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# Eco-friendly biological degradation and detoxification of congo red dye indigoes monospecies and mixed culture of bacterial strains isolated from textile industrial effluents

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**Abstract**

The textile industry is known to generate significant amounts of wastewater that contain various pollutants, including dyes, which can be harmful to the environment and public health. Therefore, effectively treating textile industry wastewater (TIWW) is crucial to ensure the environment's safety and human health. In this study, a mixed bacterial strain (RKS9) was isolated from TIWW samples, which showed potential for the biodegradation of Congo red (CR) dye. The bacterial isolates decolorized 99% of Congo red dye (60 mg/L) at 7.5 pH within 12 h from the salt broth solution, indicating their effectiveness in degrading the CR dye. Using bacterial consortia for the bioremediation of CR contamination is a promising approach, as it can effectively reduce pollution parameters. However, selecting bacterial strains and optimizing growth conditions are crucial to ensure the successful bioremediation of contaminants. Additionally, the bacterial consortium must be protected from toxic environments to ensure its effectiveness. Further research is needed to fully understand the potential of bacterial consortia in the bioremediation of CR and other contaminants. This will help develop effective and sustainable strategies for treating TIWW and other industrial wastewater.

***Keywords*:** Textile waste, congo red, decolorization, dyes biodegraders**,** COD

## 1- Introduction

The textile sector is a significant contributor to environmental pollution and health risks and a major driver of the global economy in developing nations. According to Biliska et al. (2019), several phases are involved in producing textiles, including sizing, bleaching, dyeing, printing, washing, and finishing. Various extremely hazardous recalcitrant coloring pollutants (residual dyes), dissolved solids, and toxic metals are in textile industry wastewater. These pollutants remain in the environment for a considerable amount of time and represent major risks to the environment, animal health, and human health (Zhan et al., 2020). Textile industry wastewater decreases dissolved oxygen (DO) levels and photosynthetic activity in the aquatic system, resulting in anoxic conditions that harm fauna and flora (Cao et al., 2019). Because of the buildup of harmful contaminants and metals in the terrestrial system decreases soil fertility (Kishor et al., 2021).

Nevertheless, the environmental harm brought on by the textile sector is primarily due to the discharge of untreated effluents into water bodies, which typically account for 80% of all emissions produced by this industry (Juárez-Hernández et al., 2021). There are comparatively significant levels of biological oxygen demand (BOD) and chemical oxygen demand (COD) in the majority of the residual fluids from the textile industry. The abundance of organic molecules that cannot degrade, particularly textiles, should receive more attention(Orts et al., 2018). The dyes are soluble organic molecules, particularly those within the reactive, direct, basic, and acid categories. They are very soluble in water, making it challenging to remove them using standard techniques. Due to the existence of chromophoric groups in its molecular structures, one of its characteristics is the capacity to color a particular

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substrate. However, the auxotrophic groups, which are polar and may bind to polarity groups of textile fibers, are associated with the ability to attach color to the material (Hassan and Carr, 2018)

The color associated with textile dyes taints water bodies and inhibits light from penetrating the water, slowing photosynthesis and lowering dissolved oxygen levels, negatively affecting the entire aquatic biota. The textile dyes also act as poisonous, mutagenic, and carcinogenic substances, linger in the environment as pollutants, and span whole food chains, giving biomagnification, causing species at greater trophic levels to exhibit more significant levels of contamination relative to their prey(Khatri et al., 2018). In this regard, azo-type textile dyes deserve special attention because, during the dyeing process, between 15 and 50 percent of them get out of wastewater frequently utilized in nations developing for irrigation in agriculture. The microbial populations in soil and plant germination and development are severely harmed by using these azo chemicals (Paździor et al., 2017).

Azo dyes are one of the main synthetic dye families with complicated aromatic structures. Due to the compound's resistance to degradation, these dyes are often quite stable. As azo dyes deteriorate, toxic compounds that cannot be broken down by natural processes are produced. The creation of several application-oriented biomaterials makes use of commercial dyes, which are ideal due to their source accessibility, ease of use as chemical auxiliaries, and coloring features of azo dyes (Crespi et al., 2019). Due to their nitro group, which is naturally poisonous, azo dyes pose the most threat to the environment due to the mineralization process, which might result in the breakdown of these azo dyes into aromatic amine (arylamine), they are both mutagenic and carcinogenic (Muliadi et al., 2021). Congo red (CR) as an azo dye type has a broad application in the world's clothing, textile, printing, culinary, and biomedical industries. Microbiology has employed it to identify curli fimbriae. The CR-containing effluent is intensely colored.

By reducing light penetration, excessive discharge of this raw wastewater into aquatic ecosystems, including rivers, ponds, and cannels significantly reduces the capacity of aquatic plants to photosynthesize (Khadhraoui et al., 2009; Reichhardt and Cegelski, 2018). By reducing dissolved oxygen, altering pH, reducing gas solubility, and raising BOD, COD, and total organic carbon values, effluent-containing CR degrades water quality. CR-mixed wastewater reduces soil fertility, biomass output, and seed germination. Therefore, before the wastewater containing CR is finally released into the natural environment, it must be processed (Kaur et al., 2023).

Scientists are becoming more interested in the removal of dyes from wastewater. The process of getting rid of colored effluents in treatment facilities relies primarily on physical or chemical processes, such as adsorption, chemical transformation, and incineration, as they are generally resistant to biodegradation. However, these techniques are extremely costly, generate extensive sludge, and create secondary by-products (Muliadi et al., 2021). Biological techniques for eliminating colors must be created because they are cost-effective, efficient, and ecologically friendly than physical or chemical processes. An appealing option looks to be biodegradation by various microorganisms, which employs bacteria as a dye-decolorizing agent (Sarkar et al., 2017).

Numerous circumstances can lead to microbial discoloration, including anaerobic, anoxic, and aerobic environments. Since the bacteria cannot use the dye as a growth substrate, natural carbon sources are frequently required to remove color under aerobic circumstances. In most cases, azo dyes undergo degradation and are decolored by bacteria throughout two phases. Reductive cleavage of the azo linkages in the dyes results in the creation of primarily colorless but potentially dangerous aromatic amines in the first step. Aromatic amine degradation occurs in the second step (Sarkar et al., 2017). Therefore, the present research's primary purpose is to select new bacterial isolates capable of promoting a complete degradation of CR dyes which could have important implications for reducing environmental contamination caused by these dyes. Optimizing the conditions under which these selected bacterial strains can degrade CR dyes was also assessed. This work can potentially contribute to the development of biotechnological tools that can mitigate the harmful effects of CR dye contamination in various settings.

## 1. Experimental

*1.1.* ***Microorganisms used and culture condition*** According to the experimental study published previously by El-Liethy et al., (2023), twenty eight bacterial strains isolated from real textile wastewater from different factories and environmental samples were tested to determine the most suitable biodegradable bacterial strains. The

identified strains were divided into four other groups. One strain of each group was chosen based on its biodegradation performance of crystal violet under stable conditions (35°C and pH 7.5). Six best biodegrading species, including *Citrobacter freundii, Klebsiella pneumonia, Enterobacter cloacae, Pseudomonas aeruginosa*, and *Aeromonas veronii* have been selected for decolorization experiments. The inoculum was prepared before each test to determine the initial counts. Stock microbial strains with 10% glycerol at −20°C were inoculated in 50 mL Tryptic Soya Broth (TSB) (Merck, Germany). The tubes were incubated at 37°C for 24h. The proliferated bacterial strain was centrifuged at 5000 rpm for 20 min, and the pellet was re-suspended in sterile distilled water and then washed three times to remove any additive nutrients. The counts between $10^5$ and $10^6$ CFU/mL were chosen by ten serial dilutions using the pour plate method. The resulting colonies were counted and expressed as colony-forming units (CFU/mL).

**Table 1. Tested bacterial strains and their accession number**

| Strain code | Strains | Accession number |
|---|---|---|
| **11** | *Citrobacter freundii* | MW485549 |
| **53** | *Enterobacter cloacae* | MW485575 |
| **142** | *Aeromonas veronii* | MW485582 |
| **203** | *Pseudomonas aeruginosa* | MW487241 |

1.2 ***Media***

In this stage of the project, mineral salts medium (MSM) was used as a synthetic solution (MSM) (g/L, Na2HPO4.2H2O (7.89); KH2PO4 (6.8); MgSO4 (0.2); Fe(CH3COO).NH4 (0.05); Ca(NO3)2.4H2O (0.05); NaCO3 (0.085) was prepared. pH of this medium was adjusted to 7.5 to test the biodegradation activities of different dyes using the previously identified bacterial strains to optimize the most suitable biodegrading conditions at lab. Scale (Deng et al., 2020).

***1.3. Preparation of dyes Standard Curve***

Dyes solutions with various concentrations (0-25 mg/l) CR were prepared to obtain the standard curves at their max absorbance wavelength. The standard curves were obtained by plotting the recorded absorbance versus the prepared concentration of the dyes.

**1.4. Factors affecting the decolorization activity for optimizing condition**

Optimization of various environmental parameters (pH, temperature, and initial dye concentration) for decolorization of CR dye was studied as follows:

***1.4.1. Effect of pH***

250 mL liquid medium was prepared. The pH of each medium was adjusted to (6, 7.5, and 9) using NaOH and HCL. Each flask was supplemented with (50-60 mg/l) CR dye separately and incubated at room temperature for intervals times (2, 4, 6, 24, 30, and 48 h). 10 mL of solutions were centrifuged after each time and the clear supernatant was measured using a spectrophotometer to identify the remaining dye concentration.

***14.2. Effect of temperature***

To determine the optimum decolorization temperature of CR dye by the bacterial strains, 50-60 mg/L of each dye was added to the media at pH 7.5 and 9. The mixtures were incubated at different temperatures, including room temperatures, 35°C, and 40°C, for different intervals time. The solutions were centrifuged after a contact time of (2, 4, 6, 24, 30, and 48hrs.) and the clear supernatant was measured.

***1.4.3. Effect of dye concentration***

The various concentrations (25, 50, and 75 mg/l) of CR dye were added separately into the medium at 35oC and pH (7.5 and 9) to examine the initial dye's effect on the decolorization. The regular procedure of centrifuge and analysis was next applied at time intervals (2, 4, 6, 24, 30, and 48 h.). Subsequently, the decolorization study of CR dye was carried out at optimum decolorization conditions by using 50 mg/L of each dye in a 250 ml flask containing media at pH 7.5. The mixture was incubated at 35oC for 24 h, solutions were centrifuged after time, and the clear supernatant was measured using a spectrophotometer.

***1.5. Physico-chemical analysis***

The Physicochemical analysis included pH, total dissolved solids (TDS), chemical oxygen demands (COD), and color were analyzed for the initial and treated dye solutions according to APHA (2017).

## 2. Results and discussion

Potential capability of dye-degrading species for degradation and decolorization of Congo red dye.

It's interesting to note that CR dye is a highly stable and toxic azo dye, and its biodegradation can be challenging. Study is focused on the efficiency of degradation through biological treatment by bacteria on stimulated industrial wastewater as a culture medium, which is an essential step towards developing an effective bioremediation strategy (Asses et al., 2018).The physicochemical operational parameters can affect the ability of bacteria to degrade Congo Red. The dye concentration, pH, temperature, and exposure time are all crucial factors that can impact the efficiency of bacterial degradation. By carefully studying these factors, you can identify the optimal conditions for the efficient degradation of CR dye by the selected bacterial strains. Ultimately, learning these factors aims to make the bacterial degradation process faster and more efficient. By optimizing the conditions under which the bacteria are grown, and the dye is degraded, you can maximize the efficiency of the bioremediation process and potentially reduce the environmental impact of CR dye contamination.

### *a. Effect of different pH on congo red decolorization activity*

The optimization of conditions for the selected bacterial strains can involve several steps, depending on the specific goals of the study. Some key factors that can be optimized include: The pH of the growth medium, can also impact the growth of the bacterial strains and their ability to degrade the CR dye. By optimizing the pH, it may be possible to create an environment that is more conducive to bacterial growth and dye degradation. The temperature at which the bacterial strains are grown can also impact their growth and activity. By optimizing the temperature, it may be possible to create conditions that enhance the bacterial growth rate and improve the efficiency of dye degradation. The length of time that the bacterial strains are incubated can also impact their growth and activity. By optimizing the incubation time, it may be possible to create conditions that maximize the bacterial growth rate and enhance the efficiency of dye degradation. Likewise, the specific optimization strategies will depend on the specific bacterial strains being studied and the research goals. By systematically testing different conditions, it may be possible to identify the optimal conditions for the efficient degradation of CR dyes by the selected bacterial strains.

The present research employed five indigenous dye biodegrading species to decolorize CR dye in mineral salt broth medium at different pH, temperatures, and dye concentrations. The decolorization of CR dye by the strains mentioned above in terms of the response decolorization efficiency as a function of pH, temperature, and dye concentrations is documented. The results of the effect of three various pH (6, 7.5, and 9), which were employed as a variable factor, on tested bacterial efficiency in the biodegrading performance of 50 mg/L of Congo red at ambient temperature within different contact time intervals. Investigational data displayed that the most significant decolorization activity after 48 h as an exposure time at 25$^{o}$C with different decolorizing levels was reported with all selected bacterial species, depending on each bacterial species used. Similarly, the decolorization rate of Congo red at pH 6 after 48hr was progressively reduced from initial concentration (50 mg/L) up to 8.5 mg/L using bacterial strain no. 11 followed by strain no. 203. Nevertheless, the lowest biodegradation rate (26.4 mg/L as residual dye amount) was found in strain No. 53 (Table 2).

**Table 2. Congo red decolorization at pH 6 and 25$^{o}$C, 50 mg/L dye concentration and different contact times**

| Strains | 2h | | 4h | | 6h | | 24h | | 30h | | 48h | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab |
| 53 | 44.3 | 0.11 | 44 | 0.11 | 39.6 | 0.1 | 28.6 | 0.08 | 30.3 | 0.11 | 26.4 | 0.09 |
| 203 | 30.4 | 0.18 | 28.7 | 0.17 | 26.5 | 0.18 | 22.4 | 0.17 | 16.9 | 0.14 | 15.8 | 0.14 |
| 142 | 35.4 | 0.18 | 31.5 | 0.18 | 28.8 | 0.15 | 23.1 | 0.14 | 17.5 | 0.11 | 17.8 | 0.09 |
| 11 | 24.5 | 0.15 | 23.5 | 0.14 | 22.1 | 0.12 | 18.6 | 0.11 | 10.3 | 0.04 | 8.5 | 0.09 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mix | 26.5 | 0.16 | 25 | 0.15 | 22.5 | 0.14 | 17.8 | 0.11 | 17.5 | 0.1 | 16.9 | 0.05 |
| 149 | 29.9 | 0.17 | 28.1 | 0.16 | 26.1 | 0.15 | 22.4 | 0.14 | 17.8 | 0.06 | 19.5 | 0.06 |
| Initial | 47.7 | | | | | | | | | | | |

Simultaneously, the results gained exhibited that the removal effectiveness of Congo red at neutral conditions (pH 7.5) with 48hr was dramatically increased. The best Congo red biodegrader was the mixed bacterial culture in which the highest decolorization rate and residual dyes concentration was reduced to 6.2 mg/L. In opposition, strain No. 53 was considered an ineffective biodegrader, where the remaining dye concentration was 20.5 mg/L after 48 h (Table 3).

**Table 3. Congo red decolorization at pH 7.5 and 25°C, 50 mg/L dye concentration and different contact times**

| Strains | 2h | | 4h | | 6h | | 24h | | 30h | | 48h | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab |
| **53** | 44.1 | 0.12 | 44 | 0.12 | 38 | 0.08 | 24.6 | 0.07 | 22.9 | 0.07 | 20.58 | 0.71 |
| **203** | 22.5 | 0.11 | 21 | 0.1 | 21 | 0.11 | 18 | 0.01 | 2.8 | 0.38 | 19.8 | 0.037 |
| **142** | 33.8 | 0.19 | 30.5 | 0.17 | 29.5 | 0.11 | 26.1 | 0.15 | 22.2 | 0.8 | 19.5 | 0.047 |
| **11** | 29 | 0.16 | 26 | 0.15 | 25 | 0.15 | 24.2 | 0.05 | 9 | 0.85 | 7.6 | 0.097 |
| **Mix** | 30 | 0.16 | 28.5 | 0.14 | 26.5 | 0.14 | 24.6 | 0.11 | 11.9 | 0.25 | 6.2 | 0.093 |
| **149** | 31 | 0.17 | 28.4 | 0.14 | 26.4 | 0.13 | 6.13 | 0.46 | 16.9 | 0.4 | 15.8 | 0.05 |
| **Initial** | 49.2 | | | | | | | | | | | |

In the case of increasing the pH over 9, meaning an alkaline condition, the results of the level of Congo red decolorization using five particular bacterial strains are displayed in Table 4.

**Table 4. Congo red decolorization at pH 9 and 25°C, 50 mg/L dye concentration, and different contact times**

| Strains | 2h | | 4h | | 6h | | 24h | | 30h | | 48h | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab |
| **53** | 32.2 | 0.12 | 30.5 | 0.12 | 28.6 | 0.18 | 23.5 | 0.15 | 18.9 | 0.057 | 17.5 | 0.042 |
| **203** | 23.6 | 0.17 | 26.2 | 0.14 | 24.5 | 0.13 | 21.8 | 0.11 | 19 | 0.057 | 17.0 | 0.042 |
| **142** | 29.9 | 0.19 | 26.6 | 0.15 | 21.2 | 0.14 | 18 | 0.11 | 14 | 0.066 | 12.6 | 0.052 |
| **11** | 24.6 | 0.16 | 22.5 | 0.15 | 22 | 0.14 | 16.8 | 0.11 | 17.5 | 0.05 | 8.5 | 0.046 |
| **Mix** | 22.8 | 0.12 | 21.1 | 0.12 | 20.2 | 0.1 | 15.3 | 0.08 | 9.8 | 0.063 | 7.5 | 0.049 |
| **149** | 28.4 | 0.17 | 26.4 | 0.16 | 23.5 | 0.15 | 18.4 | 0.12 | 16.5 | 0.1 | 17.2 | 0.036 |

The results revealed that the mixture of bacterial strains had been the most potent biodegrader, with a residual dye concentration of 7.5 mg/L. On the contrary, strain No. 53 needed a relatively long adaptation interval because the greatest residual dye was 17.5 mg/L, implying that the biodegradability of this strain using this strain was low compared to all others. Results could be cleared that the bacterial

activity and biodegradation rate at pH 7.5 was in the best way. Further, the results exhibited that the highest biodegradability of congo red dye using selected bacterial species was documented at pH 7.5. Besides, the results demonstrated that the mixture of bacterial multispecies had outstanding decolorization ability when compared with the mono-species.

***b. Effect of Congo red decolorization on some physicochemical parameters at different pH***

Table 5 and Fig.1, illustrated the effect of different pH on decolorization of Congo red at 50mg/L and 25°C. The results showed that the best decolorzaition was observed at pH 7.5, which reached 53 and 69% for removal of color and COD, respectively.

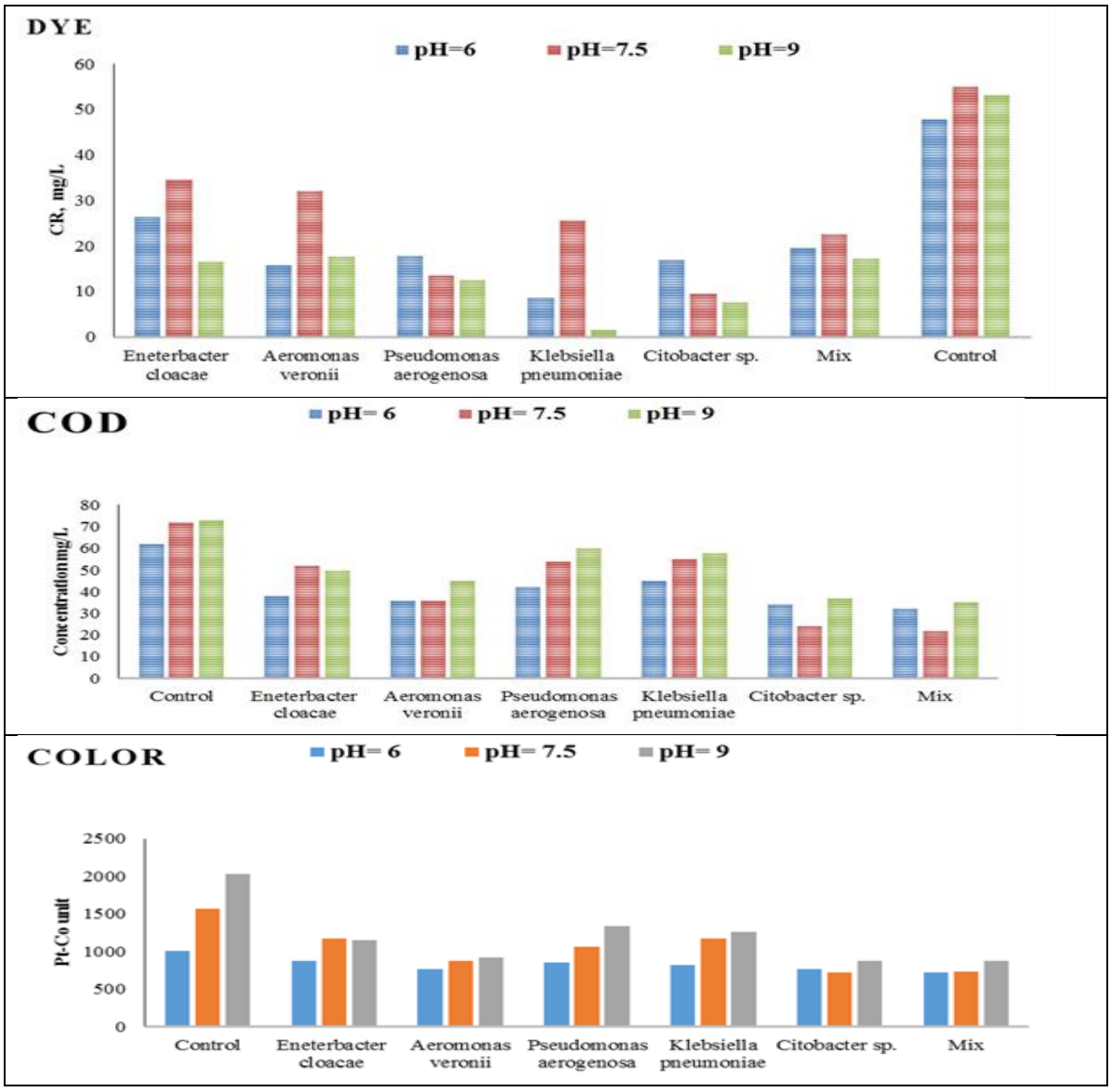



**Fig. 1. Effect of different pH levels (6, 7.5, and 9) on Congo red decolorization in terms of dye, COD and color removal.**

**Table 5. Some physicochemical analysis for congo red decolorization at different pH**

| | **pH= 6** | | | | | |
|---|---|---|---|---|---|---|
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH= 6** | Control | 6.29 | 1010 | 62 | 5800 | 9667 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 53 | 6.24 | 880 | 38 | 5510 | 9183 |
| | 142 | 6.28 | 760 | 36 | 5460 | 9100 |
| | 203 | 6.26 | 850 | 42 | 5490 | 9150 |
| | 149 | 6.25 | 820 | 45 | 5460 | 9100 |
| | 11 | 6.28 | 760 | 34 | 5360 | 8933 |
| | Mix | 6.26 | 720 | 32 | 5210 | 8683 |
| **pH= 7.5** | | | | | | |
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH= 7.5** | Control | 7.6 | 1570 | 72 | 7540 | 12567 |
| | 53 | 7.62 | 1170 | 52 | 7160 | 11933 |
| | 142 | 7.66 | 880 | 36 | 6890 | 11483 |
| | 203 | 7.46 | 1060 | 54 | 7230 | 12050 |
| | 149 | 7.45 | 1177 | 55 | 7250 | 12083 |
| | 11 | 7.48 | 720 | 24 | 6980 | 11633 |
| | Mix | 7.44 | 730 | 22 | 6750 | 11250 |
| **pH= 9** | | | | | | |
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH= 9** | Control | 9.11 | 2030 | 73 | 11130 | 18550 |
| | 53 | 9.01 | 1150 | 50 | 10310 | 17183 |
| | 142 | 9.2 | 918 | 45 | 10070 | 16783 |
| | 203 | 8.99 | 1340 | 60 | 10420 | 17367 |
| | 149 | 9.12 | 1259 | 58 | 10510 | 17517 |
| | 11 | 9.1 | 880 | 37 | 10050 | 16750 |
| | Mix | 9.2 | 870 | 35 | 10100 | 16833 |

**c. Effect of different temperatures on Congo red decolorization activity**

To evaluate the impact of temperature on CR dye degradation activity using selected bacterial strains, three different temperatures (25, 35, and 40°C), were chosen to determine the optimal temperature for promoting bacterial growth and higher biodegradability with keeping the pH constant at the optimum value of 7.5, which allows you to isolate the effect of temperature on the degradation process. Maintaining a constant CR dye concentration of 50 mg/L ensures that the bacterial strains are exposed to the same level of contaminant throughout the experiments. From the above-mentioned experimental work, optimum pH (7.5) was applied as a constant environmental condition (25$^{o}$C). Estimating suitable temperatures for promoting bacterial growth and higher biodegradability was performed under constant conditions, including pH (7.5) and 50 mg/L of Congo red.

The observed results suggest that the Congo red decolorization performance employing all bacterial species was at its peak after 24hrs with various removal grades, where the biodegradation rate was boosted in durations of time up to 24hrs, whereby it became steady. The findings demonstrate that the minor residual dye concentration equals the highest dye biodegradability, with a mixture of bacterial strains being the strongest Congo red decolorizer (9.2 out of 50 mg/L), followed by strain No.11 (Table 6). While, at 9 pH and 40 $^{o}$C mixed culture degraded Congo red to 12.8 out of 50 mg/L (Table 7). Strain no. 53, on another side, seemed to have the most minor decolorization frequency, with a persisting dye concentration of 25.8 mg/L (Table 26). This was confirmed by the results of physic-chemical analyses (Tables 8 and 9, Fig.2)

**Table 6. Congo red (50 mg/L) decolorization at 9 pH and 35$^{o}$C and different contact times**

| Strains | 2h | | 4h | | 6h | | 24h | |
|---|---|---|---|---|---|---|---|---|
| | **Conc.** | **Ab** | **Conc.** | **Ab** | **Conc.** | **Ab** | **Conc.** | **Ab** |
| 53 | 34.1 | 0.17 | 30.8 | 0.172 | 26.3 | 0.138 | 25.8 | 0.122 |
| 203 | 41.2 | 0.14 | 30.4 | 0.13 | 28.3 | 0.148 | 24.3 | 0.119 |
| 142 | 32.3 | 0.18 | 26 | 0.13 | 18.4 | 0.138 | 14.7 | 0.167 |
| 11 | 38.5 | 0.15 | 30.4 | 0.149 | 29.5 | 0.1 | 14 | 0.12 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mix | 31.5 | 0.14 | 20.8 | 0.14 | 15.8 | 0.16 | 9.2 | 0.16 |
| 149 | 31.8 | 0.0139 | 24.4 | 0.14 | 19.5 | 0.17 | 12.6 | 0.12 |

**Table 7. Congo red (50 mg/L) decolorization at pH 9 and 40°C, at different contact times**

| Strains | 2h | | 4h | | 6h | | 24h | |
|---|---|---|---|---|---|---|---|---|
| | Conc. | Ab | Conc. | Ab | Conc. | Ab | Conc. | Ab |
| 53 | 33.7 | 0.179 | 30.8 | 0.12 | 27.1 | 0.148 | 23.5 | 0.119 |
| 203 | 34.1 | 0.141 | 31.5 | 0.13 | 28 | 0.112 | 23 | 0.096 |
| 142 | 32.6 | 0.186 | 28.5 | 0.16 | 22.7 | 0.117 | 17.5 | 0.089 |
| 11 | 38.5 | 0.16 | 37 | 0.141 | 33.1 | 0.112 | 23.3 | 0.101 |
| Mix | 26.4 | 0.14 | 20.1 | 0.123 | 18.7 | 0.136 | 12.8 | 0.112 |
| 149 | 27.1 | 0.133 | 22.6 | 0.14 | 22.7 | 0.118 | 16.7 | 0.098 |

**Table 8. Some physicochemical analysis for Congo red decolorization at pH 7.5 and 9 and 25°C**

| | Temperature, 35°C | | | | | |
|---|---|---|---|---|---|---|
| | | pH | Color | COD | TDS | EC |
| **pH= 7.5** | Control | 7.6 | 1570 | 72 | 7540 | 12567 |
| | 53 | 7.62 | 1170 | 52 | 7160 | 11933 |
| | 142 | 7.66 | 880 | 36 | 6890 | 11483 |
| | 203 | 7.46 | 1060 | 54 | 7230 | 12050 |
| | 149 | 7.45 | 1177 | 55 | 7250 | 12083 |
| | 11 | 7.48 | 720 | 24 | 6980 | 11633 |
| | Mix | 7.44 | 730 | 22 | 6750 | 11250 |
| | **Temperature, 40°C** | | | | | |
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH= 9** | Control | 9.11 | 2030 | 73 | 11130 | 18550 |
| | 53 | 9.01 | 1150 | 50 | 10310 | 17183 |
| | 142 | 9.2 | 918 | 45 | 10070 | 16783 |
| | 203 | 8.99 | 1340 | 60 | 10420 | 17367 |
| | 149 | 9.12 | 1259 | 58 | 10510 | 17517 |
| | 11 | 9.1 | 880 | 37 | 10050 | 16750 |
| | Mix | 9.2 | 870 | 35 | 10100 | 16833 |

Control = without adding bacterial strains

***d .Effect of different dye concentrations on Congo red decolorization activity***

As seen in Table 10, this was created with a dye concentration of 25 mg/L after 24hrs. A mixed bacterial culture with a decolorizing value of 17.3 mg/L had the highest decolorization rate of Congo red after 24 h with mixed culture, whereas the lowest decolorizing rate of 25 mg/L was found in strain No. 203. Table 11 shows that the dye concentration (75 mg/L) was applied to be degraded using tested bacterial species at stable conditions (pH 9 and 35oC). Results exhibited the maximum decolorization rate that the highest decolorizing rate was 29 mg/L for mixed bacterial culture, while the lowest decolonizing rate (52 mg/L) was recorded for strain No. 203.

The physico chemical analyses indicated that, regarding to the dye concentrations (25 mg/L) of Congo red, it was found that the highest removal efficiency was observed with the mixed culture followed by strain no. 149 (Table 12 and Fig. 3). Concerning the dye concentrations (75 mg/L) of CR dye, it has given the same results as Table (11).

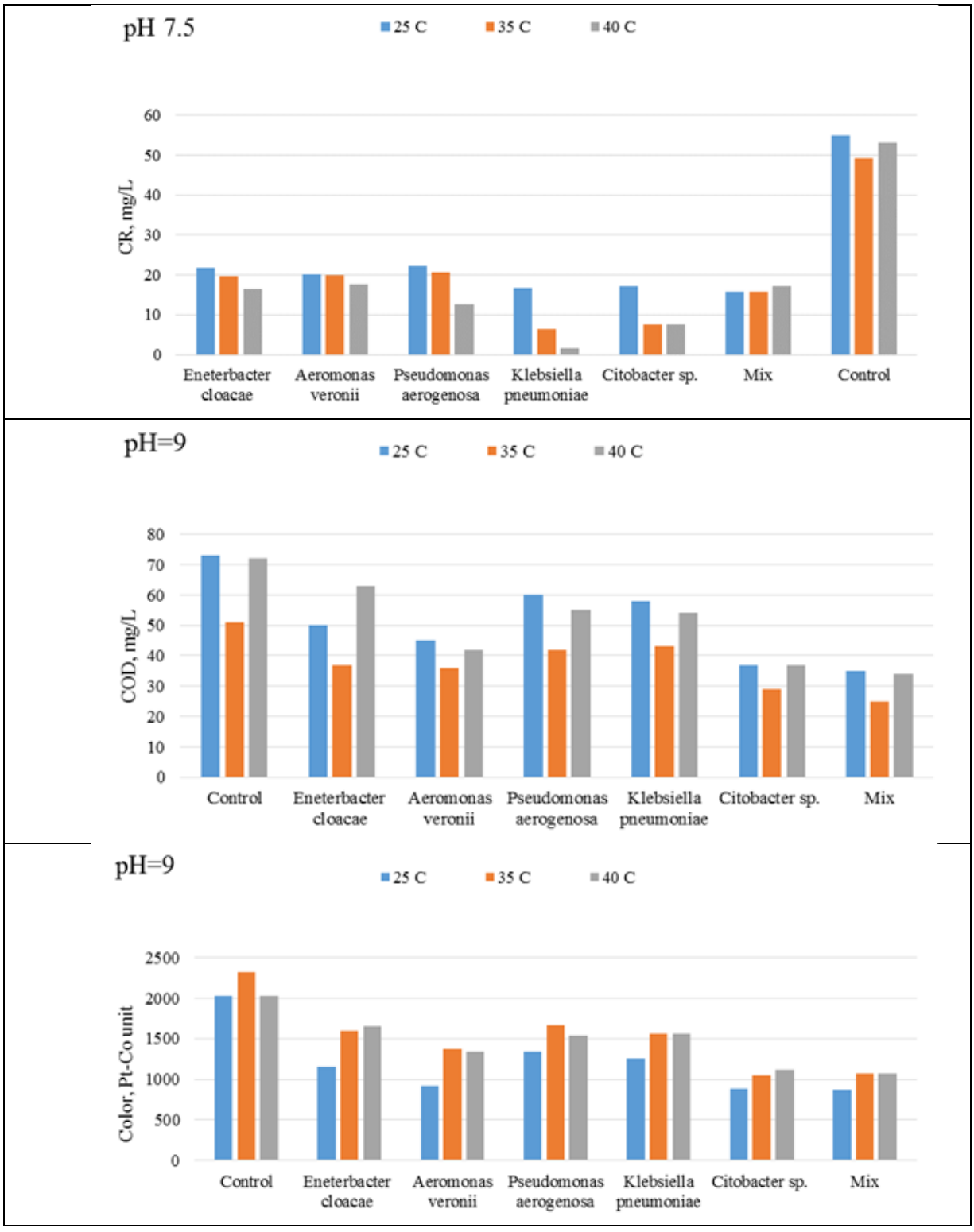



**Fig. 2. Effect of different temperature (25, 35, and 40°C) on Congo red decolorization in at different pH 7.5 and 9.**

**Table 9. Some physicochemical analysis for congo red decolorization at pH 9 and 35 and 40°C**

| | | | | | | |
|---|---|---|---|---|---|---|
| | **Temperature, 35°C** | | | | | |
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH = 9** | Control | 8.88 | 2320 | 51 | 7780 | 12966.7 |
| | 53 | 8.91 | 1600 | 37 | 7550 | 12583.3 |
| | 142 | 8.91 | 1369 | 36 | 7470 | 12450 |
| | 203 | 8.9 | 1670 | 42 | 7700 | 12833.3 |
| | 149 | 8.91 | 1560 | 43 | 7780 | 12966.7 |
| | 11 | 8.95 | 1050 | 29 | 7260 | 12100 |
| | Mix | 8.97 | 1070 | 25 | 7300 | 12166.7 |
| | **Temperature, 40°C** | | | | | |
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH = 9** | Control | 8.87 | 2030 | 72 | 7620 | 12700 |
| | 53 | 8.9 | 1650 | 63 | 7610 | 12683 |
| | 142 | 8.95 | 1340 | 42 | 7230 | 12050 |
| | 203 | 8.9 | 1540 | 55 | 7560 | 12600 |
| | 149 | 8.91 | 1560 | 54 | 7500 | 12500 |
| | 11 | 8.91 | 1120 | 37 | 7140 | 11900 |
| | Mix | 8.89 | 1070 | 34 | 7230 | 12050 |

Control = without adding bacterial strains

**Table 10. Decolorization of Congo red at pH 9 and 35°C, 25 mg/L**

| **Strains** | **2h** | | **4h** | | **24h** | |
|---|---|---|---|---|---|---|
| | **Conc.** | **Ab** | **Conc.** | **Ab** | **Conc.** | **Ab** |
| 53 | 32 | 0.13 | 27.7 | 0.107 | 22.6 | 0.10 |
| 203 | 1.8 | 0.11 | 18 | 0.12 | 25 | 0.112 |
| 142 | 22.7 | 0.129 | 29.2 | 0.117 | 19.2 | 0.10 |
| 11 | 21.4 | 0.119 | 21.8 | 0.115 | 22.6 | 0.10 |
| Mix | 15.6 | 0.132 | 18.5 | 0.11 | 17.3 | 0.108 |
| 149 | 20.2 | 0.089 | 19.2 | 0.067 | 18.5 | 0.05 |

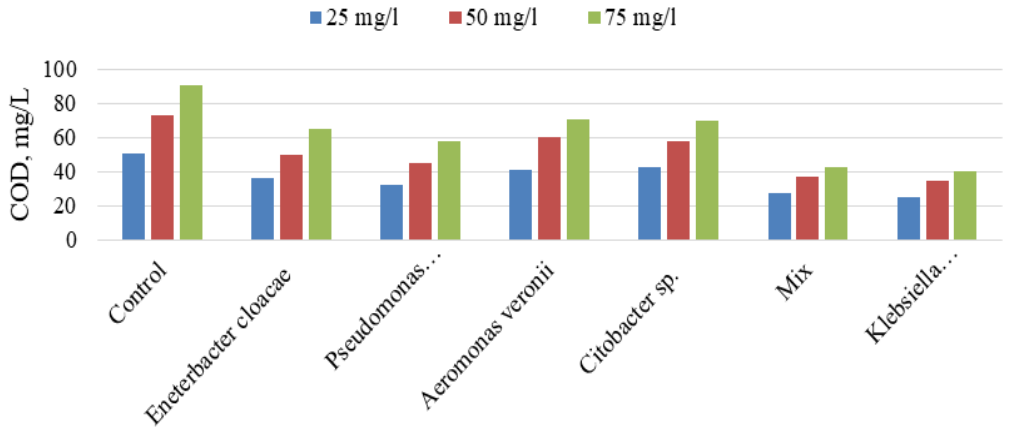
**Table 11. Decolorization of Congo red at pH 9 and 35°C, 75 mg/L**

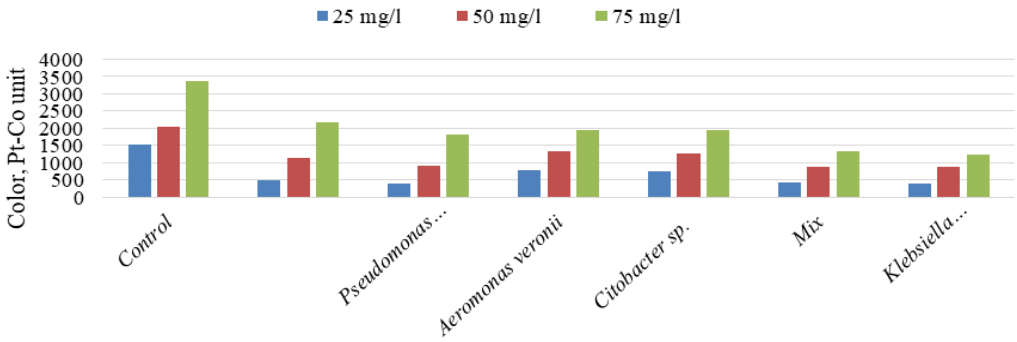
| Strains | 2h | | 4h | | 24h | |
|---|---|---|---|---|---|---|
| | Conc. | Ab | Conc. | Ab | Conc. | Ab |
| 53 | 43 | 0.51 | 53 | 0.42 | 43 | 0.4 |
| 203 | 66 | 0.42 | 52 | 0.52 | 52 | 0.48 |
| 142 | 55 | 0.5 | 45 | 0.42 | 39 | 0.39 |
| 11 | 48 | 0.49 | 53 | 0.49 | 32 | 0.46 |
| Mix | 48 | 0.42 | 35 | 0.36 | 29 | 0.30 |
| 149 | 46 | 0.46 | 48 | 0.46 | 37 | 0.41 |
| initial | 64 | 0.57 | | | | |

**Table 12. Some physicochemical analysis for Congo red decolorization at fixed pH 9 and at 35°C with different dye concentrations (25, 50, and 75 mg/L)**

| | **25 mg/L** | | | | | |
|---|---|---|---|---|---|---|
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH=9** | **initial** | 8.93 | 1519 | 51 | 8150 | 13583 |
| | **11** | 8.91 | 760 | 43 | 7159 | 11932 |
| | **53** | 8.96 | 400 | 32 | 6990 | 11650 |
| | **203** | 8.93 | 770 | 41 | 7100 | 11833 |
| | **149** | 8.91 | 430 | 28 | 6940 | 11567 |
| | **124** | 8.99 | 490 | 36 | 6980 | 11633 |
| | Mix | 9.95 | 380 | 25 | 6170 | 10283 |
| **50 mg/L** | | | | | | |
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH=9** | **initial** | 9.11 | 2030 | 73 | 11130 | 18550 |
| | **11** | 9.01 | 1150 | 50 | 10310 | 17183 |
| | **53** | 9.2 | 918 | 45 | 10070 | 16783 |
| | **203** | 8.99 | 1340 | 60 | 10420 | 17367 |
| | **149** | 9.12 | 1259 | 58 | 10510 | 17517 |
| | **124** | 9.1 | 880 | 37 | 10050 | 16750 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Mix | 9.2 | 870 | 35 | 10100 | 16833 |
| **75 mg/L** | | | | | | |
| | | **pH** | **Color** | **COD** | **TDS** | **EC** |
| **pH= 9** | **initial** | 8.89 | 3370 | 91 | 8040 | 13400 |
| | **11** | 8.93 | 2180 | 65 | 7130 | 11883 |
| | **53** | 8.91 | 1830 | 58 | 7090 | 11817 |
| | **203** | 8.9 | 1960 | 71 | 7250 | 12083 |
| | **149** | 8.92 | 1930 | 70 | 7320 | 12200 |
| | **124** | 9 | 1320 | 43 | 6980 | 11633 |
| | Mix | 8.97 | 1230 | 40 | 6900 | 11500 |



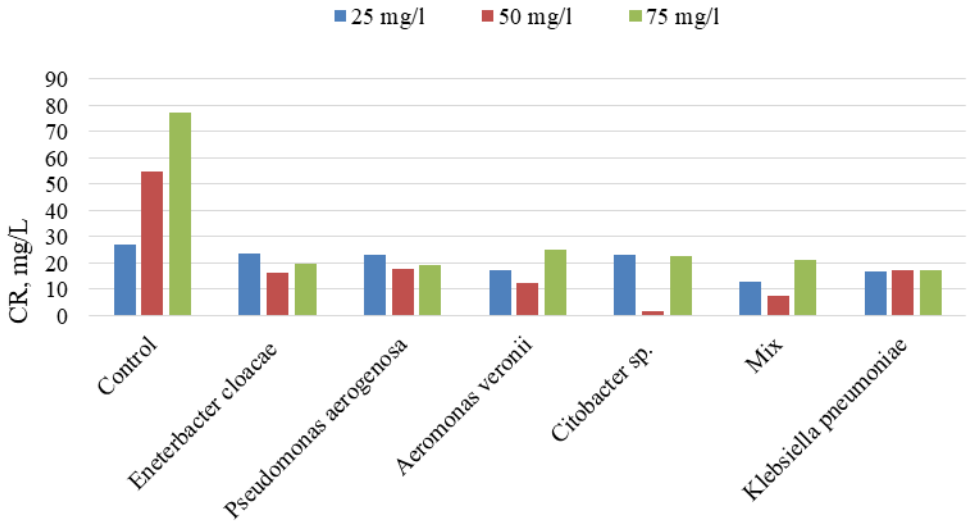



**Fig. 3. Decolorization of different CR concentrations at pH=7.5 and Temperature 35°C**

Interestingly, a bacterial consortium, which is a mixture of different bacterial strains, can be more effective at degrading Congo Red than a single bacterial strain. This is because each bacterial strain in the consortium may attack the dye molecule at different points or use the metabolites generated by co-existing strains for further degradation, leading to faster and more complete degradation of the contaminant (Lade et al., 2015). However, while bacterial consortia can effectively degrade Congo Red, it's important to note that they can also be vulnerable to toxic environments. Free-living planktonic bacterial consortia, in particular, may be susceptible to lowered defense, low metabolic activity, and reduced bioavailability of contaminants, which can limit their ability to degrade Congo Red efficiently (Sotelo et al., 2022). It is important to carefully select the appropriate bacterial strains for the consortium and optimize the growth conditions to maximize their ability to degrade Congo Red to overcome these limitations. Additionally, immobilizing the bacterial consortium in a matrix such as activated carbon or alginate beads can help protect them from toxic environments and enhance their stability and efficiency (Moyo et al., 2022). Bacterial consortia have shown great promise in degrading Congo Red and other contaminants, but much research is still needed to understand their potential and optimize their use fully. The ability of a bacterial consortium to attack the dye molecule at various places or utilize metabolites produced by co-existing strains for further degradation is undoubtedly advantageous. Still, the vulnerability of free-living planktonic bacterial consortia to toxic environments is also a concern. Further research is needed to identify suitable bacterial consortia that can effectively degrade Congo Red into less hazardous by-products even in harsh conditions. This may involve careful selection of bacterial strains, optimization of growth conditions, and immobilization of the bacterial consortium in a protective (Jamee and Siddique, 2019). Additionally, more research is needed to understand how bacterial consortia degrade Congo Red and other contaminants and the potential risks associated with using bacterial consortia in bioremediation applications. Generally, using bacterial consortia for the bioremediation of Congo Red and other pollutants is a promising approach that requires continued research and development to fully realize its potential and ensure its safety and effectiveness (Khan et al., 2021).

## Conclusion

Congo red is a synthetic diazo dye that is commonly used in the textile industry. It is known to be toxic to aquatic life and can also have adverse effects on human health. Therefore, it is important to develop eco-friendly and sustainable methods for the removal of Congo red from textile wastewater. One approach to the removal of Congo red from wastewater is through the use of biological degradation. This involves the use of microorganisms,

such as bacteria, to break down the dye molecules into less harmful substances. Mixed bacterial strains have been found to be effective in the degradation of Congo red, as they can work together to break down the dye more efficiently. To isolate bacterial strains for the degradation of Congo red, wastewater samples were collected from textile industries and screened for their ability to degrade the dye. The isolated strains were then tested for their ability to degrade the dye under different conditions, such as different temperatures and pH levels. This approach is eco-friendly and sustainable, as it does not require the use of harsh chemicals or energy-intensive processes. Overall, the use of mixed bacterial strains for the biological degradation of Congo red is a promising approach for the eco-friendly and sustainable removal of this toxic dye from textile wastewater. The present study could recommend that once the most effective bacterial strains have been identified, they were used in a bioreactor system for the large-scale removal of Congo red from textile wastewater.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

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## 5. References

APHA, AWWA, WEF, 2017. Standard Methods for the Examination of Water and Wastewater (23rd ed.), American Public Health Association, Washinton, D.C., USA.

Asses, N., Ayed, L., Hkiri, N., Hamdi, M., 2018. Congo Red Decolorization and Detoxification by Aspergillus Niger: Removal Mechanisms and Dye Degradation Pathway. Biomed Res. Int. 2018. https://doi.org/10.1155/2018/3049686

Bilińska, L., Blus, K., Gmurek, M., Ledakowicz, S., 2019. Coupling of electrocoagulation and ozone treatment for textile wastewater reuse. Chem. Eng. J. 358, 992–1001. https://doi.org/https://doi.org/10.1016/j.cej.2018.10.093

Cao, J., Sanganyado, E., Liu, W., Zhang, W., Liu, Y., 2019. Decolorization and detoxification of Direct Blue 2B by indigenous bacterial consortium. J. Environ. Manage. 242, 229–237. https://doi.org/10.1016/j.jenvman.2019.04.067

Crespi, S., Simeth, N.A., König, B., 2019. Heteroaryl azo dyes as molecular photoswitches. Nat. Rev. Chem. 3, 133–146. https://doi.org/10.1038/s41570-019-0074-6

Deng, D., Lamssali, M., Aryal, N., Ofori-Boadu, A., Jha, M.K., Samuel, R.E., 2020. Textiles wastewater treatment technology: A review. Water Environ. Res. 92, 1805–1810. https://doi.org/https://doi.org/10.1002/wer.1437

El-Liethy, M.A., Hemdan, B.A., El-Taweel, G.E., 2023. New insights for tracking bacterial community structures in industrial wastewater from textile factories to surface water using phenotypic, 16S rRNA isolates identifications and high-throughput sequencing. Acta Trop. 238, 106806. https://doi.org/https://doi.org/10.1016/j.actatropica.2022.106806

Hassan, M.M., Carr, C.M., 2018. A critical review on recent advancements of the removal of reactive dyes from dyehouse effluent by ion-exchange adsorbents. Chemosphere 209, 201–219. https://doi.org/https://doi.org/10.1016/j.chemosphere.2018.06.043

Jamee, R., Siddique, R., 2019. Biodegradation of Synthetic Dyes of Textile Effluent by Microorganisms: An Environmentally and Economically Sustainable Approach. Eur. J. Microbiol. Immunol. (Bp). 9, 114–118. https://doi.org/10.1556/1886.2019.00018

Juárez-Hernández, J., Castillo-Hernández, D., Pérez-Parada, C., Nava-Galicia, S., Cuervo-Parra, J.A., Surian-Cruz, E., Díaz-Godínez, G., Sánchez, C., Bibbins-Martínez, M., 2021. Isolation of Fungi from a Textile Industry Effluent and the Screening of Their Potential to Degrade Industrial Dyes. J. Fungi. https://doi.org/10.3390/jof7100805

Kaur, J., Mudgal, G., Negi, A., Tamang, J., Singh, S., Singh, G.B., Bose K., J.C., Debnath, S., Wadaan, M.A., Farooq Khan, M., Ruokolainen, J., Kesari, K.K., 2023. Reactive Black-5, Congo Red and Methyl Orange: Chemical Degradation of Azo-Dyes by Agrobacterium. Water. https://doi.org/10.3390/w15091664

Khadhraoui, M., Trabelsi, H., Ksibi, M., Bouguerra, S., Elleuch, B., 2009. Discoloration and detoxicification of a Congo red dye solution by means of ozone treatment for a possible water reuse. J. Hazard. Mater. 161, 974–981. https://doi.org/10.1016/j.jhazmat.2008.04.060

Khan, S., Bhardwaj, U., Iqbal, H.M.N., Joshi, N., 2021. Synergistic role of bacterial consortium to biodegrade toxic dyes containing wastewater and its simultaneous reuse as an added value. Chemosphere 284, 131273. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.131273

Khatri, J., Nidheesh, P. V, Anantha Singh, T.S., Suresh Kumar, M., 2018. Advanced oxidation processes based on zero-valent aluminium for treating textile wastewater. Chem. Eng. J. 348, 67–73. https://doi.org/https://doi.org/10.1016/j.cej.2018.04.074

Kishor, R., Purchase, D., Saratale, G.D., Saratale, R.G., Ferreira, L.F.R., Bilal, M., Chandra, R., Bharagava, R.N., 2021. Ecotoxicological and health concerns of persistent coloring pollutants of textile industry wastewater and treatment approaches for environmental safety. J. Environ. Chem. Eng. 9, 105012. https://doi.org/https://doi.org/10.1016/j.jece.2020.105012

Lade, H., Kadam, A., Paul, D., Govindwar, S., 2015. Biodegradation and detoxification of textile azo dyes by bacterial consortium under sequential microaerophilic/aerobic processes. EXCLI J. 14, 158–174. https://doi.org/10.17179/excli2014-642

Moyo, S., Makhanya, B.P., Zwane, P.E., 2022. Use of bacterial isolates in the treatment of textile dye wastewater: A review. Heliyon 8, e09632. https://doi.org/10.1016/j.heliyon.2022.e09632

Muliadi, F.N., Halmi, M.I., Wahid, S.B., Gani, S.S., Zaidan, U.H., Mahmud, K., Abd Shukor, M.Y., 2021. Biostimulation of Microbial Communities from Malaysian Agricultural Soil for Detoxification of Metanil Yellow Dye; a Response Surface Methodological Approach. Sustainability. https://doi.org/10.3390/su13010138

Orts, F., del Río, A.I., Molina, J., Bonastre, J., Cases, F., 2018. Electrochemical treatment of real textile wastewater: Trichromy Procion HEXL®. J. Electroanal. Chem. 808, 387–394. https://doi.org/https://doi.org/10.1016/j.jelechem.2017.06.051

Paździor, K., Wrębiak, J., Klepacz-Smółka, A., Gmurek, M., Bilińska, L., Kos, L., Sójka-Ledakowicz, J., Ledakowicz, S., 2017. Influence of ozonation and biodegradation on toxicity of industrial textile wastewater. J. Environ. Manage. 195, 166–173. https://doi.org/https://doi.org/10.1016/j.jenvman.2016.06.055

Reichhardt, C., Cegelski, L., 2018. The Congo red derivative FSB binds to curli amyloid fibers and specifically stains curliated E. coli. PLoS One 13, e0203226.

Sarkar, S., Banerjee, A., Halder, U., Biswas, R., Bandopadhyay, R., 2017. Degradation of Synthetic Azo Dyes of Textile Industry: a Sustainable Approach Using Microbial Enzymes. Water Conserv. Sci. Eng. 2, 121–131. https://doi.org/10.1007/s41101-017-0031-5

Sotelo, D.C., Ornelas-Soto, N., Osma, J.F., 2022. Novel Magnetic Polymeric Filters with Laccase-Based Nanoparticles for Improving Congo Red Decolorization in Bioreactors. Polymers (Basel). https://doi.org/10.3390/polym14122328

Zhan, B.J., Li, J.-S., Xuan, D.X., Poon, C.S., 2020. Recycling hazardous textile effluent sludge in cement-based construction materials: Physicochemical interactions between sludge and cement. J. Hazard. Mater. 381, 121034. https://doi.org/https://doi.org/10.1016/j.jhazmat.2019.121034