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Removing Toxic Dyes from Aqueous Medium by Trichoderma-Graphain Oxide Aerogel

Shaimaa Satae M Ali*¹  Rana Hadi Hameed Al-Shammari²  Ayad M.J. Al-Mamoori³ 

¹Environmental Studies and Research Center, University of Babylon, Iraq.

²Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.

³Department of Biology, College of Science, University of Babylon Babil, Iraq.

*Corresponding author: shamaasatea@gmail.com

E-mail addresses: dr.rana@uomustansiriyah.edu.iq , sci.ayad.mohammed@uobabylon.edu.iq

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Abstract:

Toxic dyes are commonly discharged into waste waters and dyes are extensively used in the textile industry so it is necessary to find out efficient and eco-friendly method for treating waste waters resulting from industrial effluences. To achieve this aim the fungus *Trichoderma sp.* is employed into two lines: first line was self – immobilized fungal pellets in (Czapek – Dox medium) to adsorb two dyes crystal violet, congo red by concentrations 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg/L to both dyes, PH 2, room temperature with shaker in (hrs.²,hrs.⁴,hrs.²⁴) , by Uv- Visible spectrum . the removal efficiency of 0.05 mg/L crystal violet by *Trichoderma sp* was 96%. but there was no removal by congo red. The second line was immobilizing fungal mycelium to Graphain oxide free – standing aerogel to increase efficiency of adsorption. The decolorization of toxic dyes solution was detected by the change in the adsorption Uv- Visible spectrum and scanning microscopy analysis which revealed that there was dye adsorption on fungal mycelium surface. After treatment of crystal violet with 20 mg Graphain oxide -fungi aerogel in the condition PH 2, room temperature with shaker in time (hrs.² ,hrs.⁴ , hrs.²⁴) removal percentage to crystal violet was increasing with to raise concentrations the dye crystal violet until reaching the maximum removal percentage 97% in hrs.4 in 0.05mg/L concentrate , and it increased the efficiency of other concentrations . In contrast, according to congo red there was no color removal in any concentration within treatment time since congo red surface carries both negative and positive charges and causes electrostatic attraction, therefor, the adsorption reduced or does not occur.*Trichoderma sp.* is considered a selective removal to basic dyes and could be employed to remove dyes from industrial effluents.

Keywords: Removal efficiency, crystal violet, Graphain oxide, *Trichoderma.*, congo red, adsorption.

Introduction:

There are currently more than one thousand types of commercial dyes, and more than 700000 tons of these dyes are produced and consumed worldwide annually like synthetic Azo dyes which are a large group of synthetic dyes that are used in various industries such as paper, cosmetics, food and especially textile industries^{1,2}. Light and oxygen cannot penetrate water because of these dyes, resulting in broad aquatic pollution³. Certain dyes pose a risk to human since they can result in cancer and tumors⁴. Physical, chemical, and biological methods are available for removing dyes from textile effluents as Flint Clay was used as an adsorbent to remove Congo red, Rhodamin B, and Dispers Blue dyes from water solutions^{5,6}. Physical,

chemical methods have disadvantages compared to biological removal that can be of high cost concerning equipment and the production of large quantities of sludge. These reasons have made biological methods of dye removal from textile effluents more appropriate than other methods due to their low cost because they are manufactured from these biomaterials. Plants, fungi, bacteria, and algae have demonstrated good dye removal capabilities^{7,8}. In the bioadsorption method, the dye in the effluent is attached to the functional groups present on the surface of the microorganism. Fungi, due to their extensive cells, are capable of removing dye by absorbing from textile effluents in waste water^{9,10}. Many fungi can produce stable mycelial pellets that can be used as self-immobilizers or by immobilize

fungi to a natural or chemical compounds¹¹. Other fungi like *Candida tropicalis* produce biosurfactants which are efficient in degradation different synthetic dyes in water, as well as exerting remarkable antibacterial and anti-biofilm activity against pathogenic bacteria¹². Fungus can be effective in the rate of adsorption or decomposition according to their ability to absorb azo dyes. The aim of this study was to prepare a novel Fungal-Graphian oxide aerogel by the biological culture method, to test the removal action.

Materials and Methods:

Fungal Isolate and Pellets Formation

Czapek–Dox medium was used to culture *Trichoderma sp.* Czapek–Dox medium that contained 1 gm potassium dihydrogen phosphate, 30 gm sucrose, 0.5 gm magnesium sulfate heptahydrate, 3 gm sodium nitrate, and 0.01 gm ferrous sulfate and 0.5 gm potassium chlorate in 1 L of water. Then came the sterilization stage of *Trichoderma sp* fungus hyphae before culturing, the medium was sterilized under high pressure for 20 minutes and at 121 °C. The shaking rate of *Trichoderma sp.* fungus hyphae was 150 r pm with incubation in 100 mL culture medium at 25 ° C a for about 3 days (Lab tech DAIHAN LABTECH CO.). When all that is available, the fungi can form big pellets (after one week), and to get a spreading fungus, strong instigation using a magnetic stirrer (J lasso INDIA) (1500 rounds per minute) was important. After storage, the spreading fungi at 4C° can be used at another time when treated directly with dyes¹³.

Another media which is (PDA)(CONDA,Pronadisa USP) was used in order to develop these fungi when dealing with the Graphian Oxide.

The Dyes

1-Crystal violet: this dye is found in aqueous media and carries a positive charge because of nitrogen atoms Fig. 1, and it takes its color according to the number of this charge and it changes according to the PH of the dye. Six concentrations from crystal violet 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 gm./l. PH 2 were prepared. Each concentration was put in a flask 250 ml in room temperature Fig. 2.

Absorbance was at wavelength 590 nm, chemical formula ($C_{25}H_{30}C_1N_3$)molar mass $407.99 \text{ g}\cdot\text{mol}^{-1}$ ¹³.

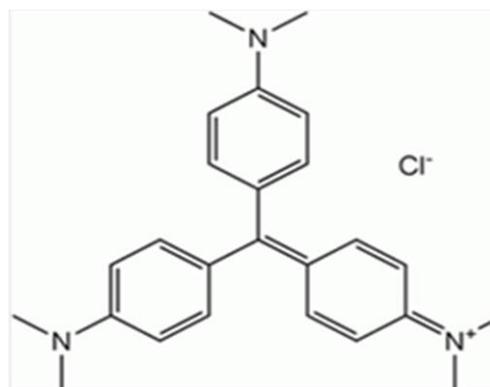


Figure 1. Molecular structure of crystal violet crystal violet



Figure 2. Concentrations of crystal violet

2- Congo red: this dye is an organic compound, the sodium salt of 3,3'- [1,1'-biphenyl]-4,4'-diyl) bis (4-amino naphthalene-1-sulfonic acid). It is an azo dye. Chemical formula is $C_{32}H_{22}N_6Na_2O_6S_2$ and molar mass is 696.665, and it is red color in condition PH 5.2 at above and contains aromatic ring which causes or gives hydrophobic interaction in some media because of its the molecular structure, Fig. 3. Six concentrations were made from this dye 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 gm./l. PH 2. Each concentration was put in flask 250 ml in room temperature Fig. 4. Absorbance was at wavelength 495 nm ¹⁴.

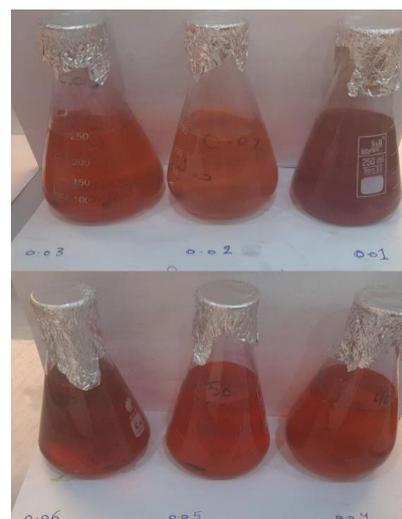
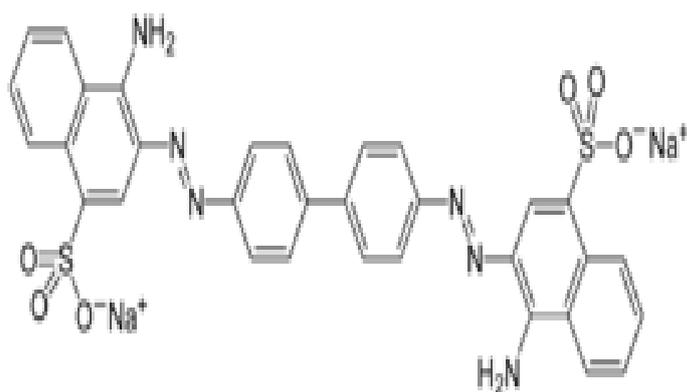


Figure 3. Molecular structure of Congo red

After all preparation, 10 ml from fungi growth was added to all concentrations from crystal violet and Congo red and put in a shaker 120 rpm (VRN 480,105480, TAIWAN) for 2 hrs, 4 hrs, 24 hrs. ,48 hrs., and readings were taken after all time by UV spectrophotometer (APEL japan PD -303S).

After taking all reading adsorption from spectrophotometer order, concentrations were taken and control reading of both dyes is calculated, the equation of removal as below:

$$\text{de coloration rate \%} = (C_0 - C_e) / C_0 \times 100\%$$

Removal efficiency.

2- Graphene Oxide (GO): by methods (Briefly) modified Hummers, 23 mL sulfuric acid 98% was added into 1gm of graphite in a 500 mL glass flask with continuous stirring. Before starting, by pre-treatment, this flask must be exposed to low heat in the ice bath at 5 C. After this, 3 gm KMnO₄ was added to the glass flask cautiously and the temperature must be below 20 C°, to get on best reaction solution, then it was converted to an oil bath 34 C° with stirring for 2 hrs. After that, 50 mL water was carefully added successively to 90 C° and kept to for 10 minutes. Another 5 mL of 30% H₂O₂ and 120 mL of water was cautiously placed into the glass flask. Soon the yellow color will appear. After this and in the ending reaction and by vacuum filter, the product was collected which is the graphene oxide. By using 250 mL of HCl (v/v ¼ 1: 10), graphene oxide was washed several times to remove the metal ions. And then using membrane dialysis, Fig. 5, for the best purification of graphene for 7 days in water. Using ultra-sonication for 6 hrs., we can get purified graphene (Lab Tech: LUC-410). To get the best concentration from the GO solution, it was freeze - dried in a vacuum dryer.^{12,15}

An amount 50 gm of fungus was taken from culture and dispersed into a 0.2 gm of dried GO pieces with

the mixture, then the mixture was stirred vigorously for several hours by magnetic stirring in room temperature overnight.

The ending production (fungus/GO free- aerogel) becomes ready for use¹⁶.

When adsorption was performed by (fungus/GO free- aerogel) color change can be detected by measuring the UV-vis spectra and by applying the equation. The removal efficiency percentage (discolorations %) appears in the equation

$$\text{discoloration rate \%} = (C_0 - C_e) / C_0 \times 100\%$$

C₀ = control.
C_e = concentration of sample .



Figure 5. Medical membrane to dialysis

Results and Discussion:

According to previous material and methods the results as below:

1-Vivo Adsorption:

The growth process of the fungus *Trichoderma* is into two lines: the first line included growth fungi in by (Czapek–Dox) order to conduct treatment with two dyes (crystal violet, Congo red) and adsorption. The second line included growth fungi that were mixed with Graphene oxide in order to improve and

raise the level of adsorption, the growth was in both cases as follows: Fig. 6.

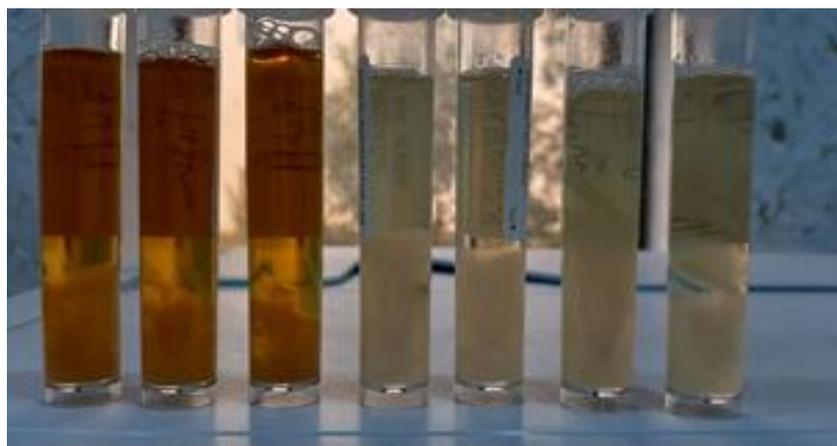


Figure 6. Trichoderma activation after shaker incubator

After adding fungi 20 gm to two dyes, the results denote that the adsorption by *Trichoderma* in crystal violet in pH 2 room, temperature was gradually increasing with increasing concentration of the dyes until the concentration of 50 0.05 mg/l. There is an increase in dye removal with the increasing amount of adsorbent till 50 gm which is attributable to an increase in surface area which in turns facilitates the adsorption and hence the

removal efficiency. Due to the driving force of the dye in all concentrations, the best time was in 4 hrs. percentage of removal efficiency was decolorization rates 96%, indicating that the dyes were adsorbed onto the fungus hyphae mainly by an electronic interaction force. This means that the *Trichoderma* has effectively worked on adsorption by its surface and by bio adsorption Figs.7,8.

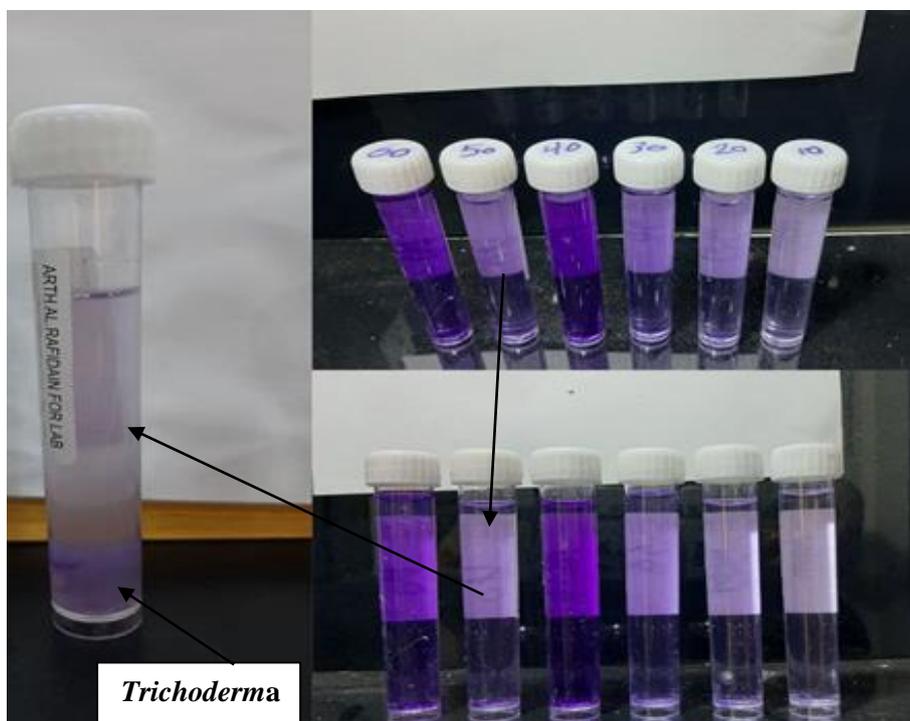


Figure 7. Crystal violet after adsorption

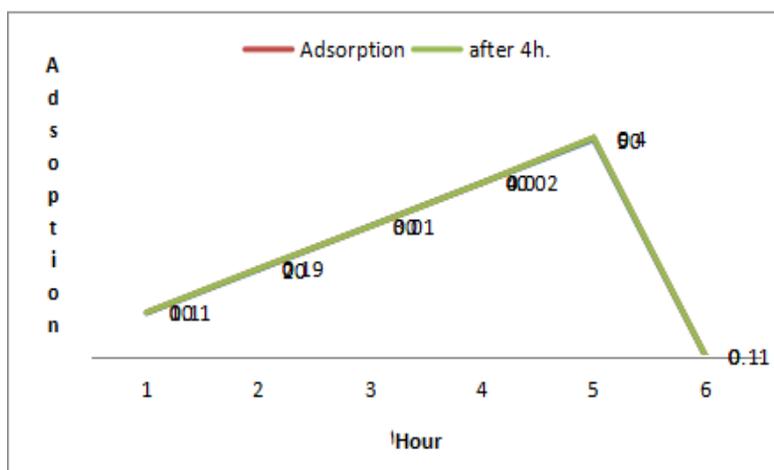


Figure 8. Highest adsorption in 0.05 in 4hrs. for crystal violet

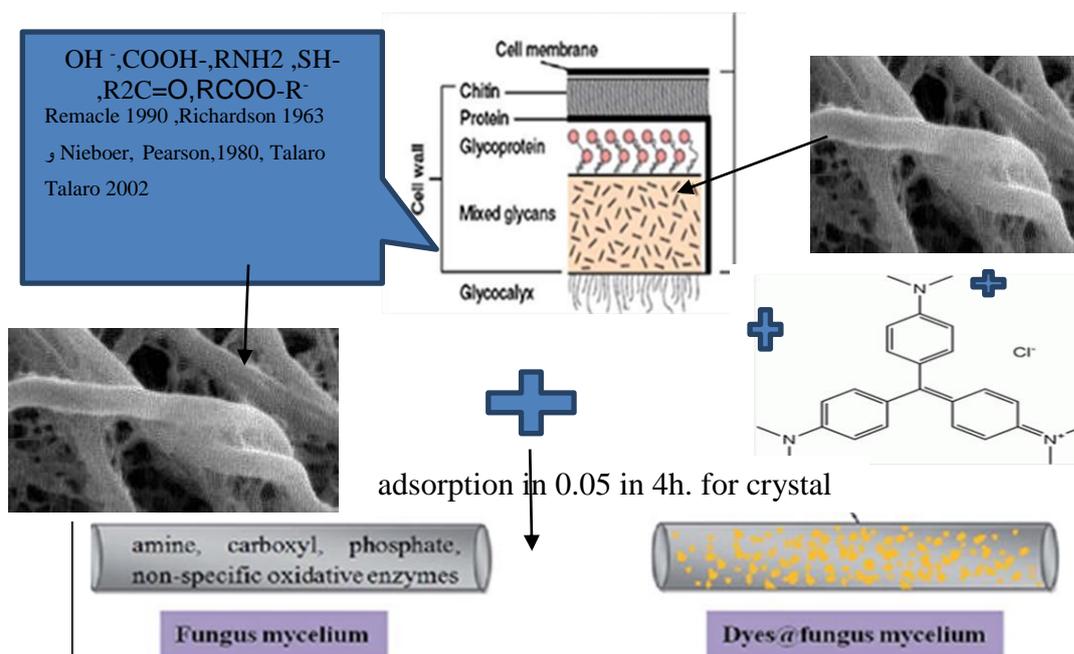


Figure 9. Electrostatic interaction (adsorption) between fungi and dye (crystal violet)

This stable removal efficiency may be explained by the fact that when the adsorbent to the aqueous dye solution, dye molecules readily adsorbed into the absorbent surface and there is no initial adsorption due to the large number of available active sites. However, as soon as equilibrium is established in 5 minutes, there will be constant adsorption and adsorption of dye molecules to and from the adsorbent surface. At this stage, the system is said to be in a steady state and henceforth steady dye removal efficiency was observed^{14,17}.

And because the surface of the fungus pellet is charged by its surface with a negative charge, this belongs to the carboxyl group (HCO₃⁻) and positive charge by H⁺, this causes the difference in ability to adsorb on its surface, because of the acidic media in

this treatment. It gives the best condition and highest to adsorb anionic acid dyes with presence and accreditation static electrical force. In addition, the treatment by a base fungus has the lowest level in potentials adsorption. Therefore, the adsorption properties became weak due to the group of HCO₃⁻. Observation for the increase in the removal efficiency was recorded at higher pH values. The percentage removal was found to increase with the time passage. The lower removal of the dye at lower acidic pH may be associated with the competition that available between the excess concentration of H⁺ ion and the cationic part of the dye for the active site on the adsorbents^{18,19}. It was observed that adsorption efficiency was higher at acidic, Fig. 9.

When the adsorption occurs, there happens increased adsorbent material concentrations, this is due to the rate of diffusion and transfer of mass on the adsorbing surface²⁰ till saturation limit is obtained on the adsorbent surface, this refers to isotherm adsorption²¹.

As for the behavior of *Trichoderma* in the Congo red, the percentage of removal 0% Fig.10. There is no color removal in any concentration or at any time. This may be due to the acidic medium in which the adsorption took place, where is the amine group found on the dyed surface which refers to the absence of acidic medium. It gains a proton in an acidic medium and converts it to ion anilinium thus, the dye surface carries both negative and positive charges and cause electrostatic attraction, therefore, the adsorption is reduced or does not occur²².

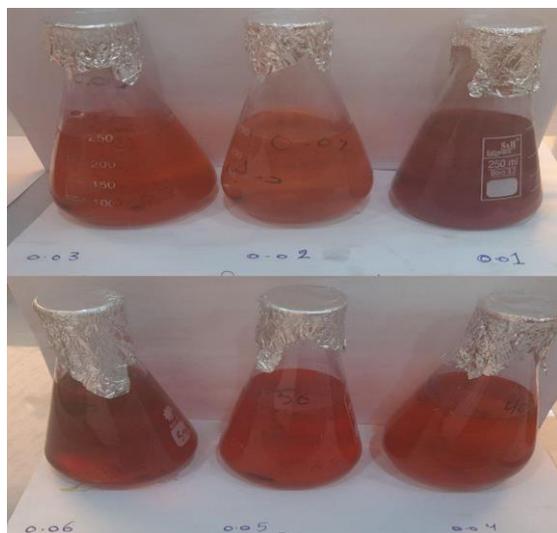


Figure 10. No discoloration in Congo red

2-Vitro Adsorption (Graphene Oxide-fungi Aerogel):

Because the continuous growth of fungus pellets was active during the biosorption, but after the time of growth, the level of adsorption decreased. By second growth line from fungi *Trichoderma* sp and mixture with G.O. Figs. 11,12 which was got from methods as flow (G.O. fungi aerogel) Fig. 13. In order to observe the mass size of the fungi pellet, it was assessed by taking pictures in SEM Fig. 14, in starting the growth of fungi, the fungi pellet was in small mass, but after the growth, the fungi pellet became big in mass and then the adoption increased. The fungi hyphae became dense and more effective and the weight and diameter of the fungus pellets would give the best adsorption. All these would increase in growth until all nutrients were used up then the adsorption would decrease. When comparison between the growth in 3 days and

4 days, this growth was unchanged and became more saturated and in a stable state^{23,24}.



Figure 11.G.O. after filtering

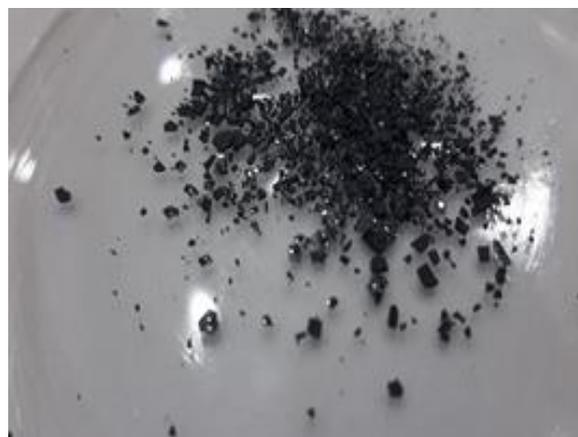


Figure 12. G.O. after drying



Figure 13. Graphene oxide-fungi aerogel

After treatment of Graphene oxide-fungi aerogel 20 mg with dye crystal violet in the condition pH 2, room temperature concentrations 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 with shaking in time 2hrs. 4hrs. 24hrs, 590 nm, the results were: the removal rate increased with the raise of concentration of the dye crystal violet until concentration was 0.05mg

removal rate 96% in 4hrs. and 97% in 4hrs, but high adsorption was in 0.05mg, and pest time was 4hrs, all these mean that the(G.O.fungi aerogel) raises activation of the fungi to adsorb the dye even in the rest of the other concentrations 0.01, 0.02, 0.03, 0.04 which is higher than in using fungi only at the same time and the same concentrations Fig. 15.

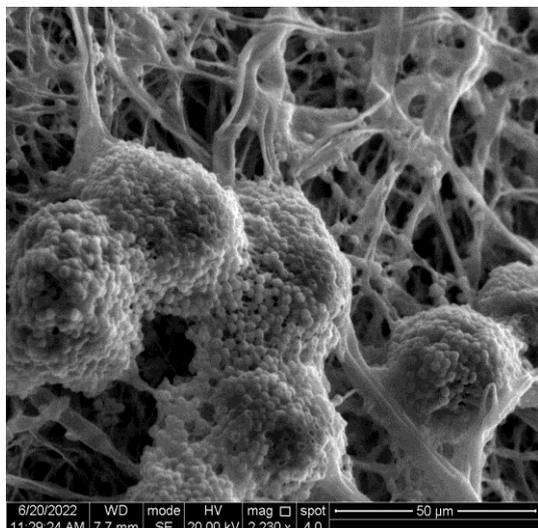


Figure 14. SEM *Trichoderma* in 3 days from growth in media

because the layer spacing of GO is expanded and successfully introduced numerous polar carboxyl groups, Fig.16.

The mechanism analysis indicated that electrostatic interactions, π - π conjugation, and hydrogen bonding are the predominant forces for adsorbing cationic dyes²⁵. The macrostructures (3D rGO) are more efficient in the adsorption of cationic dyes rather than the anionic dyes because of strong specific interactions in their structures²⁶. But in comparison with Ag_3PO_4 /graphene oxide aerogel, the graphene oxide aerogel resulted from a higher percentage of adsorption^{27,28}.

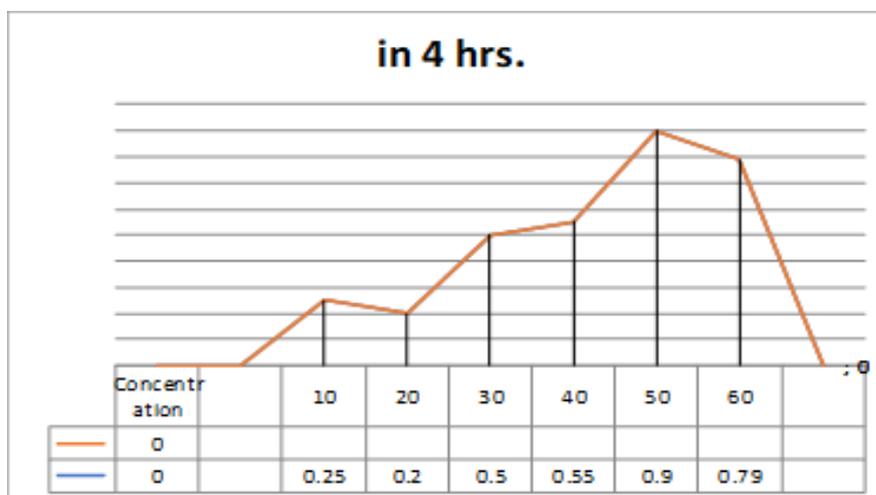


Figure 15. Highest adsorption in 0.05 in 4hr. for crystal violet by G.O. fungi aerogel

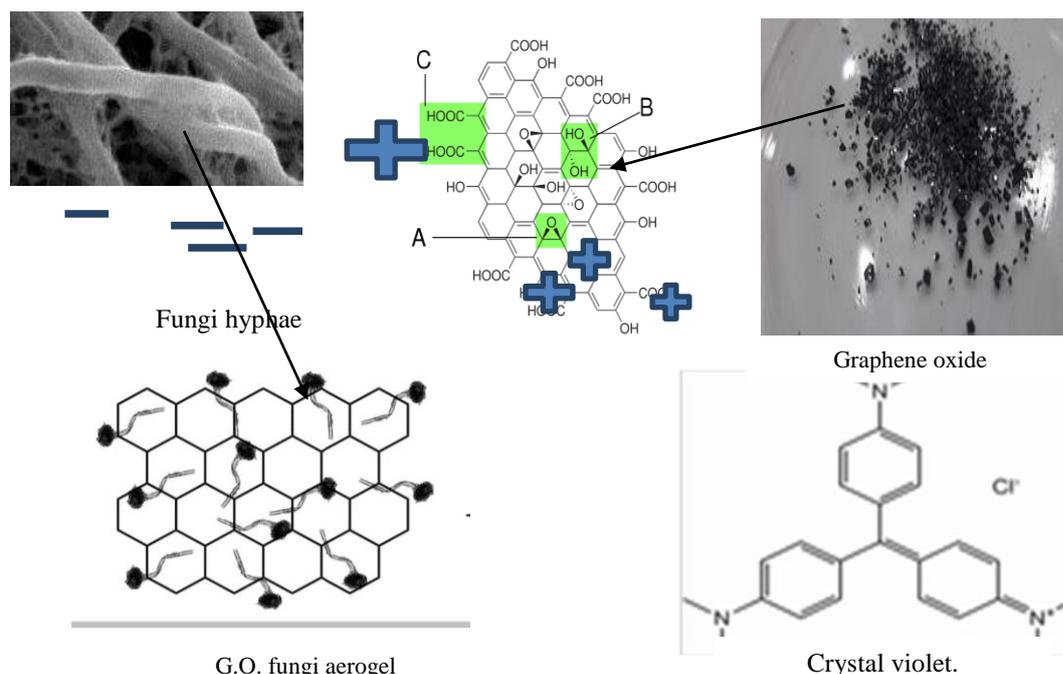


Figure 16. Graphene oxide fungi aerogel and interaction with crystal violet

Then a comparison between fungus/GO aerogels and only fungus aerogels will require an observation of removal efficiency in discoloration in fungus/GO aerogels of around 2–3 times higher than that of the fungus aerogels^{28, 29}.

Conclusion:

In general, the removal rate raised more clearly at all concentrations and all times by (G.O.fungi aerogel) than the same concentration and time in treatment only fungi. However, the distinct difference in the removal rate was clear between the two cases. The removal occurred faster in 4hrs. with only fungi. While it took more time and a higher percentage in 4 hrs. with (G.O. fungi aerogel), due to their excellent mechanical force and extended surface areas. Due to the results of the adsorption in the removal of synthetic dyes and heavy metal ions from aqueous media, in the same way it is possible to use the graphene oxide over 90% to remove dye pollutants and heavy metal ions from wastewater. The similarity between the two cases were in removal rate in 0.05 mg/l : 96%,97%

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Authors' Declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of babylon

Authors' Contribution Statement:

This work carried out in collaboration between all authors. R H. H. A conceived the idea and designed the experiments. S S M. A performed the experiments and analyzed data and wrote the initial draft of the manuscript. A M.J.A reviewed and validated the experiment. All authors revised and approved the manuscript

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إزالة الصبغات السامة من الوسط المائي بواسطة جل ترايكوديرما- اوكسيد الجرافين

شيماء ساطع محمد علي¹ رنا هادي حميد الشمري² اياد محمد جبر المعموري³

¹مركز البحوث والدراسات البيئية، جامعة بابل، بابل، العراق.
²قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق.
³قسم علوم الحياة، كلية العلوم، جامعة بابل، بابل، العراق.

الخلاصة:

عادة ما يتم تصريف الأصباغ السامة الى مياه الصرف الصحي اذ تستخدم الأصباغ على نطاق واسع في صناعة النسيج ، لذلك من الضروري إيجاد طريقة فعالة وصديقة للبيئة لمعالجة مياه الصرف الناتجة عن النفايات الصناعية السائلة. لتحقيق هذا الهدف انجزت هذه الدراسة وذلك بتوظيف الفطر ترايكوديرما بطريقتين الأولى عبارة عن كريات فطرية نامية في الوسط المغذي (Czapek – Dox) الصلب وامتزاز الصبغتين الكريستال البنفسجي ، أحمر الكونغو ، باستخدام التركيز 0.01،0.02،0.03،0.04،0.05،0.06 لكليهما و الرقم الهيدروجيني = 2 ، درجة حرارة الغرفة ، مع الهزاز للفترات (2 ساعة و 4 ساعات و 24 ساعة) من الزمن . تم قياس تركيز الصبغة بواسطة طيف الأشعة فوق البنفسجية المرئي. حيث كانت كفاءة الإزالة بواسطة فطر ال *Trichoderma sp.* في التركيز 0,05 لصبغة الكريستال البنفسجي هو 96%، ولكن لا يوجد إزالة لصبغة احمر الكونغو. اما الخط الثاني كانت بدمج خيوط الفطريات إلى جل نقي وحر من أوكسيد الجرافين لزيادة كفاءة الامتزاز. إزالة اللون من محلول الأصباغ السامة بواسطة الكشف عن الامتزاز بواسطة طيف الأشعة فوق البنفسجية المرئية وتحليل فحص المجهر الإلكتروني للكشف عن امتزاز الصبغة على سطح الخيوط الفطرية. وبعد معاملة (صبغة الكريستال البنفسجي) بـ 20 ملغم من جل الفطريات -لاوكسيد الجرافين في ظروف pH = 2 ، درجة حرارة الغرفة مع الهزاز للفترات (2 ساعة و 4 ساعات و 24 ساعة) من الزمن ، زادت نسبة كفاءة الإزالة للكريستال البنفسجي مع زيادة تراكيز الصبغة حتى الوصول إلى الحد الأقصى لنسبة الإزالة 97% في الساعة 4 في تركيز 0.05 ملغم / لتر ، وزادت الكفاءة في التراكيز الأخرى.. على النقيض من احمر الكونغو اذ لم تتم إزالة اللون في أي تركيز خلال وقت المعالجة لأن سطح احمر الكونغو تحمل شحنات سالبة وإيجابية ويحدث جذبًا إلكتروناتيكياً ، وبالتالي قل الامتزاز ولم تحدث في تراكيز منها. يمكن استخدام الفطر ترايكوديرما في الإزالة الانتقائية للأصباغ الأساسية ويمكن استخدامها لإزالة الأصباغ من النفايات السائلة الصناعية.

الكلمات المفتاحية: كفاءة الإزالة ، كريستال فايوليت ، كرافيين اوكسايد ، ترايكوديرما ، احمر الكونغو ، الامتزاز.