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Biodegradation of Safranin-O and Crystal violet dyes by Using Some Fungi Species

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Abstract:

The current study aims to remove Safranin-O and Crystal Violet from aqueous solutions using some species of fungi, including *Aspergillus niger* and *Alternaria spp*. as an ideal alternative to expensive traditional industrial methods and an alternative to chemicals that have a detrimental effect on the environment. The effect of the initial dye concentration and the size of the inoculum was studied in different incubation periods. The results of this study showed that *Aspergillus niger* is more efficient than *Alternaria spp.* in removing the Safranin-O dye more than the percentage of removing the crystal violet at a concentration of 25% during three days with a removal rate of 75%, followed by a removal rate of 71%, but when the concentration was 5%, it was the lowest percentage for removing crystal violet, and during one day of treatment, where the percentage of removal was 43%.When using *Alternaria spp.*, its effectiveness in removing dyes was equal, as the highest removal rate was 65% when using the two dyes at a concentration of 25% within three days of treatment, followed by a removal rate of 61% for both dyes at a concentration of 15% during two days of treatment, and the lowest removal rate reached 43% for both. dyes at a concentration of 5% during one day of treatment.

Keywords: Crystal violet, Safranin-O, Decolorization , *Aspergillus niger* , *Alternaria spp*.

Introduction

Textile waste are considered as one of the biggest sources of water contaminants [1;2] , industrial dyes are more than 100,000 different type such as acidic, basic, reactive, azo, diazo, Crystal violet, Safranin, as well as, anthraquinone based meta complex dyes, These dyes are being produced with more than seven hundred metric tons annually for commercial uses [3;4;5]. More industrial effluents containing synthetic dyes are released in large quantities, which have a significant and dangerous impact on the environment and health [6;7]. Industrial dyes are characterized by the difficulty of biodegradation and more stable in the environment due to their complex aromatic composition [7;8] Most of these dyes are carcinogenic and toxic and have a great impact on aquatic organisms because of the toxic substances that result from the breakdown of these materials, as well as their impact on the photosynthesis process of aquatic organisms due to the reduction of light penetration [9;10;11]. Because of the seriousness of these materials and the problems they cause in different environments, they must be removed from the environment or their impact should be reduced. There are several methods physical, chemical and biological used for treatment. [11;12]. It is not preferable to use chemical and physical methods to remove industrial effluents, due to the high costs, their negative impact on the environment, the need for manpower [13;14]. The use of environmental friendly methods, which are biological methods using fungi, bacteria and algae cultures, are being considered for their low costs, easy to use, and are highly efficient for dye removal[15;16] Researches are focused on the importance of using biological methods such as the using of fungi to remove dyes because of their large biomass and high ability to decolorization and degradation of synthetic dyes [16;17]. Basic dyes including the Crystal violet (CV) and Safranin-O (S) have been the focus of many studies due to its frequent presence in soil and river sediments, since it been disposed-off unproperly [18,19]. In the present study, several freshwater fungi were isolated from streams in Babylon, Iraq, were tested for their ability for decolorizing multiple synthetic dyes was evaluated.

Material and Methods

Preparation fungus suspension

Fungi isolates were taken from the fungal *Aspergillus niger, Alternaria spp.* previously diagnosed and kept in the refrigerator and the isolates were activated by growing them again on Potatoes Dextrose Agar (PDA) medium, after a 7 days of incubation period at 28 °C and PH 5.5, the fungal inoculum was taken by using the cork polar was 5 mm in diameter ,added with distilled water(10 ml) to the fungus disc to make a fungus suspension, With shaken rigorously and continuously, the particles of the adsorbent was separation by centrifuged from solution to obtain the equilibrium concentration [20].

Preparation Dyes Solution

1- **Crystal violet**

Crystal violet $C_{25}N_3H_{30}Cl$, IUPAC name Tris(4- dimethyl lamino) phenyl),methylium chloride

2- Safranin-O

$C_{20}H_{19}ClN_4$IUPACname, 3,7-Diamino-2,8-dimethyl-5-phenylphenazin-5-ium chloride

The dyes were prepared by taking a certain weight of the dyes powder and making a standard solution of 1000 ml of distilled water. Three concentrations (5, 15, and 25) % were prepared of the standard solution crystal violet and safranin-O. The liquid culture media PDA were placed in 250-ml conical flask; the dyes solution (crystal violet, safranin-O) were added

to give the desired concentration (5,15,25) % and final volume (100 mL); then, the pH was adjusted at 5.5 before sterilization. After cooling, spore suspension 5 mm was added. Each experiment was performed in triplicate. All conical flasks were incubated at 30 °C under static condition for various periods (24,48 and 72) hours, Samples were collected, centrifuged, filtered, washed, completed to a constant volume for measuring remaining concentration dyes [21].

Measuring The absorbance of Dyes

Using different concentrations of dyes including 5 %, 15 % and 25 %, and incubated for 24 h ,48 h and 72 h. The final concentration of crystal violet and safranin-O were estimated for each sample spectrophotometrically at the wavelength corresponding to maximum absorbance for safranin-O (λmax = 490 nm) and crystal violet (λmax = 585nm) by using the Spectrophotometer (PD-303). The following equation was used to calculate the amount of dye removal:

$$\text{Removal \%} = (A° - A) / A° \times 100$$

Ao: (absorbance) concentration of dye before decolorization A : (absorbance) concentration of dye after decolorization [22; 23]. The effect of initial dye concentration and the inoculum volume on the rate of removal was evaluated with different concentrations of dyes (5 %, 15 % and 25 %) and incubated for 24 h ,48 h and 72 h.

Statistical Analysis

Differences between two groups were explanation by IBM SPSS Statistics 23 both Mean and standard deviation for three identical replicates was also studied, with possibility value of $p \leq 0.05$.

Results and Discussion

In the figures (1,2) showed that *Alternaria spp*. was more effective than *Aspergillus niger* in removing safranin-O dye, where the percentages of dye removal by *Alternaria spp*. with concentration of 25% on the first, second, and third days were 56%, 58% and 61%, respectively, compared to *Aspergillus niger*, where the percentage of dye removal at the same concentration for three days was 52%,53% and 58% respectively. The results of the current study agree with the results of previous studies on the efficiency of fungi in removing industrial dyes that may cause side effects or carcinogenicity to humans, Previous research showed that fungi have the ability to remove environmental pollution resulting from chemical dyes and their damages to human health and the environment alike [24,25,26]. Previous studies showed that *Aspergillus niger* has a high efficiency in removing chemical dyes, and the maximum color removal from the primary fuchsia dye was (81.85%)[24].

The results of the present study also showed that the dye crystal violet can be removed better *using Aspergillus niger*, where the percentage of removal of the dye at a concentration of 25% on the first, second and third days was 67%, 71% and 75%, respectively, while the efficiency of *Alternaria spp*. was lower in removing the dye of concentration for three consecutive days, where the percentages were 59%, 61% and 65%, respectively(figures 3,4). The ability of fungi to remove chemical dyes is due to adsorption and biodegradation. The adsorption of dyes on the fungal cell surface is the main mechanism for color removal [24].

Aspergillus niger is more efficient than *Alternaria spp*. in removing the crystal violet dye more than the percentage of removing at a concentration of 25% during three days with a removal rate of 75%, followed by a removal rate of 71% with the same concentration during two days of treatment, and removal percentage 67% at concentration of dye 25% at one day ,

As for the removal percentage at concentrations of 5% were 52%, 61%, 66% and at concentrations of 15% crystal violet dye were 55%, 59% and 66% during 24 hour, 48 hour and 72 hour respectively. While the concentration was 5%, it was the lowest percentage for removing crystal violet, and during one day of treatment, where the percentage of removal was 43% during 24 hour, followed 50% and 54% in 48 house and 72 hour respectively .while in the concentrations 15%,25% during 48 hour and 72 hour of treatment period, the removal percentage reached,53%,61%,59% and 65% respectively. When using *Alternaria spp.*, its effectiveness in removing dyes was equal, as the highest removal rate was 65% when using the two dyes at a concentration of 25% within three days of treatment, followed by a removal rate of 61% for both dyes at a concentration of 15% during two days of treatment, The two dyes at a concentration of 5% during one day of treatment, *Aspergillus niger* (Figures 1,2,3,4).

Previous studies showed that fungi decompose or decolor chemical dyes either through enzymatic activities or adsorption. Fungal enzymes including lignin peroxides, manganese peroxides also play an active role in the decomposition of toxic by-products formed by chemical dyes [27,28,29,30]. There is an agreement with previous studies, which showed that absorption of stains to the microorganism surface cell is the main technique of decolorization. it was found to be significant technique that participate in the decolorization method, potential to primary conversion of the pigment degradation [31, 32,33]. Previous studies show that microscopic scanning displayed that the fungal germs, in place of the fungal surface were truly absorbing the stain, A hydrophobic -hydrophilic connection between the fungus and the pigment participated to the absorption phenomenon[34]. When the concentration of the fungal cell mass increases, the dye color concentration decreases due to the enzymatic action of the fungal cell, as well as due to the adsorption of the dye on the surface of the fungal cell [35,36,37]. So this method can be used commercially for color removal and disposal of various dye wastes in industries and biotechnology laboratories.

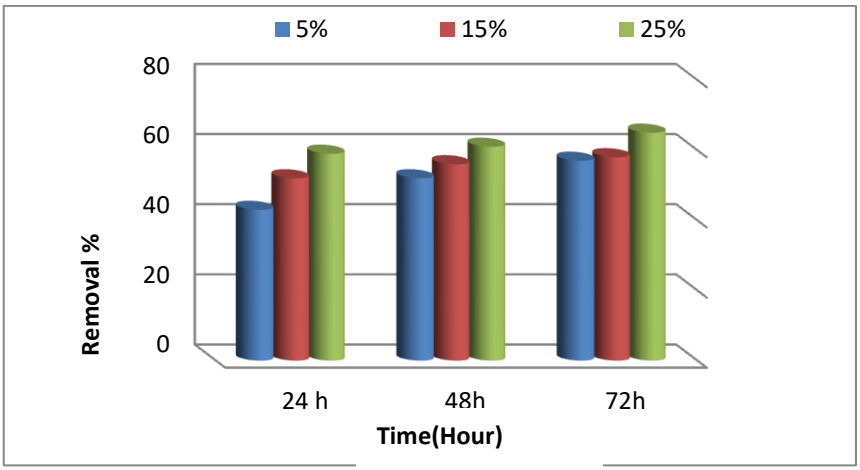

Fig.1: Removal Percentage of safranin-O dye by *Alternaria spp.*

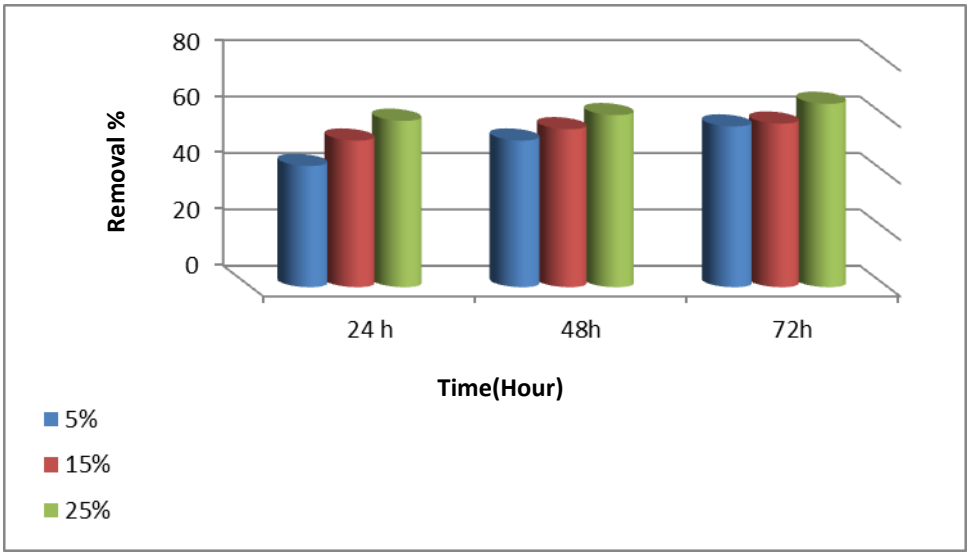

Fig. 2: Removal Percentage of safranin-O dye by *Aspergillus niger*

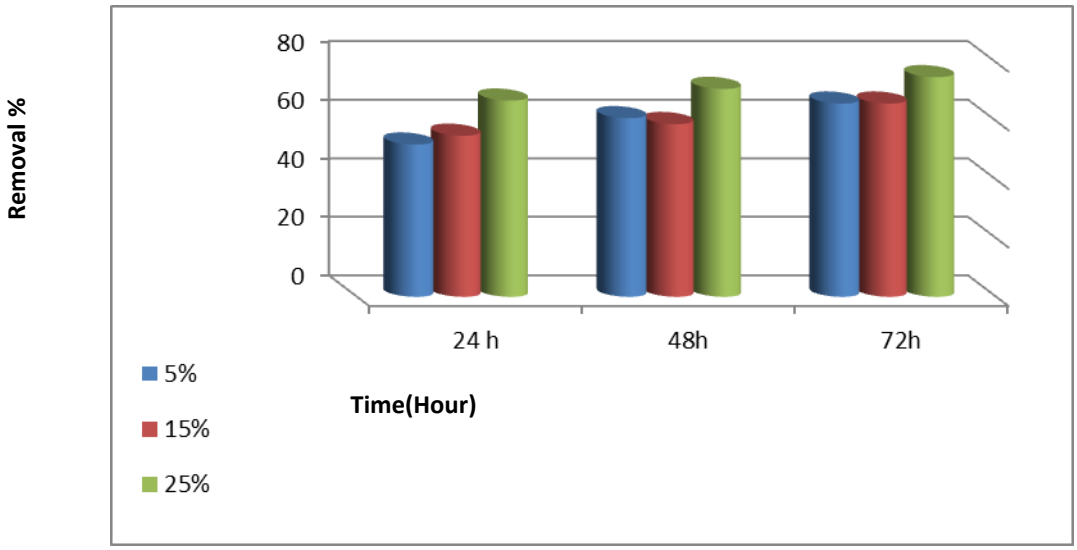

Fig. 3: Removal Percentage of crystal violet dye by *Aspergillus niger*

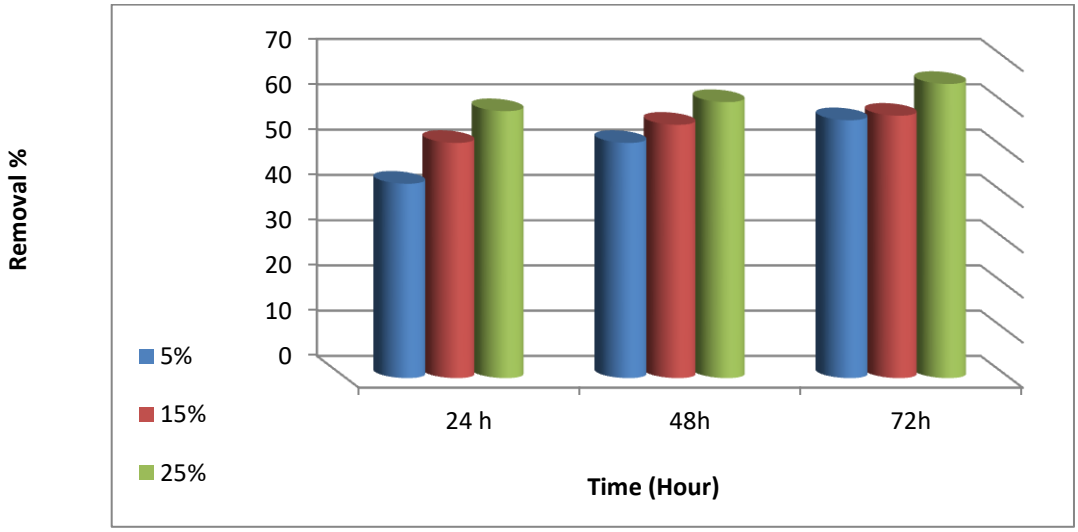

Fig. 4: Removal Percentage of crystal violet dye by *Alternaria spp.*

Conclusions

The study showed the ability of some fungi, including *Aspergillus niger* and *Alternaria spp* in removing dyes, showed that *Alternaria spp*. was more effective than *Aspergillus niger* in removing safranin-O dye, while *Aspergillus niger* was more efficient than *Alternaria spp.* in removing the crystal violet dye, So biological methods are considered as alternative and successful solutions in removing industrial dyes.

Author Contributions Statement

Sarab A.Juda , , Huda Abbas Mohammed: working steps of the experimental and write the research

Noor S. Naji : Results analysis and statistics

Declaration of competing interest

The author confirms that there was no competing interest with others.

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