Effect of pH variation on the activity of green algae (*Chlorella Vulgaris*) and bacteria (*Bacillus subtilis*) in remediation of toxic dye (Congo red)

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**Abstract.** The present study used two species of microorganism, algae (*Chlorella Vulgaris*) and bacteria (*Bacillus subtilis*), in the removal of toxic industrial dye (Congo red) and deal with the effect of pH variation on the activity of these organisms to remediation and reduces of dye in vitro. Algae (*C.vulgaris*) also bacteria (*B.subtilis*) isolated from habitats and cultured under controlled laboratory conditions and, using different concentration of dye (50, 150 and 250 mg/l) used in different pH (4, 7 and 9) during different period times (3, 7, 9, 11 and 13 days) to algae and 7 days to bacteria to testing the ability of bioremediation. The results showed that the best pH for removal by *Chlorella Vulgaris* was (7) for a period of (13 days) at concentration (150) mg/l and with the complete removal (75%) and *Bacillus subtilis* best pH (7) complete removal (98%) at concentration (50) mg/l. The efficiency of the *Chlorella Vulgaris* and *Bacillus subtilis* in the removal of pollutants increased with the high value of many environmental factors such as pH.

**Keywords:** pH, Congo red, *Chlorella Vulgaris*, *Bacillus subtilis*, Bioremediation, Removal

1. **Introduction**

Bioremediation is a degraded waste, especially organic compound by microorganisms such as bacteria, algae, and mycorrhiza, to remove toxic materials from the surrounding environment in a field or laboratory [1]. The biotreatment process may reduce the effect and transport pollutants through different media [2]. Biotreatment issues convert organic pollutants into harmless metabolites or mineralise the pollutants into carbon dioxide and water [3]. Microalgae have many roles in biotreatment and are widely used to remove different aquatic pollutants [4]. Algae is autotrophic organisms that have the potential portability to bioremediation of much hazardous waste in eco-friendly methods to reduce treatment costs [5]. *Chlorella Vulgaris* was selected due very strong compared to other species and can perform well in wastewater and remove pollutants. It can also tolerate a wide range of nutrient concentrations, temperature, and pH, making it versatile for wastewater remediation [6]. The technique of degradation of azo dyes by microorganisms may involve the reductive cleavage of azo bonds (−N=N−), and reduction of enzymes in the anaerobic state maybe lead to colourless solutions containing hazardous-aromatic amines [7; 8]. The use of bacteria and other microorganisms in removing this group of dyes has been of considerable interest since it can achieve a higher degree of biodegradation and mineralisation [9].

Azo dyes are synthetic aromatic compounds that contain one or more (N=N) groups and (-SO3-) groups [10; 11]. Azo dyes are water-soluble synthetic organic compounds containing one, two or three azo linkages, linking phenyl, naphthyl rings that are usually substituted with some functional groups, including triazine amine, methyl, nitro, chloro, hydroxyl, and sulphonate [12]. They have toxic effects like lethal effect, genotoxicity, mutagenicity, and carcinogenicity to plants and animals [15].

Congo red (CR) is generated from many industrial activities such as textiles, printing, paper, rubber and plastics [14].
The cango red is carcinogenic material due to cleavage of one or more azo groups and high risk in small concentration [15]. The current study deal with used one species from algae and bacteria in the bioremediation of cango red with a variation of pH.

2. Materials and Methods

2.1. Culture of Algae
The unicellular green microalga *C. Vulgaris* was grown on Chu-10 media for 15 days under control laboratory conditions at 25 ± 2 °C and a light system of 16: 8 hours(light/ dark)(Pinheiro et al., 2004). This culture was transported into 1000 ml of media and incubated for 14 days to increase algal biomass [16].

2.2. Experimental Design
*Chlorella Vulgaris* (100ml) was cultured in 1 litter from Chu-10 medium and left for at least two weeks before starting the experiment under constant laboratory conditions. This alga is exposed to Congo red (1000mg/L) and transported to new growth media to prepare the experiment's concentrations (50, 150 and 250 mg/l). This experiment is prepared in 250 ml of liquid culture media and using culture media with algae as the control without adding congo red under the same constant conditions to compared with effect algae by congo red, another control using culture media and Congo red without algae in the same concentration to exam the effect of light on dye under study and the compared to treatment by algae. A controlled sample (0.1% v/v) was prepared for each experiment [17], three ml of samples were taken at different intervals under control conditions for examination. Light intensity and percentage of reduction rates were calculated after being compared with control [18].

2.3. Bacterial growth & culture
Bacteria (*Bacillus subtilis*) were collected from soil samples in different sites urban and agriculture area. Serial dilutions (up to 10^-7) from samples in the nutrient agar medium by spread plate technique and the isolated colonies were isolated and, Bacteria were diagnosed by identifying phenotypic and properties of the shape and edges of colonies in the dish. The experimental studied under different variation of pH (4.7 and 9). Under stable incubation condition with (50 ml of the nutrient broth mixed with 50, 150 and 250 mg/L) from Congo red and McFarland media and incubated at 37 C for 168 h.[19]. The samples were centrifuged at 4000 g for 15 minutes to exclude biomass, and the percentage removal was calculated as per the method documented [20].

Culture media was used as a control sample without algae incubated in the same conditions to exam specimens. A spectrophotometer (UV and visible) used to find out maximum absorbance (λmax) and wavelengths for Congo red found residual concentration was measured after period treatment [21].

The following formula determined the concentration of residual congo red:
Removal Efficiency RE (%) =  \( \frac{A - B}{A} \times 100 \)
A = Initial absorbance of Congo red
B= Congo red absorbance after treatment.

2.4. Statistical Analysis
Data were analysed by using the Statistical Package for social sciences (SPSS) program (version 21); the least significant difference (LSD ) was used to compare the significant difference between means at p<0.01.

3. Result and Discussion

3.1. Biotreatment of Congo red by algae (*Chlorella Vulgaris*)
The results showed that *C. Vulgaris* had a high ability to removed congo red after 3, 5, 7, 9, 11 and 13 days of treatments. Decolourisation by the algal cell may be due to chromophores' utilisation in biomass
production in algal culture. Also, photosynthesis's assimilation of CO2 by photosynthesis may help convert several species of Chlorella sp. and Oscillatoria sp. are used to degrading hazardous azo dyes by metabolising the aromatic amines to simpler organic compounds or dioxide carbon [23; 22]. The results observed significant differences (p ≤ 0.01) between treatments and residual concentration. Some researchers reported that more than 30 azo compounds could be biodegraded and decolourised by many algae species (C. pyrenoidosa, C. Vulgaris and Oscillatoria tenuis) [24].

The study showed significant differences (p ≤ 0.01) between different treatments and residual concentration after (3, 5, 7, 9, 11,13) days (see Figures 1 and 2) shows removed of Congo red in pH4 compared with control. After 13 days of treatment, the higher removal percentage efficiency in concentration 50ppm the removal percentage was 75%. Minimum removal percentage efficiency showed after 3 day of treatment, was 13%.

Figure 1: Effects of Chlorella vulgaris on removal of congo red at pH 4 and concentration (A=50 ,B=150 and C=250 mg/L).

Figure 2: Removal Efficiency (%) of Congo Red dye at pH 4 and concentration (A=50 ,B=150 and C=250 mg/L) by Chlorella vulgaris.

While in concentration 150 mg/L, the higher removal percentage recorded in 13 days of treatment was 60%, but the minimum removal percentage efficiency showed 3 days (8%). The result showed higher removal percentage efficiency was 54% recorded by concentration 250 mg/L after 13 days and lowest removal percentage was 5% after 3.

The maximum removal percentage efficiency after 13 days in concentration 50 mg/L was 70% as the maximum value, but the minimum removal percentage efficiency was 20% recorded after 3 days of treatment (see Figures 3 and 4).

While in the concentration 150 mg/L, the result showed a maximum removal percentage in 13 days of treatment, 75%, while minimum removal percentage efficiency after 3 days of treatment was 16%.

The maximum percentage in the concentration of 250 mg/L was recorded after 13 days to 66%, but the minimum percentage was recorded after 3 days of treatment (12%).
Figure 5 and 6. showed the result of Congo red inpH9. In concentration 50 mg/L, the maximum removal efficiency recorded after 13 days of treatment 66% but the minimum removal efficiency recorded after 3 days of treatment was 12%.

In concentration 150 mg/L, the maximum removal percentage showed in 13 days of treatment was 49%, the minimum removal percentage efficiency after 3 days of treatment, and the removal percentage was 8%.

Minimum removal percentage efficiency in concentration 250 mg/L was 39% after 13 days, but the removal percentage after 3 days of treatment was 5%.

The results showed that *C. vulgaris* appear higher ability to removal congo red after 3, 5, 7, 9, 11 and 13 days of treatments. In most results, there is a significant difference between treatments and control in all days of exposure.

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**Figure 3:** Effects of *Chlorella Vulgaris* on removing Congo red at temperature pH 7 and concentration (A=50, B=150 and C=250 mg/L).

**Figure 4:** Removal Efficiency (%) of Congo Red dye at pH 7 and concentration (A=50, B=150 and C=250 mg/L) by *Chlorella vulgaris*

**Figure 5:** Effects of *Chlorella Vulgaris* on (removal of Congo red at pH 9 and concentration (A=50, B=150 and C=250 mg/L).
Three values for pH (4, 7 and 9) were selected to test moss’s ability to remove congo red dye. The most noticeable results were that the preferred pH of algae in the removal was 4. The removal ratio after the last day of treatment at concentration 50 mg/L was 75% ; They were 70, 66% for pH 7 and 9 values, respectively.

The study shows a clear variation to pH over a range of (4,7,9) at initial dye concentrations (50,150,250 mg/L). The dye’s pH plays an important role in the adsorption process because it directly influences these techniques [25]. The colour of the examine dye’s solution was changed from red to dark blue at a pH of more than 9. The removal efficiency is recorded as a maximum value in an alkalinity medium, but the dye's solution becomes as cationic form [26][27]. Also, there are competing between anionic dye and –OH in alkalinity condition to attraction with adsorption site [28].

The results showed an increase in dye removal at low pH. They showed the effect of pH on congo red removal has varied between (4-9) and computable with [29], while removal efficiency is reduced from 90% to 77.7% gradually, like other studies [30, 24].

The maximum removal percentage efficiency in concentration 50 ppm recorded after 13 days of treatment (75%). Still, the low-value removal percentage recorded after 3 days of treatment was (20%).

In concentration 150 ppm, the result was recorded maximum removal percentage in 13 days of treatment (75%) and minimum removal percentage efficiency after 3 days of treatment (16%).

The maximum value of removal percentage efficiency in concentration 250 ppm recorded after 13 days (66%), but the low-value removal percentage recorded after 3 days of treatment was (12%). Figures (11 and 12) showed the Congo red removal in pH 9. In concentration 50 ppm, the maximum percentage efficiency was (75%) and minimum removal percentage efficiency after 3 days of treatment was (20%).

3.2 Biotreatment of Congo red by Bacteria (Bacillus subtilis):

pH is also an essential factor with regard to removal; the pH has a major effect on dye removal efficiency.

Removal of azo dye by B. subtilis was showed significant differences (p ≤ 0.01) between treatments after (3, 5, 7, 9, 11, 13) days (Figures 7 and 8) shows removed result of Congo red removal is pH 4. The result showed a maximum removal percentage in concentration 50 ppm efficiency after 13 days of treatment (75%). Still, the minimum removal percentage efficiency after 3 days of treatment was 13%. While in concentration 150 mg/L, the maximum removal percentage after 13 days of treatment was(60%), while (13%) the minimum removal percentage efficiency after 3 day of treatment was (8%). The maximum removal percentage efficiency in concentration 250 ppm was (54%) after 13 days, but the removal percentage after 3 days of treatment was 5%. Figures (9 and 10) showed the congo red removal in pH 7. In concentration 50 ppm, the maximum removal percentage efficiency after 13 days of treatment, the removal percentage was( 70%) and the minimum removal percentage efficiency after 3 day of treatment was( 20%).

In concentration 150 ppm, the result was recorded maximum removal percentage in 13 days of treatment (75%) and minimum removal percentage efficiency after 3 days of treatment (16%).

The maximum value of removal percentage efficiency in concentration 250 ppm recorded after 13 days (66%), but the low-value removal percentage recorded after 3 days of treatment was (12%). Figures( 11 and 12) showed the Congo red removal in pH 9. In concentration 50 ppm, the maximum percentage efficiency was (75%) and minimum removal percentage efficiency after 3 days of treatment was (20%).
Figure 7: Effects of *Bacillus subtilis* on removing Congo red at pH 4 and concentration (A=50, B=150 and C=250 mg/L).

Figure 8: Removal Efficiency (%) of Congo Red dye at pH 4 and concentration (A=50, B=150 and C=250 mg/L) by *Bacillus subtilis*

Figure 9: Effects of *Bacillus subtilis* removal of Congo red at pH 7 and concentration (A=50, B=150 and C=250 mg/L).
For this study, pH is an important factor affecting removing dye from an aqueous solution [32]. pH variation is directly associated with the overall biochemical processes and the growth of microorganisms related to dye molecules' transport across the cell membrane, which was considered the limiting factor in decolourisation performance. The effect of pH values was studied at 4, 7 and 9. The pH 7 showed maximum decolorization of CR dye that was 98.86 and 79%, while pH 4 was 71.59 and 46% and in pH 9 was 71, 65 and 49 in concentration 50, 150, and 250 mg/L respectively. This study agrees with [32], which showed the increases removal percentage from 80.5% to 87.5% as the pH increases between (4-6) and reaches the higher percentage of 96.5% in pH 7. The biochemical reactions by bacteria for degradation of dye was essential and increased significantly in pH 7; the study...
of Kumar and Sawhney [19] showed the maximum removal values (95.67%) in pH 8 and temperature (370°C) throw (60 h.).

4. Conclusion

The capacity of *Chlorella Vulgaris* and *Bacillus subtilis* to survive and grow in high concentrations of the Congo red dye, showed that these species might possess the potential to be used in the bioremediation of dyes contaminated environments.

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