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Biodegradation of the Azo Dye Airedale Yellow CHD: Understanding using residuals

Vamshi Krishna Mukkera*, Srivani Katuri

Department of Chemical Engineering, National Institute of Technology, Warangal-506004, Telangana, India

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ABSTRACT

Textile industries are heavy users of water and also produce lots of contaminated effluents. The main contaminants are azo dyes. Hence, the effluents are to be treated before leaving in the environment. In this study, the azo dye Airedale Yellow CHD was biodegraded using two bacteria *Thalassospira frigidphilosprofundus* (NCIM no 5438) and *Erwinia chrysanthemi Burkholder* (NCIM no 5213) in shaking conical flasks. Effect of Various parameters like pH, temperature, agitation, and concentration of dye solution on its decolorization was investigated. The biodegradation was statistically worked out using MINITAB software for the ANOVA. The residual plots along with the scatter plots for the decolorization of Airedale Yellow CHD using *T. frigidphilosprofundus* and *E. chrysanthemi Burkholder* are also obtained and included in this work. The maximum percent removal of the azo dye was obtained by using *T. frigidphilosprofundus* (77.41%) whereas it was reported at 74.64% by using *E. chrysanthemi Burkholder*. The obtained results formed a good fit according to the obtained normal residual plot which can conclude that the findings of the study are accurate and satisfactory.

* Corresponding author

E-mail: kvamshi93@gmail.com (Vamshi KrishnaMukkera)

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1 Introduction

Less than one percent of the planet's water is available as clean water and can be used for drinking, but by 2050, its consumption is predicted to increase by more than 35%. Waterways such as lakes, waterways, and ponds, are amassed with rubbish, garbage, and pollutants (Melissa Denchak 2018). Textile industries utilized around $2.8X10^8$ kg of dyes per year across the world (Jin et al. 2007; Sen et al. 2016). The effluent from textile dyeing and fabric industries accounts for 15–25% of the total textile wastewater (Garg et al. 2020). The textile sector is a significant segment when it comes to the global budget. The textile business accounted for 8.54 percent of the global business and reported an increase in its value every year (World trade statistics 2019; World trade statistics 2020). Meanwhile, in 2020 global exports of textiles grew by 16 percent, driven by an increase in the production of personal protective equipment (PPE). Most types of manufactured goods saw significant gains in the second half of 2020, notably textiles, pharmaceuticals, computers, and telecommunications equipment (World trade statistics 2021). Textile demand is increasing every year, resulting in a huge volume of effluent being dumped into freshwater streams. According to Subramani (2020) from the mid-2000s through the end of 2019, the textiles division got 3.41 million dollars in FDI, whereas India's overall FDI was 456.79 million dollars. The textile division has a high amount of FDI as compared to other divisions. Small textile factories with high manufacturing costs can be found in India. Due to a change in the MSME (Ministry of Micro, Small and Medium Enterprises) scenario, a substantial number of textile exporters will now be classified as MSME and get a 5% interest equalization rebate (Subramani 2020). This will increase the competitiveness of Indian textile products on the global market, boosting India's textile exports and, in turn, employment creation which results in an increase in textile production. Water is also utilized in industries to make a variety of products, with a significant concentration in the textile industry. These businesses produce a huge volume of polluted (mostly Azo dye-adulterated) water. Azo dyes fall under the category of organic molecules consisting of -N=N- (azo group). Because of the presence of the azo group, they are electron deficient genotoxic substances and are water soluble. Further, these azo dyes are most likely to be absorbed into the bodies of living creatures by inhalation, swallowing, or skin contact. Azo dye polluted wastewater discharge from the textile industries into water bodies without significant treatment or removal, and it harms the aquatic living beings (Hassan and Carr 2018; Bharathiraja et al. 2019).

The azo dye's physical structure has an azo link, which allows various groups such as methyl (–CH3), chloro (–Cl), nitro (–NO2), hydroxyl (–OH), amino (–NH2), and carboxyl (–COOH) to be substituted for the phenyl groups to produce a broad range of azo dyes that are electron acceptors (Saratale et al. 2011). Further, when visible light rays strike the azo dyes, they absorb them (Chang et al. 2000). Moreover, the mixing of these azo dyes in water bodies increases the BOD, COD, and TOC of the water bodies (Saratale et al. 2011). Many researchers have been motivated to create more effective wastewater treatment technologies as a result of the rigorous laws demanding the remediation of effluents before they are discharged into the environment. Many techniques for the treatment of wastewater like physical, chemical, and biological methods are available (Figure 1).

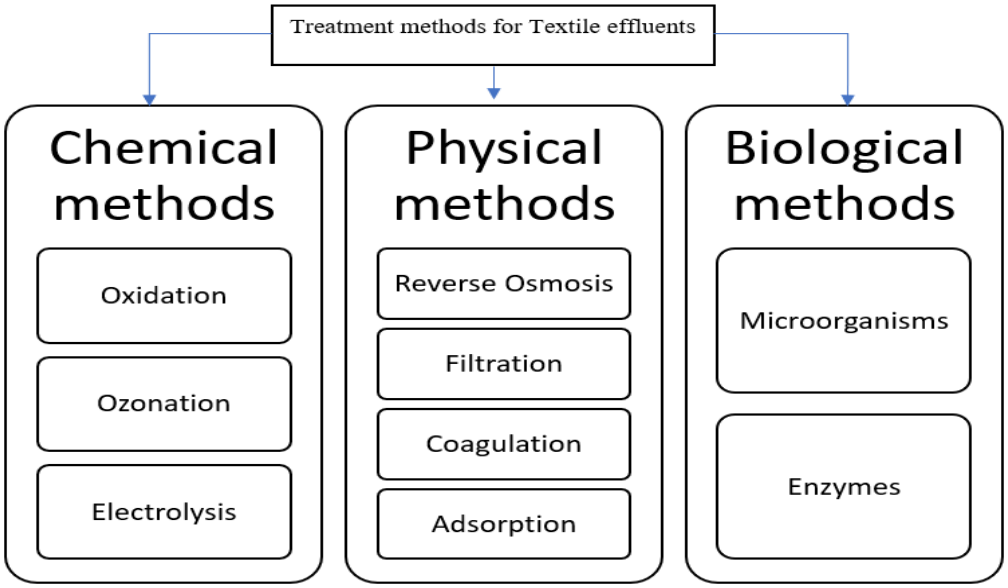


Figure 1 Various methods of treatment of Textile effluents.

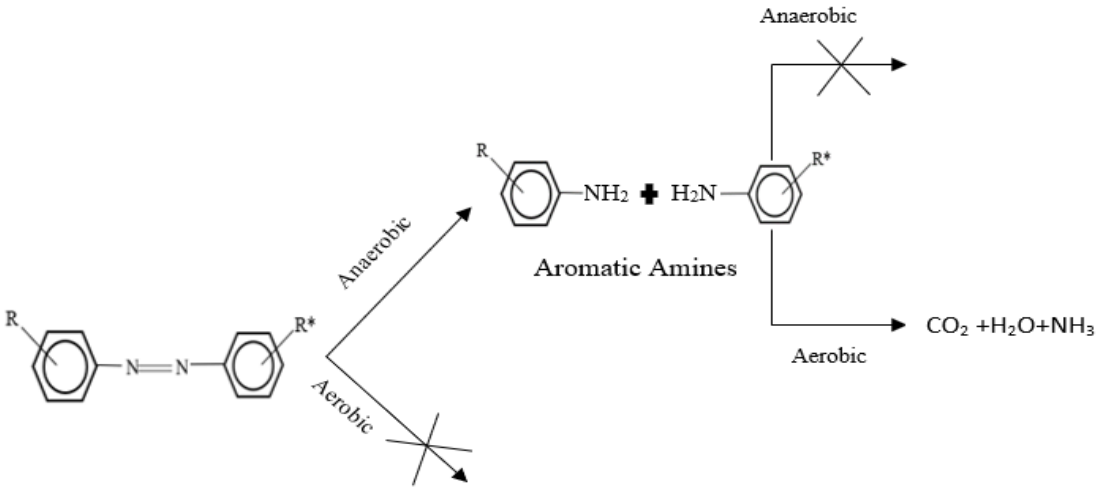


Figure 2 Common outline of the breakage of azo bonds

Various membranes can be used in industry to decolorize and lower the values of parameters like COD, BOD, color, etc (Dos Santos et al. 2007). However, their use has some drawbacks such as expensive investment, membrane fouling, and additional sludge production (Dos Santos et al. 2007). Like this, chemical decolorization techniques have also several drawbacks, including increased operating costs and difficulties scaling up processes to an industrial scale (Atalay and Ersöz 2016). As a result, biological methods for treating azo dying effluents can be utilized. The bioremediation process utilizes microorganisms such as fungi and bacteria to remove or modify pollutants (Varjani et al. 2020; Jain et al. 2022). Although dyes are difficult to degrade, but biological methods are used to complete the process and provide other benefits such as ecologically friendly end products, lower costs, reduced sludge, and nontoxic end products (Rai et al. 2005).

There would be no effect on azodye degradation if aerobic conditions were employed for metabolism in biodegradation. Generally, the breakage of azo bonds follows two phases (Figure 2). The initial phase should be anaerobic, which breaks the azo link and produces aromatic amines, which are carcinogenic and harmful to both flora and animals. In the second phase of biodegradation, aerobic conditions are utilized to convert the aromatic amines into carbon dioxide, water, and other by-products (Van der zee and Villaverde 2005). As a result, utilizing microbes to break down azo dyes may require a combination of anaerobic and aerobic conditions.

Bacteria are required since they can break down a wide range of dyes and have a high level of adaptability to changing environmental circumstances (Dos Santos et al. 2007). Because bacteria are ecologically favorable, several researchers employed them for biodegradation (Saratale et al. 2011). These bacteria can break down a wide range of dyes and have a high level of adaptability to changing environmental circumstances (Dos Santos et al. 2007). This can be the reason why a lot of work involved bacteria but not fungi which may have a long development period and will be a restriction to growing by nitrogen gas (Stolz 2001).

There is very limited information available related to the biodegradation of Airedale yellow CHD (Hema and Suresha 2014). In the current study, an attempt has been taken for the biodegradation of Azo dye Airedale Yellow CHD with the help of two bacterial species i.e. *Thalassospira frigidphilosprofundus* and *Erwinia chrysanthemi Burkholder*. The novelty involved here is the utilization of the selected bacterial strains which were never used anytime for the bio decolorization of dye contaminated wastewater. The goal of this study was to examine the quantity of the dye biodegradation using two distinct microorganisms and to understand the biodegradation of dye using residual plots. The residual plots along with the scatter plots of the decolorization are obtained and included in this work.

2 Materials and Methods

2.1 Airedale Yellow CHD

The dye Airedale Yellow CHD is also known as Cotton Yellow CH, Chrysophenine, Chrysophenine GX with an empirical formula $C_{30}H_{26}N_4Na_2O_8S_2$, and a molecular weight of 680.66 is taken for the study. It's a red-light yellow powder that belongs to the double azo class and is used for dyeing cellulose fiber, cotton, viscose fabric painting, silk, wool, PVA, polyamide fiber, leather, paper, biological shading (World Dye variety 2012). According to the International Union of Pure and Applied Chemistry, it is sodium, 5-[(4-ethoxyphenyl)diazenyl]-2-[(*E*)-2-[4-[(4-ethoxyphenyl) diazenyl]-2-sulfophenyl]ethenyl]benzenesulfonic acid. It is commonly accepted since it has high purity, high effectiveness, accurate composition, and longer shelf life (Figure 3).

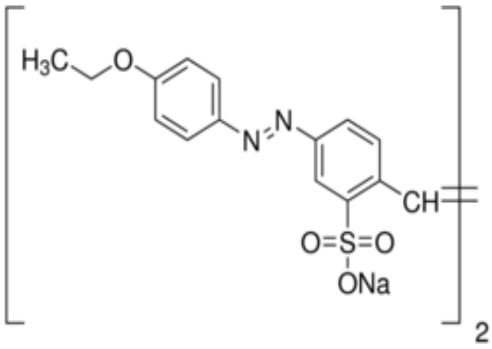


Figure 3 Molecular structure of Airedale Yellow CHD

2.2 Microbes (Bacteria) and medium compositions employed in the experiment

The pure bacterial cultures of *T. frigidphilosprofundus (NCIM 5438)* and *E. chrysanthemi Burkholder (NCIM no 5213)* were obtained from NCL Pune as pure cultures. *T. frigidphilosprofundus* is an aerobic gram-negative curving rod with a single polar flagellum bacteria that belong to the family rhodospirillaceae proteobacteria and is found in a warm, humid environment (Kaneshiro et al. 2008; Pulicherla et al. 2013; Adapa 2018). The obtained pure culture of bacteria was maintained on the medium supplemented with 5 gl^{-1} bactopeptone, 1 gl^{-1} yeast extract, 0.1 gl^{-1} FeCl3, 5 gl^{-1} MgCl2, 12 gl^{-1}NaCl, 1.8 gl^{-1} CaCl2, 0.55 gl^{-1} KCl employed in Nutrient Medium 1 (NM1) having pH 7.6.

E. chrysanthemi Burkholder also known as *E. cartovora*, and it's a Gram-negative found either alone or in clusters and can survive under anaerobic environments. Due to its mesophilic nature, it can survive between 27 and 30^{o} C temperature (Kaneshiro et al. 2008). Nutrient agar medium supplemented with 10 gl^{-1} Beef extract, 5 gl^{-1} NaCl, and 10 gl^{-1} Peptone was used for the bacterial multiplication. The pH of the medium was adjusted to 7.5 and the cultures were multiplied at 20^{o}C and 30^{o}C temperatures regularly and after multiplication stored at 4^{o}C in a refrigerator.

2.3 Preparation of Azo dye contaminated water to be treated

After making a standard dye solution with a concentration of 1000 mg/l, the samples with the appropriate concentrations were generated by diluting the standard dye solution with deionized water using volumetric proportions. The amount of standard solution to be mixed with deionized water is calculated using the idea of concentration equality, which is described below in equation 1.

$$C_1V_1 = C_2V_2 \quad (1)$$

Where C_1=1000 mg/l, V_1=volume of standard solution required, C_2=Concentration of wastewater sample, V_2= 100ml. All of the tests were done in conical flasks with a capacity of 250 mL.

Experiments were carried out in a temperature-controlled incubator shaker with 100 mL nutrient medium with dyestuff in a 250 mL shake flasks under various circumstances. After 48 hours of incubation, the samples were tested for decolorization. For this, samples were taken and centrifuged for 7.5 minutes at 13,000 rpm. The analysis of supernatants was carried out and the dyestuff and nutrition present in the control flasks and were devoid by microorganisms were tested and the percentage decolorization of the dye is calculated from the below equation 2.

$$y(\%) = \frac{(IA - FA)}{IA} x100 \quad (2)$$

Where y(%): % decolorization, IA: Initial Absorbance, FA: Final absorbance

2.4 Analytical methods

Analytikjena SPECORD 205 spectrophotometer was used to measure absorbance. For dye, the wavelengths that resulted in the highest absorption were selected. The dye solution is produced in seven different concentrations of 10mg/l, 30 mg/l, 50 mg/l, 90 mg/l, 150 mg/l, 200 mg/l, 250 mg/l. With distilled water as the blank solution, the solutions were evaluated using a UV-Visible spectrophotometer. The wavelength with the highest absorption was 398nm.

2.5 Response surface methodology (RSM)

RSM is a statistical technique for modeling and analyzing studies in which multiple input independent factors impact the output response. When running different experiments, the RSM finds the optimum experimental design, which saves a lot of time and work. RSM uses a variety of techniques to design experiments, including Central Composite Design (Said et al. 2015; Olawoye 2016). The decolorization percentage y is the output variable, which is a dependent selected variable. The independent variables (4) chosen for the studies are the sample concentration (mg/l), pH of the medium, temperature (^{o}C), and agitation (rpm) in the incubator shaker. The number of tests to be performed is specified by the formula $N=2^{n}+2*n+n_c$, where N is the total number of runs to be performed, n is the number of independent variables, and n_c is the number of center points. Based on the applied formula total of 26 tests can be run. Using the software Minitab 14, the data of 26 tests were analyzed to generate residual and scatter plots.

3 Results and Discussion

3.1 Calibration experiments

The wavelength with the highest absorption was 398nm. The maximum absorbance that is obtained at a wavelength at a particular weight of dye are shown in Table 1.

Table 1 Maximum absorbance obtained at a particular weight of the dye

S. N.	Concentration (mg/l)	Wave Length (nm)	Absorbance
1	10	398	0.3186
2	30	398	0.429
3	50	402	0.611
4	90	398	1.41
5	150	398	2.037
6	200	398	2.35
7	250	398	2.859

The values in table 1 are obtained from the UV Vis spectrophotometer. The dye used here had its standard calibration curve (shown as 'a') and Absorbance vs Concentration (shown as 'b') drawn as shown in figure 4. The standard calibration plot can give us the wavelength at which maximum absorbance is attained and the Absorbance vs Concentration plot gives the concentration of the dye that is treated.

3.2 Decolorization of Airedale Yellow CHD with *T. frigidphilosprofundus*

In the work done by Hema and Suresha (2014), the biodegradation of the dye Airedale Yellow CHD required the addition of NH_4Cl

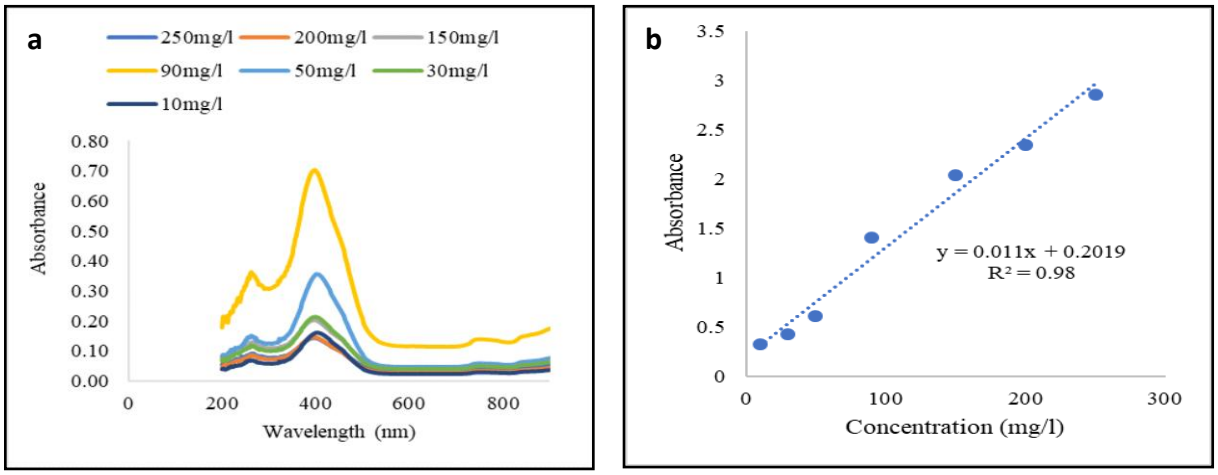


Figure 4 (a) Standard calibrations plot and (b) Plot of absorbance vs concentration of the dye taken

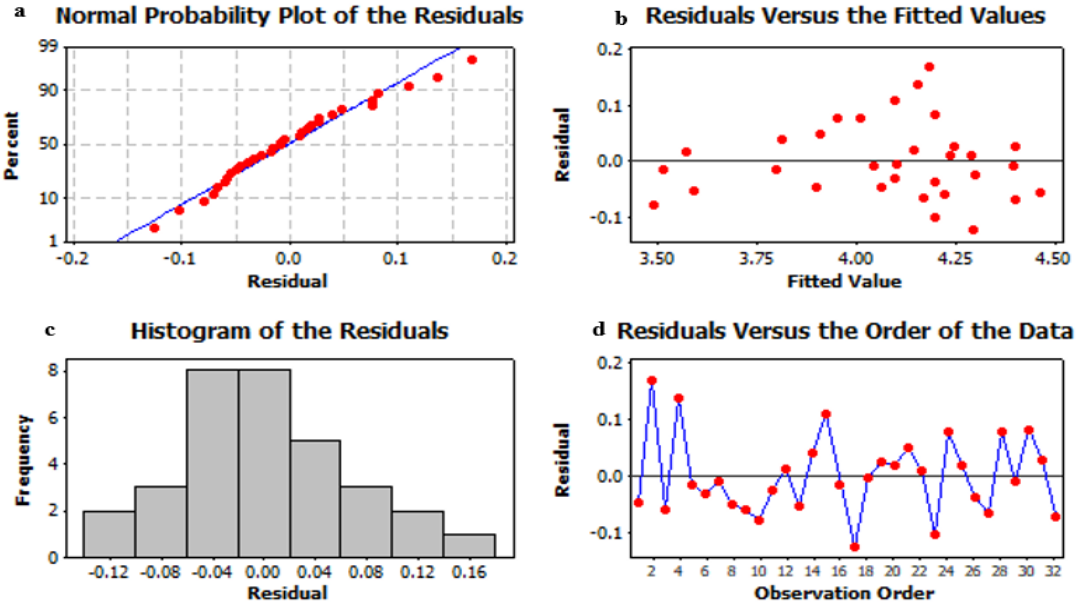
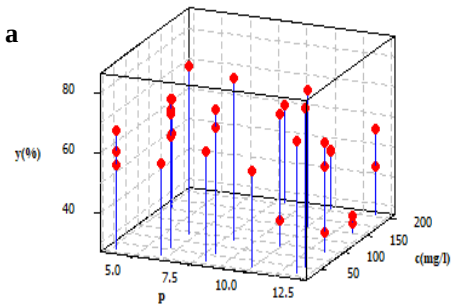


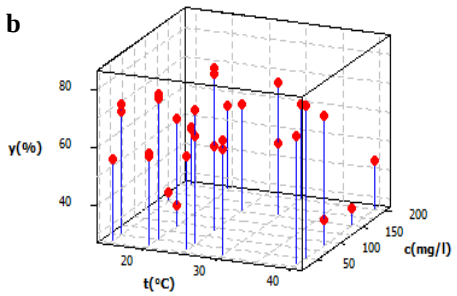
Figure 5 plots showing (a) Normal probability plot of the residuals, (b) Residuals versus the order of the data, (c) Histogram of the residuals, (d) Residuals versus the fitted values of N1D

and starch to get a percent decolorization of 63.15%. Whereas, in this current study, while using the bacteria *T. frigidphilosprofundus* the percent decolorization of the dye was 77.41% and there is no need for the external addition of NH_4Cl and starch. The residual plots of degradation of Airedale Yellow CHD using *T. frigidphilosprofundus* are shown in Figure 5.

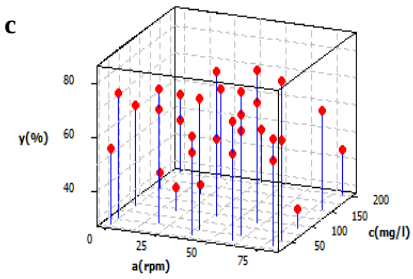
A residual is a difference between the predicted value and the obtained value. The obtained value is the experimental result and the predicted value is of the software (Olawoye 2016). The normal probability plot was shown if the acquired data follows the normality distribution and whether the gathered data from the trials form a good fit. It depicts the deviation between points and detects deviation. Figure 5a explains that residuals obtained from experiments form a nearly straight line which implies that the errors were distributed normally. The acceptable limit for the residual value is $\leq \pm 2$ (Ibrahim et al. 2022) and the obtained residual is very less and are within the limit. Hence it can be stated that the values didn't depart from the approved limitations (Ibrahim et al. 2022). The assumption of constant variance is tested by plotting the residuals against the ascending fitted response values. According to Olawoye (2016), the plot should be random scatter (constant range of residuals across the graph). This has been seen in plot b above. The number of trials that match the residual product is shown in the histogram of the residuals graph, and when the frequency is connected by a straight line, it follows a bell curve, which can describe it as a good fit. There will be a random dispersion on the plot (Olawoye 2016). From c, the residuals don't



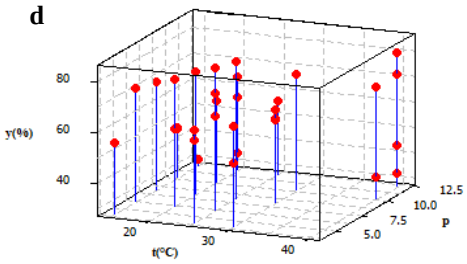
3D Scatter plot of y% vs c (mg/l) vs p



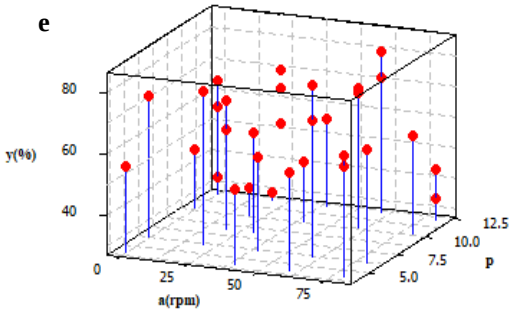
3D Scatter plot of y % vs c (mg/l) vs t (°C)



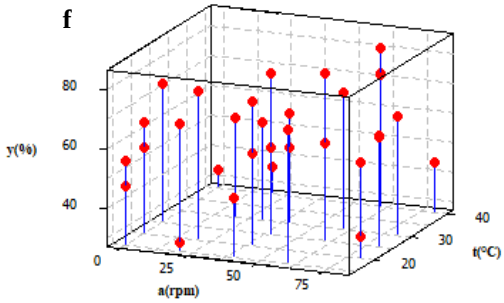
3D Scatter plot of y% vs c (mg/l) vs a (rpm)



3D Scatter plot of y% vs p vs t (°C)



3D Scatter plot of y% vs p vs a (rpm)



3D Scatter plot of y% vs t (°C) vs a (rpm)

Figure 6 Scatter plots of biodegradation of Airedale Yellow CHD using T. frigidphilosprofundus; here, (a) denote the figure of scatter plot of y% vs c (mg/l) vs p, (b) denote the figure of scatter plot of y% vs c(mg/l) vs t(°C), (c) denote the figure of scatter plot of y% vs c(mg/l) vs a(rpm), (d) denotes the figure of scatter plot of y% vs p vs t(°C), (e) denotes the scatter plot of y% vs p vs a(rpm), (f) denote the scatter plot of y% vs t(°C) vs a(rpm); From the above plots, the majority of the data points tend to rise or fall.

show a clear form. It implies that there is very little error in the collection of results of the experiment order (Patidar and Sharma 2021). Since from the above points, the work done in this study complies with the work to be done, to obtain very few errors, this is said to be a valid work to understand the biodegradation of Airedale yellow CHD using *T. frigidphilosprofundus*.

The scatter plots were also obtained as shown in figure 6. They denote the correlation between the result obtained and the input variables (Barker and Westfall 2022). Here 3D plots were obtained, where the points indicate the percentage decolorization obtained at two particular input variables.

3.3 Decolorization of Airedale Yellow CHD with *E. chrysanthemi Burkholder*

The maximum amount of decolorization obtained was 74.64 %. The biodegradation of the dye Airedale Yellow CHD required the addition of NH_4CL and starch in the work of Hema and Suresha (2014) to achieve a percent decolorization of 63.15 percent. The percent decolorization of the dye was 74.64 % that is also without any addition of NH_4Cl or starch from the outside. The residual plots of the runs for degradation of Airedale Yellow CHD using *E. chrysanthemi Burkholder* are shown in figure 7.

The disparity between the predicted and actual value is called a residual. The obtained value is the result of the experiment, whereas the predicted value is the result of the software (Olawoye 2016). The Normal Probability plot illustrates if the acquired data follows a normal distribution and the data from the trials fits together well. It shows the difference between two locations and detects the difference. Our model's residual values did not deviate from approved limitations ($\leq \pm 2$) and fell on a straight line with only a few exceptions, implying that the errors were distributed normally, as the residuals form a nearly straight line (Ibrahim et al. 2022). By displaying the residuals against the ascending fitted response values, the assumption of constant variance is checked and the plot should be random scatter (Olawoye 2016). This can be seen in plot b. The histogram of the residuals graph showed the number of trials that match the residual created, and when the frequency is connected by a straight line, it follows a bell curve, which can be described as a good fit. On the plot, there will be a random dispersion (Olawoye 2016). The residuals shown in plot c don't have a distinct form. It suggests that the experiment order's results are collected with relatively minimal inaccuracy (Patidar and Sharma 2021). Because the work done in this study corresponds with the work that needs to be done to achieve very few errors, it is considered a valid study to investigate the biodegradation of Airedale yellow CHD utilizing *E. chrysanthemi Burkholder*.

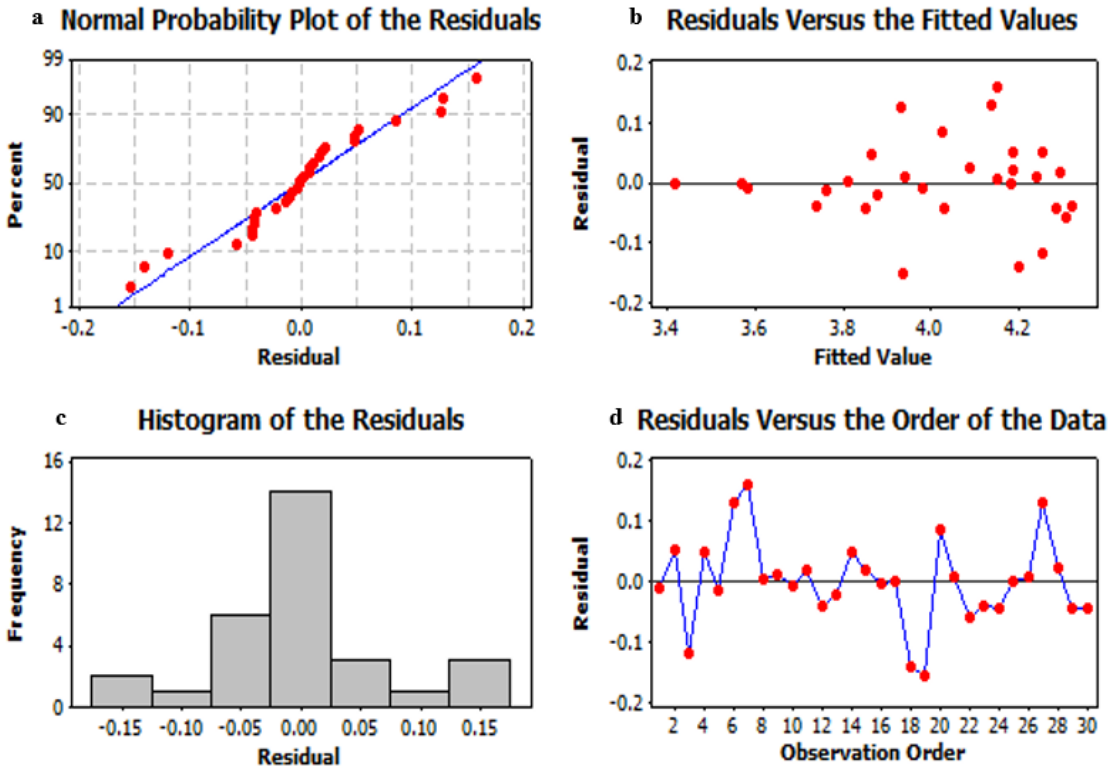
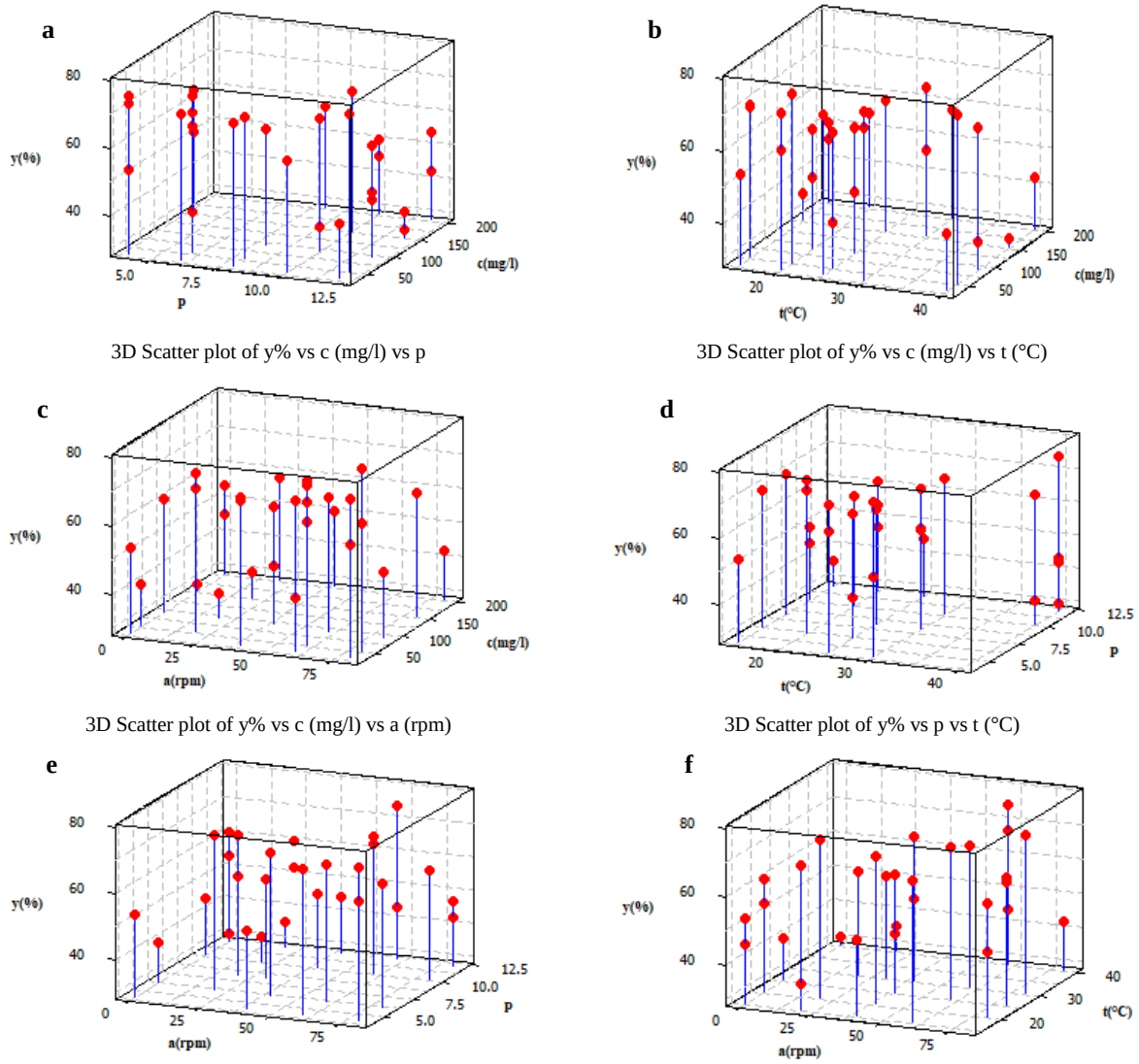


Figure 7 plots showing (a) Normal probability plot of the residuals, (b) Residuals versus the order of the data, (c) Histogram of the residuals, (d) Residuals versus the fitted values of N2D



3D Scatter plot of y% vs c (mg/l) vs p

3D Scatter plot of y% vs c (mg/l) vs t (°C)

3D Scatter plot of y% vs c (mg/l) vs a (rpm)

3D Scatter plot of y% vs p vs t (°C)

3D Scatter plot of y% vs p vs a (rpm)

3D Scatter plot of y% vs t(°C) vs a (rpm)

Figure 8 Scatter plots of N2D; here (a) denote the figure of scatter plot of y% vs c (mg/l) vs p, (b) denote the figure of scatter plot of y% vs c (mg/l) vs t (°C), (c) denote the figure of scatter plot of y% vs c (mg/l) vs a (rpm), (d) denotes the figure of scatter plot of y% vs p vs t (°C), (e) denote the scatter plot of y % vs p vs a (rpm), (f) denote the scatter plot of y% vs t (°C) vs a (rpm); From the above plots, majority of the data points tend to rise or fall.

Figure 8 shows the scatter plots that represent the relationship between the acquired result and the input variables (Barker and Westfall 2022). The points reflect the percent percentage decolorization obtained at two specific input variables in these 3D plots.

Conclusions

The ability of *T. frigidphilosprofundus* and *E. chrysanthemi Burkholder* to decolorize the azo dye Airedale yellow CHD contaminated wastewater is discussed in this work. The maximum percentage removal of the azo dye was obtained using *T. frigidphilosprofundus* (77.41%), and this was followed by *E. chrysanthemi Burkholder* (74.64%). The use of the bacteria can be considered the novelty of the work. The experimental results are compared with those of the modeled result, represented as residual plots. The obtained normal plots of the Residual plots for decolorization of the dye contaminated wastewater using the bacteria showed the formation of a nearly straight line which can conclude that experimental runs formed a good fit. Moreover, scatter plots showed the interaction between the input variables and the obtained result.

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Conflicts of Interest

The authors report there are no conflicts of interest exist.

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