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Biodegradation of Congo Red Dye by a Mixed Culture of Escherichia coli and Pseudomonas aeruginosa under Anaerobic Conditions

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Abstract: *The decolorization of azo dye; Congo Red (CR), in use in textile industries, were investigated using two facultative microorganism (Escherichia coli and Pseudomonas aeruginosa) under anaerobic and aerobic conditions. Forty milligram per liter of dyes and 10 g of yeast extract contained in a synthetic medium were used and incubated for 7 days. Simultaneous biomass activity and colour removal performance was monitored during batch assays. The effects of two different microorganisms and aerobic/anaerobic conditions on decolourization were recorded with the monitoring of pH, color and concentration. The aromatic amines (as benzidine) and sulphate arising from the metabolites of anaerobic bio-degradation of dyes and the recoveries of these aromatic amines were also monitored. High benzidine recoveries indicated the accumulation of aromatic amines under aerobic conditions. The colour of the Congo red dye was removed up to 90% and 86%, respectively, by Escherichia coli and Pseudomonas aeruginosa at the end of anaerobic incubation*

Keywords*: Azo dye, decolourization, color, microorganism*

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1.0 Introduction

Textile industries is one of the oldest industry in Nigeria. Taking into account the volume and composition of effluent, the textile wastewater is rated as the most polluted among all others in the industrial sector (Awomeso *et a*l., 2010).. The textile industry remains a significant source of water pollution due to the discharge of colored effluents containing synthetic dyes (Sheng et al., 2020). These dyes pose environmental threats by hindering light penetration in water bodies, disrupting aquatic ecosystems, and potentially being toxic (Ghaly *et al*., 2011). Conventional treatment methods like coagulation and adsorption often have limitations, including high costs, inefficiency with a variety of dyes, and generation of secondary waste (Eddy *et al*., 2023; Garg *et al*., 2022). Toxic impact of dyes have been significantly study, especially when they are present in aqueous medium. Dyes can hindered the light penetrating ability to a water body and could record low primary productivity in aquatic system (Eddy *et al*., 2023a). Some dyes are also carcinogenic while some can also combine with heavy metals in the water to form a stable compound with high toxicity (Eddy *et al*., 2022).

Biological treatment methods offer a more sustainable and eco-friendly approach for dye removal from wastewater. Various bacteria have been shown to degrade dyes, including *Escherichia coli*, *Pseudomonas spp*., and others (Zhao et al., 2018). This research investigated the potential of a mixed culture of *E. coli* and *Pseudomonas aeruginosa* for the anaerobic decolorization of Congo red dye, a commonly used azo dye. Also, anaerobic

decolorization has emerged as a promising strategy for dye removal due to its potential for lower energy consumption compared to aerobic processes (Das *et al*., 2019). The exact mechanisms underlying bacterial decolorization can involve biosorption onto the cell surface or biodegradation by intracellular enzymes (Harish *et al*, 2023). This study aims to elucidate the primary mechanism for decolorization by *E. coli* and *P. aeruginosa*.

Several studies have explored the potential of *E. coli* and *Pseudomonas spp*. for dye degradation. For instance, Li et al. (2021) demonstrated the ability of engineered E. coli strains to decolorize various azo dyes under aerobic conditions through laccase enzyme expression. Similarly, *Pseudomonas strains* have been shown to effectively degrade azo dyes anaerobically, with studies by Wang et al. (2021) reporting high decolorization rates for Congo red using a newly isolated Pseudomonas sp. However, research on the combined use of *E. coli* and *P. aeruginosa* in a mixed culture for anaerobic decolorization of Congo red is scarce. This study aims to address this gap by investigating the synergistic effects of these two bacterial strains under anaerobic conditions.

2.0 Materials and Methods

2.1 Sample collection

The azo dye – congo red was purchased from a chemical dye shop in Zaria. Two facultative cultures; E coli and *619 seudomonas aeruginosa* were obtained from the microbiology laboratory of the faculty of pharmacy, A.B.U Zaria and inoculated in the autoclave media.

2.2 Reagent Preparation

2.2.1 Azo Dye Preparation

The Congo red was dissolved with distilled water. The wavelength of the maximum absorption was 497 nm (Sugiura *et al*., 2006). After which 10, 20, 30, 40 and 50 mg of the dye were dissolved to give 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L concentrations respectively.

2.2.2 Synthetic Media

Chemical mineral salt medium was used in all batch experiment at pH 7. To prepare the media, 2 g of K_2HPO_4, 2 g of KH_2PO_4, 2 g of $CaCl_2$, 2 g $MgSO_4$, 10 g of $(NH_4)_2SO_4$, and 10 g of yeast extract were dissolved in 2 liters of distilled water. The chemical media and dye concentration were autoclaved at 121^0C at 15 min to sterile and dissolve the media

2.2.3 Decolourization of Congo Red at Different Concentration at a Physiological Temperature Using Pseudomonas Aerugiosa and Escherichia Coli

To assess the ability of mixed bacteria strain of *Pseudomonas aerugiosa* and *Escherichia coli* to decolourize various concentration of Congo red dye, the following procedure was carried out. 250 cm^3 of already prepared minimal media 30 mg/L, 40 mg/L and 50 mg/L were used. The flasks were plugged with sterile cotton wool and then covered with aluminium foil. The flasks and its content were sterilized in a autoclave (Gallankamp, Japan), for about 15 min; after which, the conical flasks were removed from the autoclave and allowed to cool to room temperature. The media in the flask were inoculated with aid of flame sterilized wire loop with mixed bacteria strain of *Pseudomonas aerugiosa* and *Escherichia coli* and then inoculated at 30^0C in an incubator. Uninoculated flasks were also prepared to serve as control.The bacteria growth in the media was evident by the degree of turbidity of the solution. At 24 hrs interval, both the sample (inoculated flasks and uninoculated flasks) were centrifuged. The supernant was transferred into 1.0 cm glass cuvette and its absorbance was taken at a wavelength of 397 nm using a Uv/visible spectrophotometer (Jenway, UK) to determine the level of decolonization. Decolorization efficiencies were calculated from absorbance measurements exclude biomass and the percentage documented (Olukanni *et a*l., 2006).

Decolorization (%) = $\frac{A_o - A_t}{A_o} \times 100$ (1)

where A_0 = Absorbance of the blank (dye solution) and A_t = Absorbance of the treated dyes solution at specific time and curves plotted between dye concentration and absorbance values

2.2.4 Determination concentration of Congo Red Dye

pH of the samples were determined at the point of samples collection using digit pH meter (Jenway pH meter) which had been initially standardized by using buffer solutions. The pH of the dissolved Congo Red were determined by dipping the pH into the effluent and the reading is recorded. The readings were taken in triplicate.

150cm^3 of the media was aliquot into five conical flasks from 250 cm^3 stock and the pH was adjusted with either NaOH or HCl to the pH value 5, 6, 7, 8 and 9 using pH meter (Jenway, UK). The flasks and the content was autoclaved at 121^{o}C for 15 min, the flasks were inoculated with the aid flamed sterilized wire loop with mixed bacterial strain of the bacteria strains. The flasks were then plugged with sterile cotton wool and were incubated at 30^{o}C, also inoculated flasks were also prepared (blanks). The bacteria growth in the minimal media was measured by taking the optical densities of the supernant from such flask at 24 hrs interval for seven days. Both the sample (inoculated flasks) and the blank (uninoculated flasks) were centrifuged.

3.0 Results and Discussion

The decolourization of Congo red dye by *Pseudomonas aerugiosa* and *Escherichia coli* was determined at different initial concentration by monitoring it's disapperance over the period of incubation. In order to observe the simultaneous colour removal and increases in cell mass of the bacteria strains the absorbance of each concentration of the in the cultures were measured everyday till the end of 7 day of incubation period respectively. Fig. 1 shows the degraded kinetics of Congo red dye under inoculated conditions varied with its initial concentration. The growth of *Pseudomonas aerugiosa* and *Escherichia coli* was measured by monitoring the optical density of the culture at λ_{497} at different time intervals, in the case of initial concentration of 10 mg/L there is no significant increase in optical density with time. Whereas substantial increase in growth of Pseudomonas aerugiosa and Escherichia coli was observed at initial concentration of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L. Maximum increase in bacterial growth was recorded at initial concentration of 40 mg/L. Further increase in Congo red intitial concentration resulted in the decrease growth of the bacteria. That is to say the initial concentration of Congo increase above 40 mg/L, is toxic to the bacteria thereby limiting the growth of the bacteria

To investigate the role of environmental factors on decolourizing activity, the percent of decolourization was measured under mixed culture under static conditions, including varying pH ranging from (5-9), dye concentration 10-50 mg/L over several time interval of 1-7 days at a physiological temperature. Congo Red decoulorization efficiency at pH 5 in Fig. 2 shows a slight growth of the bacteria, which give a small percentage of decolourization. However, the best pH and color removal was observed at pH 7. Monitoring of the pH indicated little significant change throughout the course of the decolourizing tests, as the dye concentrations decreases in the mixed colour medium. Decline in color removal was observed in Fig. 2 after a pH of 8, the time required for decolourization decreased with decrease in dye concentration. This might be attributed to the toxicity of the dye to bacterial cells through the inhibition of metabolic activities, saturation of cell with dye blockage of active site of azoreductase enzyme by the dye molecule (Mabrouk and Yusef, 2008). Similar growth pattern reached a stationary phase within 28-48 hours then

sharply declined. Growth inhibition was observed after 24 hrs of incubation. Therefore in the acidic and basic medium there was growth in both cases but the best pH that enable the bacteria growth was pH 7 at a physiological temperature and optimum concentration which aid colour decolourizing efficiency.

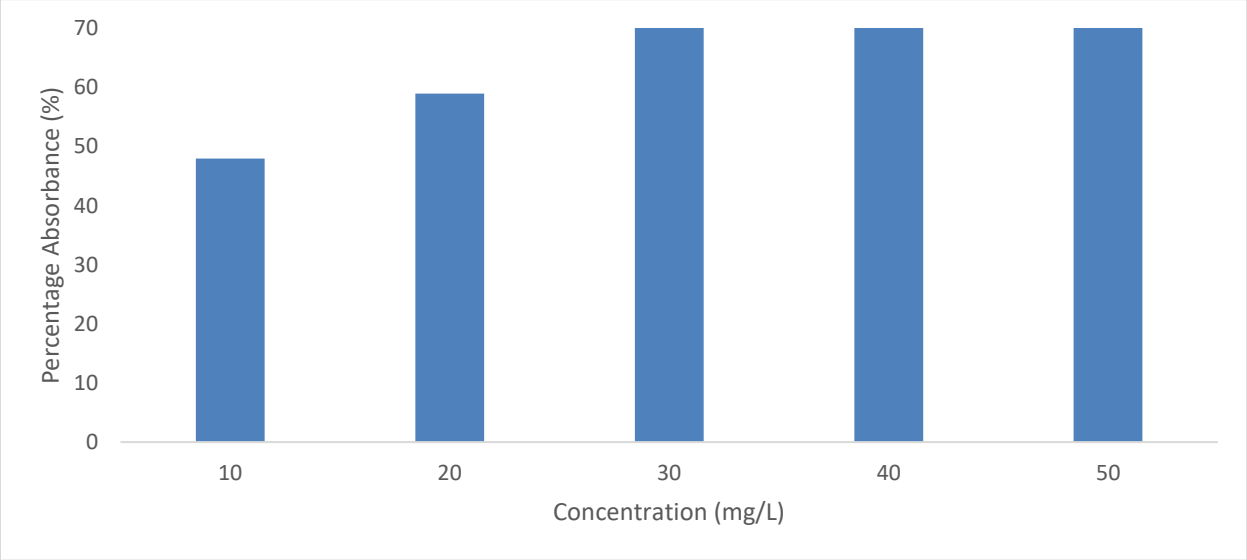

Fig. 1: The effect of different concentration of Congo red with time using *Pseudomonas aerugiosa* and *Escherichia coli*

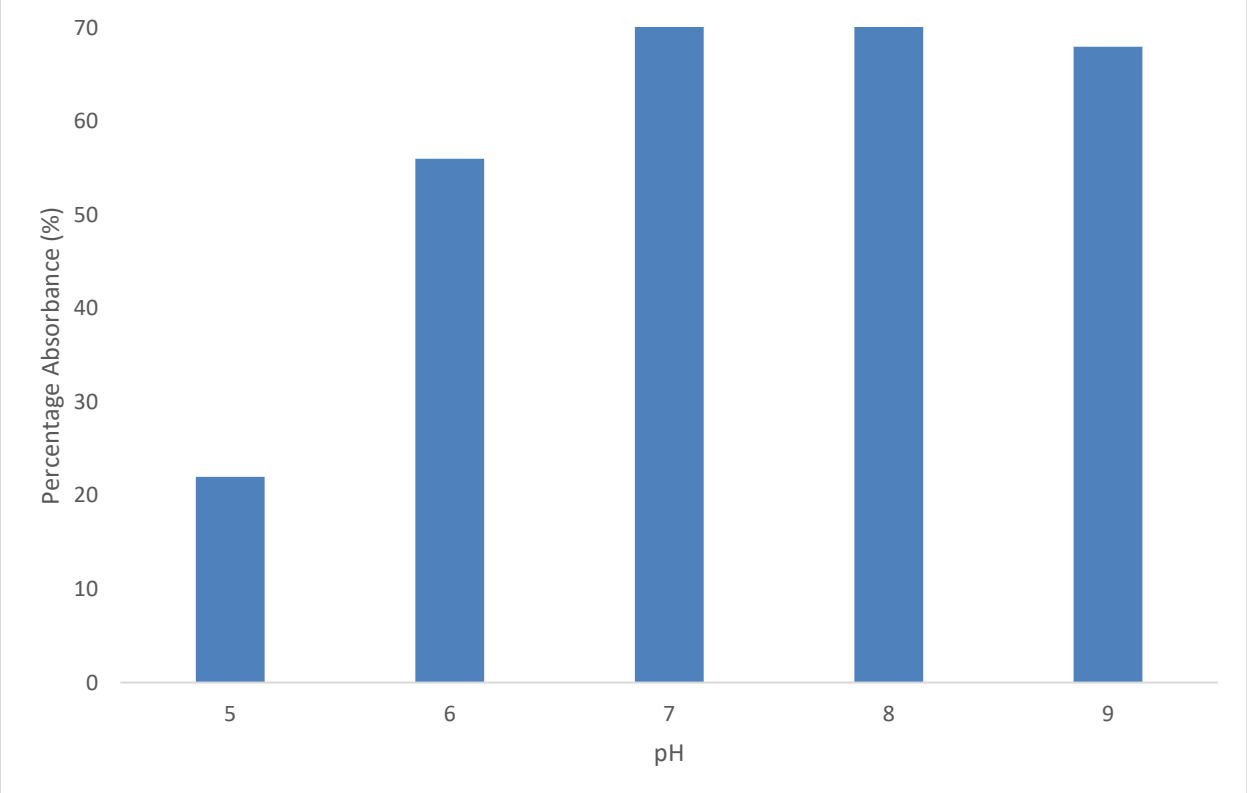

Fig. 2: The effect of different conecntration of Congo red with time using *Pseudomonas aerugiosa and Escherichia coli*

Decolourization of dye solution by bacteria could be due to adsorption to microbial cells or to bioderadation. In adsorption, examination of the absorption spectrum would reveal that all peaks decreased approximately in proportion to each other. If dye removal is attributed to biodegradation either the major visible light absorbance peak would completely disappear or a new peak would appear. Dye adsorption would result in cell materials, which are deeply coloured because of adsorbed dye, where as those retaining the original colour are accompanied by the occurrence of decolourization. In this study the abiotic decolorization studies with autoclaved, dead *Pseudomonas aerugiosa and Escherichia coli* cells showed that no significant colour removal were observed under anearobic conditions for Congo red dye. In addition, as the Congo red levels were reduced, the minimal media returned to its original yellow- white colour. The absorbance peak at 497 nm completely disappeared after cultivation for decolourization of Congo red. In other words, when comparing the UV-vis scans of dye and incubated dye samples it was observed the aforementioned spectra were different, suggesting that Congo red dye was degraded and exhibited maximal absorbance peak at 350 nm. In addition, as the Congo red dye was reduced the synthetic medium returned to its original colour. The anearobic breakdown of Congo red dye has a peak at 350 nm while the original dyes showed maximal absorbance at 497 nm. These results indicates that the colour removal by *Pseudomonas aerugiosa and Escherichia coli* may be largely attributed to bidegradation under anearobic conditions through intracellular enzymes, since the decolourization in the supernatant samples were not significant while the colour in the cell-mass containing samples completely disappeared. This fact indicates that dye decolourization involves constitutive intracellular enzymes.

Table 1: The effect of different concentration on the decolourization of Congo red by strains of mixed cultured bacteria *Pseudomonas aerugiosa and Escherichia coli*

C (mg/L)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean	%
10	0.204	0.312	0.336	0.446	0.555	0.696	0.779	0.480	48
20	0.246	0.307	0.380	0.530	0.664	0.920	1.110	0.590	59
30	0.388	0.421	0.538	0.692	0.814	0.904	1.220	0.710	71
40	0.589	0.697	0.789	0.849	0.947	1.084	1.345	0.900	90
50	0.467	0.581	0.674	0.799	0.801	0.949	1.329	0.800	80

Table 2: The effect of different pH on the decolourization of Congo red by strains of mixed cultured bacteria *Pseudomonas aerugiosa and Escherichia coli*

pH	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean	%
5	0.028	0.034	0.141	0.250	0.318	0.385	0.445	0.230	23
6	0.156	0.201	0.389	0.581	0.782	0.920	0.961	0.57	57
7	0.501	0.674	0.740	0.864	0.980	1.101	1.160	0.860	86
8	0.245	0.389	0.687	0.787	0.898	0.902	1.202	0.73	73
9	0.286	0.357	0.480	0.578	0.697	0.845	1.587	0.69	69

Therefore, it is postulated that the mechanism of colour reduction by E Coli and decolourization can be explained as follows: the dye was adsorbed to the cell surface, some amounts of the dye was degraded enzymically inside cells and the degraded products diffused out to the minimal solution. In this study, at dye concentration of 40 mg/L, almost all of the dyes were degraded completely by the reductase enzyme inside the cells

4.0 Conclusion

This study demonstrates that a mixed culture of Escherichia coli and Pseudomonas aeruginosa can effectively decolorize Congo red dye under anaerobic conditions, with a maximum removal of 90% achieved at 40 mg/L dye concentration and a neutral pH (7). Biodegradation appears to be the primary mechanism for decolorization, suggesting potential for this bacterial consortium as a bioremediation tool for Congo red-contaminated wastewater. However, further research is needed to optimize the process for industrial applications, including investigating the specific enzymes involved, exploring strategies to enhance decolorization efficiency, and evaluating the toxicity of the biodegradation products. Based on the findings of the study, the following recommendations are made,

(i) Exploring the specific enzymes involved in the biodegradation process by *E. coli* and *P. aeruginosa.*

(ii) Optimization study on the decolorization process for industrial applications, potentially including factors like nutrient supplementation and aeration strategies.

(iii) Investigation of the toxicity of the biodegradation products formed.

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Declarations

The authors declare that they have no conflict of interest.

Data availability

All data used in this study will be readily available to the public.

Consent for publication

Not Applicable

Availability of data and materials

The publisher has the right to make the data Public.

Competing interests

The authors declared no conflict of interest.

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Authors' contributions

All the authors contributed to the benchwork and the reporting of the work.