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Research Article
Comparative Decolouration of Crystal Violet Dye using Chicken Feather Fibre, Chemical Oxidation and Bacterial Cells

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Abstract

Background and Objective: Due to the substantial contribution of feather waste to the total solid waste between efforts aimed at countering the attendant environmental impact of waste feathers are urgently required. This investigation was aimed at comparing selected chemical, physical and biological methods in the decolouration of crystal violet dye from wastewater. **Materials and Methods:** The study, which was carried out under batch experimental conditions, made use of Hydrogen Peroxide (HP), Fenton (FT), Raw Chicken Feather fibre (RCF), Carbonated Chicken Fibre (CCF) and two bacterial species (*Pseudomonas aeruginosa* and *Bacillus subtilis*). The parameters studied were the effect of feather quantity/chemical quantity/inoculum size, pH and initial crystal concentration. **Results:** Data obtained from this study showed that after 144 h of treatment, 92% decoloration of the crystal violet dye was achieved using the RCF as biosorbent compared to 63 and 10% decolouration using *Bacillus subtilis* and *Pseudomonas aeruginosa* cells, respectively and 93 and 100% decolouration using HP and FT, respectively. The lowest quantity (2 g) of the RCF was observed the most effective while *B. subtilis* was best at 2 mL inoculum size. The HP and FT were effective over the various concentration tested. Maximum decolouration was achieved at pH 4, 6 and 12 with the RCF, pH 6 with the bacterial cells and over pH ranges between 2 and 12 with HP and FT. **Conclusion:** Based on these results, the RCF was observed to compare favourably with HP and FT and better than the bacterial cells in crystal violet decolouration. Considering the environmental side effects of HP and FT as decolourants, the RCF could offer a better alternative as biological biosorbent. Application of the findings of this study could enhance further scale up studies for more efficient treatment of dye effluents.

Key words: Decolouration, carbonated chicken fibre, dye effluents, feather waste, crystal concentration, bacterial species, crystal violet dye, raw chicken feather fibre

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dyes are substances that possess color and have the ability to affect the color of any substrate added to it. The production of textile dye was invented to improve the economy and fashion, it has been used for bags, shoes, leather, nylon several other products. Dyes are grouped or classified based on their uses and chemical structure, they possess chromophores which is responsible for the colouration of dye, substances with considerable colouring ability are used widely in several industries such as textile, pharmaceutical, food, cosmetics, plastics, photographic and paper[1].

In water, the presence of any colouration is usually the first contaminant that is noticeable due to the fact that even a very minute quantity of synthetic dye present in water will be highly visible, thus affecting the aesthetic quality, transparency and gas solubility of the water[2]. Generally, colour is released into the environment as a result of incomplete exhaustion of dyes onto textile fibre from an aqueous dyeing process. This has resulted in the need to reduce the concentration of residual dye in textile eflluent[3].

The presence of extreme concentrations of dyes in water bodies is indicated to have effect on re-oxygenation process, thus obstructing sunlight and prevention of photosynthetic activity processes. In addition, some of these dyes are capable of reacting with cellular macromolecules such as DNA and proteins to form reactive adducts which are known to trigger gene mutation and destruction of DNA thus initiating carcinogenesis known to trigger gene mutation and destruction of DNA can lead to several health conditions[4,5]. Most reactive dyes are indicated to cause respiratory problems in persons who inhale dye particle. These dye particles accumulate in the lungs of the individual and obstruct the free flow of oxygen. In some cases, they could lead to allergies in susceptible individuals[6].

Several methods (physical, chemical and biological) have been employed in the decolouration of dyes. None of the methods for dye decolouration is indicated to be 100% efficient, with each having several limitations. This study was therefore aimed at investigating the effectiveness of chemical oxidation (Fenton and hydrogen peroxide), adsorption with chicken feather fibre (raw and carbonated) and bacterial (*Pseudomonas aeruginosa* and *Bacillus* subtilis) in the decolouration of crystal violet in water.

MATERIALS AND METHODS

Preparation of chicken feather fibre: This study was carried out between January and May, 2018. The chicken feathers used for the study was obtained from the Landmark University Commercial Farm in Omu-Aran, Kwara State, Nigeria. The feathers were first washed with detergent and disinfected with 5% hypochlorite and thoroughly rinsed with copious water. After which, it was sun-dried for a week and pulverized to increase the surface area.

For preparation of the carbonated feather, known quantities of the pulverized feathers were placed in crucibles and transferred to a laboratory furnace at 250°C for 2 h. After carbonation, the crucibles were allowed to cool, after which the contents were scooped out, powdered and stored in an airtight container until when needed.

Wastewater source: The wastewater used for the investigation was obtained from the Landmark University Commercial Farm in Omu-Aran, Kwara State, Nigeria. Prior to use, the wastewater was filtered using Whatman No. filter paper. For studies using bacterial cells, the wastewater was supplemented with 5 g L^{-1} of sodium acetate (to serve as carbon sources) and 0.5 g L^{-1} of sodium nitrate (to serve as nitrogen source).

Preparation of crystal violet standard curve: To prepare the standard curve for the crystal violet, 0.1% solution of it was prepared as stock solution. In order to ascertain the ideal wavelength for use, aliquot sample of the diluted stock solution was scanned over several wavelengths in a spectrophotometer. The wavelength which gave maximum absorbance with no further increase in absorbance was taken as the ideal. In this study, the ideal wavelength was observed to be 520 nm.

After obtaining the ideal wavelengths, several concentrations of the dye were prepared from the stock solution and their corresponding absorbance readings obtained. A calibration curve was obtained by plotting absorbance readings against the corresponding concentrations. From the calibration curve, an equation of a line was obtained.

Dye decolouration studies: Decolouration experiments were carried out following the protocol reported by Zablocka-Godlewska *et al.*[7]. For the dye decolouration studies, the effect of the quantity of decolouration agent [hydrogen peroxide and Fenton (iron (II) chloride and hydrogen peroxide, at 1:1 v/v), raw and carbonated chicken feather fibre and bacterial cells] and the effects of pH and initial crystal violet concentration were investigated.

In the study on the effects of quantity of decolouration agents on crystal violet decolouration, 2, 4, 6, 8 and 10 g of the

respective raw and carbonated feathers were used for investigation. For the Fenton and hydrogen peroxide, 2, 4, 6, 8 and 10 mL were used for investigation while for the *Pseudomonas aeruginosa* and *Bacillus subtilis*, 1, 2, 3, 4 and 5 mL of the broth cultures were used.

In the study on the effect of pH, before sterilization, the pH of the wastewater was adjusted to the pH of interest. In this study, the pH used for investigation were 2, 4, 6, 10 and `12. Adjustment of pH of the wastewater was carried out using either 1 M NaOH or 1 M HCl to obtain alkaline and acidic ranges, respectively. For the effect of initial crystal violet concentration on decolouration potential of the decolouration treatments, five different initial concentrations were investigated.

In all experimental setups, after inoculation with the respective treatment agents, aliquot samples were obtained from each flask immediately after inoculation and every 24 h for 144 h for the estimation of crystal violet concentration in the wastewater. All experimental procedures were carried out in duplicates.

For statistical analysis, comparison of means was carried out using the one-way analysis of variance (ANOVA) test at confidence level of 95 %.

RESULTS

As shown in Fig. 1, in presence of the respective concentration of raw feathers in the wastewater, no decolouration was observed within the first 24 h of incubation. However, progressive increase in decolouration was observed with time after 96 h of incubation. This trend was irrespective of the raw feather quantity used (Fig. 1). Generally, in the raw feather treated sample, the results obtained indicated that the effect of variation in the amount of the biosorbent (raw feather) on the extent of decolouration of crystal violet dye was not statistically significant as the quantity of the biosorbent did not determine the effective decolouration rate ($p = 0.05$).

For the Carbonated Feather (CF) set up, the presence of varying concentration of the carbonated proved to be effective in the rate of decolouration of the crystal violet dye. Results obtained showed that the carbonated feather at 6 g produced significant decolouration of the crystal violet dye compared to 8 and 10 g indicating a significant reduction from an initial value of 290.00-125.7 mg L^{-1}. However, the decolouration in crystal violet dye concentration observed with 6 g carbonated feather was not significantly different from that recorded for the 2 and 4 g carbonated feather (Fig. 1).

As indicated in Fig. 2, there was no significant decrease in crystal violet dye decolouration in the presence of 1, 3, 4 and 5 mL broth culture of *Bacillus subtilis*. However, 2 mL culture broth of *Bacillus subtilis* showed a significant and more appreciable decolouration trend compared to 1, 3, 4 and 5 mL. This trend was observed at 120 h of the incubation period which resulted to a concentration value of 99.28 mg L^{-1} and at 144 h, 85 mg L^{-1} from an initial value of 290.00 mg L^{-1}.

For *Pseudomonas aeruginosa*, it was observed that the highest cell mass of the microorganism (5 mL) showed a significant trend of decolouration compared to lower cell mass (1, 2, 3 and 4 mL) this indicated that the increase in the cell mass of the organisms aided the increased rate of decolouration of the dye (Fig. 2).

When different volumes of hydrogen peroxide were used for decolouration, significant decolouration of the crystal violet was observed within 24 h incubation. After 24 h of decolouration, crystal violet concentration decreased from 290.00-65.71, 60.00, 89.28, 106.42 and 80.00 mg L^{-1}, when 2, 4, 6, 8 and 10 mL of hydrogen peroxide were used for decolouration, respectively (Fig. 3).

In media containing different volumes of the Fenton, significant decolouration of the crystal violet was observed

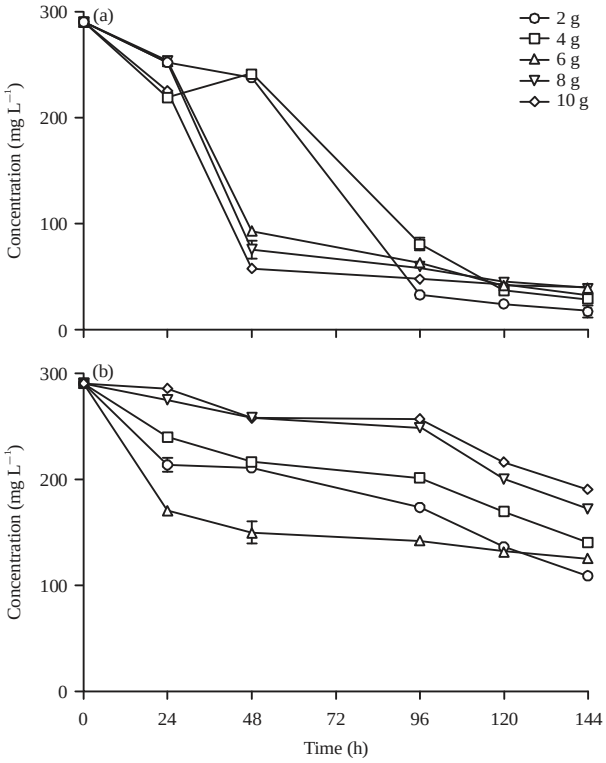

Fig. 1(a-b): Effect of feather quantity on crystal violet decolouration, (a) Raw feather and (b) Carbonated feather

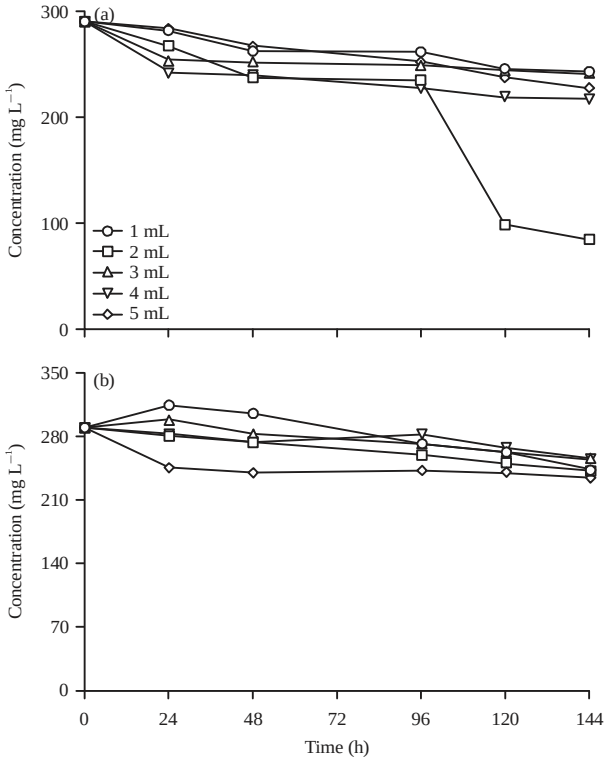

Fig. 2(a-b): Effect of initial inoculum size on crystal violet decolouration, (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa*

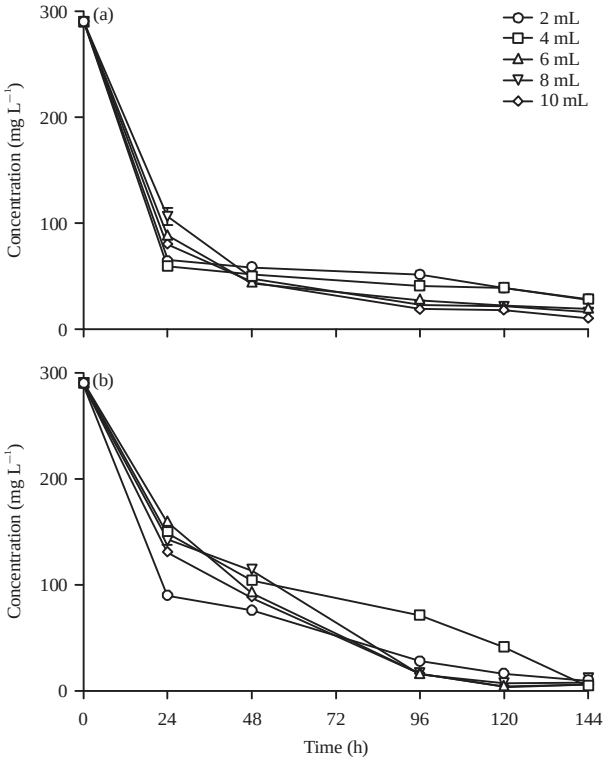

Fig. 3(a-b): Effect of quantity of chemical on crystal violet decolouration, (a) Hydrogen peroxide and (b) Fenton

after 24 h of incubation. This trend was irrespective of the Fenton that was used. Generally, continuous reduction in the concentration of crystal violet was observed till the end of 144 h incubation period. However, the reduction rate of the dye in all varied volumes were not statistically significant, indicating that increase in volume of the Fenton did not determine the rate (Fig. 3).

As illustrated in Fig. 4, when the initial dye concentration in the wastewater was varied, the rate of decolouration in presence of the raw feather was observed to be more effective at low initial crystal violet concentration, when compared to those at high initial crystal concentrations. At the termination of the 144 h incubation period, concentrations were observed to decrease from 465, 604, 664, 684 and 714 mg L^{-1} to 51.42, 78.57, 26.42, 93.57 and 24.28 mg L^{-1}, respectively (Fig. 4).

In the carbonated feather set up, the rate of decolouration observed was insignificant. However, a colour change was observed after 24 h of incubation which was as a result of the colouration of the carbonated feather. The media containing lower concentration of the crystal violet dye showed some level of decrease after 96 h of incubation and this was sustained till the end of the 144 h incubation period (Fig. 4).

In the presence of Fenton, at the different initial concentration of the crystal violet, significant decolouration was observed at the different concentrations investigated. In set up containing hydrogen peroxide, the reduction rate observed during the first 24 h of incubation was remarkable. The decolouration rate varied according to the concentration of dye added to wastewater, those with lower concentration of dye had an increased rate of reduction compared to others. The trend in reduction was determined by the concentration of dye added to the wastewater. At the end of the incubation period, complete decolouration of the crystal violet was observed at the different initial concentrations used for investigation (Fig. 5).

In the presence of *Bacillus subtilis*, remarkable decolouration was only observed within 24 h of incubation in media containing the lowest concentration of the crystal violet. The reduction rate was monitored through a continuous trend. Additionally, the wastewater contained sources of nitrogen and carbon required by the micro-organisms to multiply. However, those with higher dye concentration showed no significant decrease in dye colour. When the *Pseudomonas aeruginosa* was used

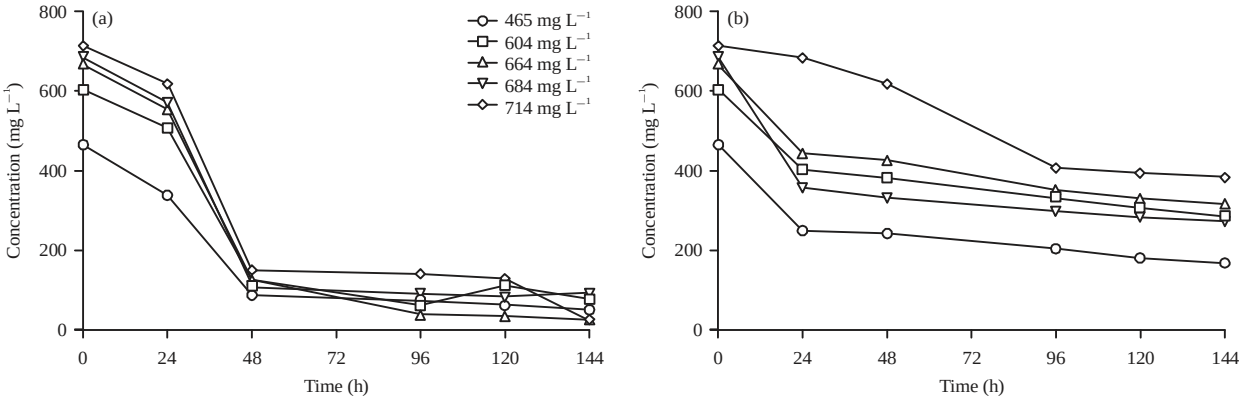

Fig. 4(a-b): Effect of initial crystal violet concentration on decolouration in presence of (a) Raw and (b) Carbonated chicken feather

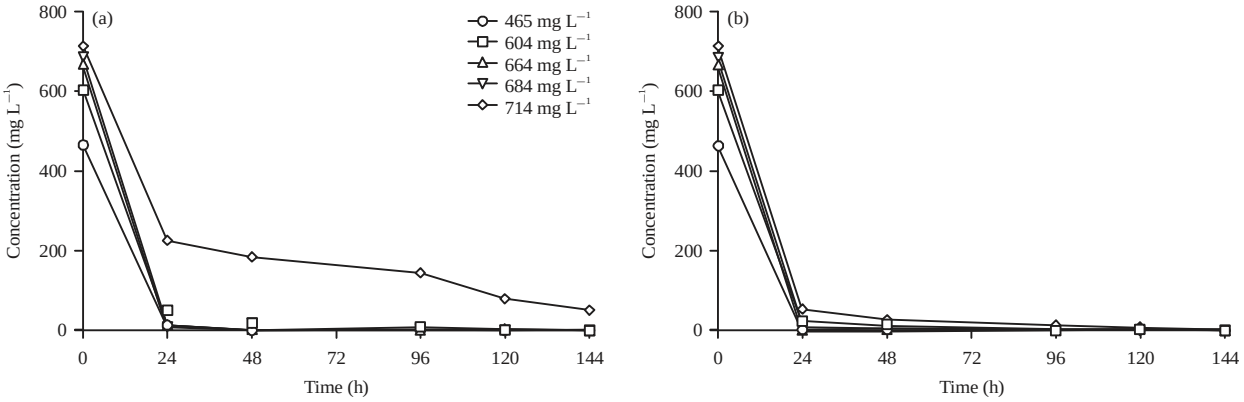

Fig. 5(a-b): Effect of initial crystal violet concentration on decolouration in presence of the (a) Hydrogen peroxide and (b) Fenton

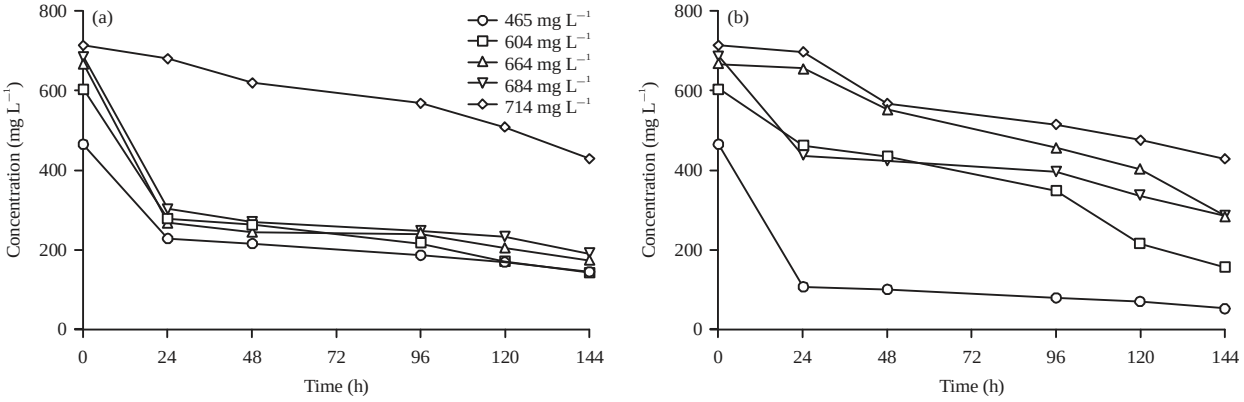

Fig. 6(a-b): Effect of cell mass of (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa* in the decolouration of varied dye concentration

for inoculation, remarkable decolouration was only observed after 96 h of incubation (Fig. 6).

At the different pH investigated, crystal violet concentration in presence of the hydrogen peroxide showed significant decolouration throughout the period of incubation. This trend was irrespective of the respective pH investigated. When the Fenton was used for investigation, crystal violet decolouration showed remarkable decreases with time. This observation was also irrespective of the pH investigated (Fig. 7).

When the raw feather was used for the study, significant decrease in crystal violet concentration was observed within

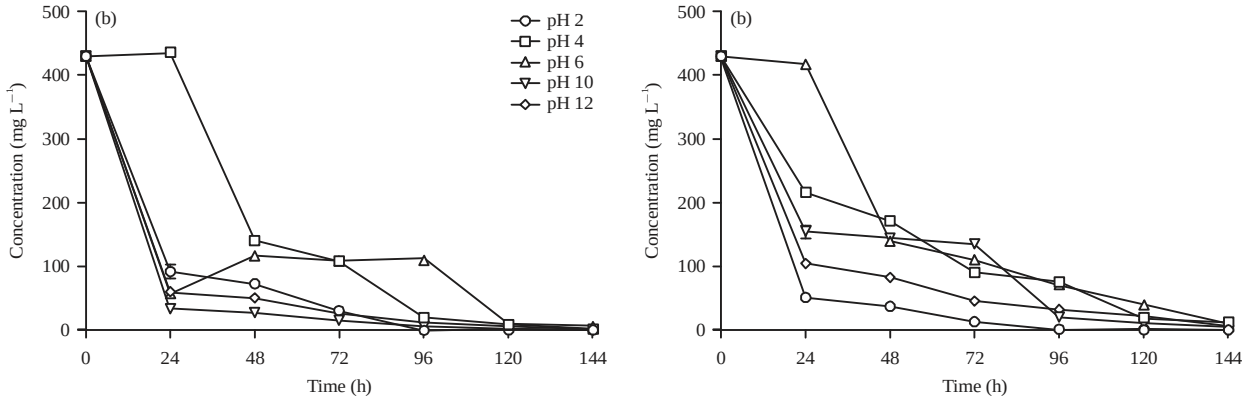

Fig. 7(a-b): Effect of pH crystal violet concentration on decolouration in presence of the (a) Hydrogen peroxide and (b) Fenton

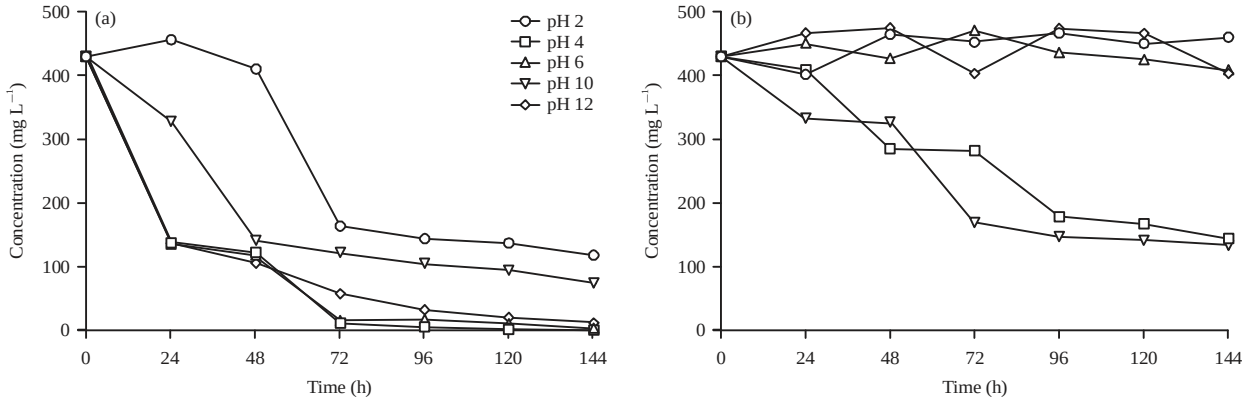

Fig. 8(a-b): Effect of pH crystal violet concentration on decolouration in presence of the (a) Raw and (b) Carbonated feathers

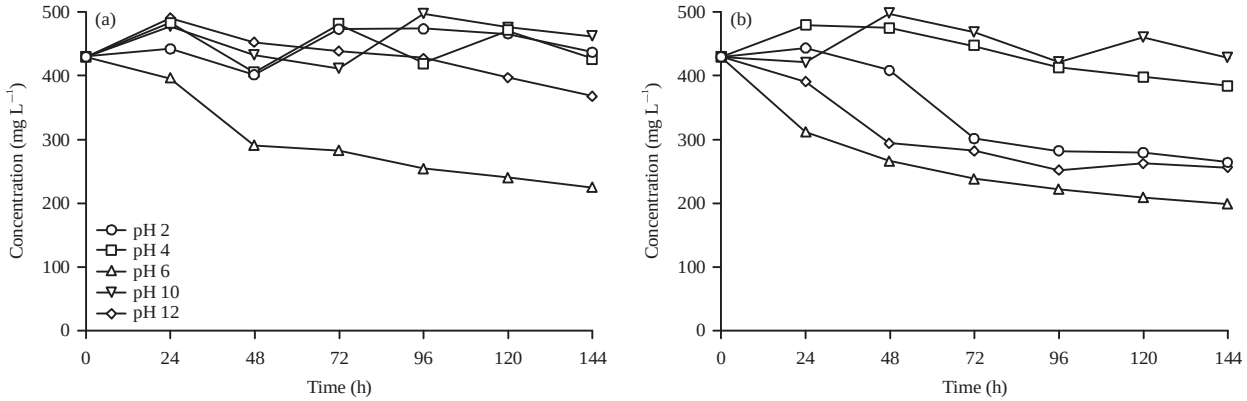

Fig. 9(a-b): Effect of pH crystal violet concentration on decolouration in presence of the (a) *Bacillus subtilis* and (b) *Pseudomonas aeruginosa*

the first 24 h of incubation. This decrease was consistent till the end of incubation and was observed at pH 4, 6 and 12. For the carbonated feathers, significant decolouration of the crystal violet was observed at pH 4 and 10, after 48 h of incubation. At the other pH investigated, no significant decrease in crystal violet levels was observed throughout the period of incubation (Fig. 8).

In presence of the *Bacillus subtilis*, no significant decolouration of the crystal violet was observed at pH 2, 4, 10 and 12, with the exception of pH 6. This trend was consistent throughout the period of incubation. The *Pseudomonas aeruginosa*, crystal violet levels at the respective pH were observed to show no significant decrease, except at pH 6 (Fig. 9).

DISCUSSION

This study explored the use of chicken feather fibre as possible biosorbent for crystal violet decolouration in water. The use of the feather fibers was deliberate. Although they are normally discarded as waste, chicken feathers are indicated to be keratinous, non-abrasive, biodegradable, renewable and ecofriendly[8]. Waste feathers have been reportedly used as efficient biosorbents for the removal of dyes, heavy metals and several other impurities in water[9].

From the findings of this study, progressive decolouration of the crystal violet was observed with time in presence of the raw feather fibre. This observation was irrespective of the quantity of feather. Similar observation has been reported by earlier investigators. In a study by Pandima Devi and Muthukumaran[10], when investigating the utilization of chicken feathers as adsorbent for the removal of acid brown dye from aqueous solutions, their finding revealed variation of the quantity of dye adsorbed with initial dosage of the adsorbent used. No further increase in adsorption capacities was observed with further increase in adsorbent dosage. Mittal *et al.*[11] also reported similar observation from their studies on the adsorption of erythrosine dye, using hen feathers.

The present study revealed significant decolouration of the crystal violet in presence of hydrogen peroxide or Fenton. The use of Fenton in dye decolouration and heavy metal removal studies in water has been reported by Pawar and Gawande[12]. In a study on the decolouration of Azo dye acid Red 18 by Fenton reagent in the presence of iron powder, it was reported that acidic pH produced a remarkable decolouration rate when compared to other pH ranges[13]. In the presence of Fenton or hydrogen peroxide, the decolouration of crystal violet was observed at the different dosages of solutions used for investigation. However, the rate of decolouration was faster at higher concentrations of Fenton or hydrogen peroxide. In a study on the decolouration of reactive black 5 using Fenton oxidation, 58.9% COD and 97% colour removal was reported after 10 min reaction by Fenton when 50 mg L^{-1} of $FeSO_4$ and 300 mg L^{-1} of H_2O_2 was used[14]. In a related study, the decolourization rate of azo dye orange was reported to remarkably increase with increase in quantity[15] of H_2O_2.

Although, remarkable crystal violet decolouration was observed at the different pH investigated in this study, no remarkable decolouration was observed at pH 4 and 6 within the first 24 h of incubation in presence of hydrogen peroxide and Fenton, respectively. This observation was at variance with the findings of Bahmani *et al.*[15]. In their report, the degree of decolouration was observed to decrease with increase in pH, with highest and lowest removal rates of 99.1 and 41.1% observed at pH 3 and 7, respectively. A similar observation has been reported by Lucas and Peres[16].

Result obtained from this study indicated that cells of *Bacillus subtilis* and *Pseudomonas aeruginosa*, showed no significant decolouration at high crystal violet concentration. It is reported that dye concentration is a vital factor that affects dye decolouration in wastewater. The rate of dye decolouration is therefore affected by a variety of factors, including toxicity of the dye, which increases as the dye concentration increases[17].

The dye-containing media used in this study for the setup involving the test bacterial species was supplemented with sodium acetate (as carbon source) and sodium nitrate (as nitrogen source). It is opined that during microbial decolouration of dyes, the presence of carbon and nitrogen sources is not limited to the metabolic activity of the micro-organisms alone but also to aid the decolouration process through the stimulation of the reduction process in the cleavage of bonds present in the dye[18,19].

In this study, the decolouration of crystal violet was observed to increase with increase in inoculum concentration of the test bacterial species. Inoculum dose is considered an important factor that is taken into consideration during microbial decolouration of dyes. In a study on the bacterial decolourization of textile dye-orange 3R, Ponraj *et al.*[20], observed that the rate of decolouration increased with increase in inoculum size.

Optimum pH for crystal violet decolouration was observed in presence of the test bacterial species to be 6. Higher and lower pH did not support decolouration of the dye. Singh *et al.*[21] have reported optimal condition for the decolouration of Acid Orange dye by *Staphylococcus hominis* to be at a pH of 7.0, after 60 h of incubation. In a study on microbial decolourization of textile wastewater, by different fungal isolates, Ali and El-Mohamedy[22] reported maximum degradation activities occurring at pH for acid dyes after 9 days and at pH 5 to for reactive dyes.

CONCLUSION

Data generated in this study showed that the RCF (biological treatment) compared favourably with HP and FT (chemical treatment) and better than the test bacterial cells in crystal violet decolouration. This study thus offers a good strategy of actualizing one of the key components of United Nation's Sustainable Development Goals (SDGs) of providing clean water, air and environment by the year 2030. However,

to validate the possible application of the findings of this study, there is need for scale-up studies in laboratory scale microcosm, which is the focus of the next study.

SIGNIFICANCE STATEMENT

The efficient decolouration of crystal violet dye in wastewater using chicken feather waste as demonstrated in this study will not only provide an environmentally friendly low-cost solution to wastewater recycling but also a viable solution to reducing the environmental hazards associated with waste feather disposal.

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