that the effectiveness of the degradation increases with the increase of temperature.

[Figure 2. The graph of temperature with different concentrations within days interval.]

Temperature is also considered to be an important environmental factor during the process of biological decolourization of azo dye. Optimal temperature to decolorise Congo red by Bacillus was around 30°C± within 5 days. From Figure 2 shown, the temperature increased from 25 to 30°C± causing the increase of degradation percentage of dye. In addition, the biodegradation activities by bacteria were affected by changes in temperature. According to a study conducted by [15], it was found that in microbial physiology, temperature changes lead to a sudden alteration of the activation energy. Furthermore, further increase in the temperature may decrease the percentage of degradation. This phenomenon might be caused by the loss of cell viability or to the denaturation of the azo reductase enzyme secretion at higher temperature [16]. It is also likely that Bacillus was mesophilic bacteria because they showed better decolourization in the temperature range of 25 to 35°C.

3.3 Degradation of Congo red Dye

Based on Table 1 below, the blank absorbance of Congo Red dye is an absence of bacteria was tabulated with a control concentration at 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm for 1 day, 2 days, 3 days, 4 days and 5 days which were analysed using UV-Vis spectrophotometer. The absorbance readings for 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm were 0.311, 0.618, 0.921, 1.227 and 1.541. It gradually increased from 0.311 to 1.541 by the increase of the Congo red dye within the increasing time.
Table 1. The blank absorbance of Congo red dye in absence of bacteria.

| Concentration (ppm) | Absorbance (nm) |
|---------------------|-----------------|
| 5                   | 0.311           |
| 10                  | 0.618           |
| 15                  | 0.921           |
| 20                  | 1.227           |
| 25                  | 1.541           |

All the values obtained from Table 1 were used throughout the experiment as the blank absorbance of the dye which was Congo red absence in bacteria. These values acted as the textile wastewater before the reaction process began with bacteria as the biological treatment. The maximum absorbance wavelength ($\lambda_{\text{max}}$) of Congo red is 497 nm. Therefore, the degradation of concentration dyes in solution as different in days can be determined by measuring the absorption intensity at ($\lambda_{\text{max}}$) 497 nm.

Table 2. The absorbance of Congo red dye in presence of bacteria in 1 day, 2 days, 3 days, 4 days and 5 days.

| Concentration (ppm) | Absorbance (nm) |
|---------------------|-----------------|
|                     | 1 Day | 2 Day | 3 Day | 4 Day | 5 Day |
| 5                   | 0.283 | 0.281 | 0.277 | 0.269 | 0.218 |
| 10                  | 0.321 | 0.301 | 0.264 | 0.205 | 0.203 |
| 15                  | 0.410 | 0.317 | 0.262 | 0.240 | 0.238 |
| 20                  | 0.416 | 0.320 | 0.283 | 0.276 | 0.268 |
| 25                  | 0.504 | 0.320 | 0.278 | 0.264 | 0.260 |

From Table 2, the absorbance readings of Congo Red dye in presence of bacteria for 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm did not increase constantly and this may be caused by the overall reaction of the degradation that has happened between Congo Red dye and bacteria. Meanwhile, the absorbance readings of Congo Red in presence of bacteria for 1 day, 2 days, 3 days, 4 days and 5 days gradually decreased and this may be due to the efficiency of degradation activities within the increasing time. Furthermore, the absorbance of the dye using the presence and absence of bacteria can be compared and the percentage of degradation can be calculated using the percent degradation formula. From the data obtained, the absorbance presence of bacteria in Congo red dye was lower than the absence of bacteria. The percentage of degradation efficiency of Congo red dye at both conditions is shown in Figure 3.
Figure 3. The percentage of efficiency of degradation at different conditions.

The graph in Figure 3 shows the percentage of efficiency of degradation at two different conditions which are pH and temperature for 5 ppm within 1 day, 2 days, 3 days and 5 days are 9.00 %, 9.65 %, 10.93 %, 13.50 %, and 29.90%. Meanwhile, for 10 ppm, the percentage of efficiency of degradation at two different conditions within 1 day, 2 days, 3 days and 5 days are 48.06 %, 51.29 %, 57.28 %, 66.83 %, and 67.15%. Next, for concentration 15 ppm, the percentage of efficiency of degradation at two different conditions within 1 day, 2 days, 3 days and 5 days are 55.48 %, 65.58 %, 71.55 %, 73.94 %, and 24.16%. Besides, 66.10 % 73.2 %, 76.94 %, 77.51 %, and 78.16 % are the percentage of efficiency of degradation at two different conditions within 1 day, 2 days, 3 days and 5 days for concentration 25 ppm. For the last concentration 25 ppm, the percentage of efficiency of degradation at two different conditions also within 1 day, 2 days, 3 days and 5 days are 67.29 %, 79.23 %, 81.96 %, 82.87 %, and 83.16 %.

Moreover, the dye concentration also influences the bacteria on the rate of degradation percentage and toxicity occurred by higher dye concentrations. A previous study by [12] observed that the rate of decolourisation decreased with increasing dye concentration. Generally, the dye concentration of textile industry effluent is commonly in the range between 16– 50 mg/L [17]. The figure shows that degradation efficiency increased with the increase of concentration of Congo red dye. Therefore, the results from this study demonstrated that higher dye concentration at 25 ppm shows that concentration was not toxic to Bacillus bacteria. According to the recent research by [16], further increase of dye concentration might be imposed to the toxicity of dye to bacterial cells through the inhibition of the bacteria ability to degrade dye by azoreductase activity or Flavin reductase, inactivation of transport system by the dye and saturation of the cells with dye products. Hence, it can effect of dye concentration on the growth of organisms. Furthermore, higher dye concentrations need more biomass concentration [12]. Next, azo dyes with reactive groups as sulfonic acid (SO\textsubscript{3}H) on their aromatic rings are greatly inhibited the growth of microorganisms at higher dye concentrations [14].

From all of the observations, the biodegradation of Congo red was carried out using Bacillus and the results showed that Congo red was effectively assimilated by the microorganism. The lowest percentage of efficiency of degradation at different conditions was 9.00 % at 5 ppm within 1 day at pH 9.40 and 26°C. Meanwhile, the highest percentage of efficiency of degradation at different conditions was 83.12 % at 25 ppm within 5 days at pH 7.55 and 30°C. The degradation of the dyes at 25 ppm showed no toxic effect on the microbe. Thus, this bacterium may hold great potential for treating other industrial wastewater containing high dye concentration. Furthermore, the effectiveness of microbial
treatment depends on survival, adaptability, and activity of the selected organisms and depends on the structure and complexity of the dyes.

4. Conclusion

The experiment has proven that Bacillus bacteria was efficient in the degradation of an azo dye, Congo red dye solution. In the present study, the Bacillus sp. showed good performance with different concentrations of Congo red dye under the effect of pH and temperature. The lowest percentage of efficiency of degradation at two different conditions was 9.00 % at 5 ppm within 1 day at pH 9.40 and 26 °C, whereas the highest percentage of efficiency of degradation at different conditions was 83.12 % at 25 ppm within 5 days at pH 7.55 and 30 °C. As the conclusion, the effectiveness of degradation of Congo red dye of Bacillus bacteria was within 5 days in 25 ppm. Thus by this present study, Bacillus could be used as a good microbial source for wastewater treatment, specifically in biological degradation of textile dye effluent in aerobic condition.

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