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Decolorization of Methylene Blue and Crystal Violet by Some Filamentous Fungi

Ihsan Flayyih Hasan AI-Jawhari*

Department of Environment and Pollution, Marshes Researches Center, Thi – Qar University, AI- Nasiriya, Iraq
*Corresponding author: dr.ihsan_2012@yahoo.com

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Abstract Five strains of filamentous fungi were studied to ability a removal of Methylene Blue (MB) and Crystal Violet (CV). These fungi were *Rhizopus stolinifer, Aspergillus fumigates, Aspergillus niger, Fusarium solani* and *Penicillium funigulosum. F. solani* and *P.funigulosum* showed that decolorization activity was higher than that of remaining 3 fungi on solid medium containing Methylene blue, although MB did not appear to interfere with the mycelia growth of *A.fumigatus*, this fungus was not able to effectively decolorize MB. In the present study all fungi strains showed a poor ability to decolorize Crystal violet, However the ability of *A.fumigatus* to decolorization of CV reached to 0.009, this fungus was higher than other fungi. Although CV did not appear to interfere with the mycelia of *R.stolinifer* and *F.solani*, these fungi that not able to effectively decolorize CV. There was a significance differences recorded with decolorization. The results showed that the dry weight of *F.solani* was higher than other fungi, the dry weight of this fungus reached to 0.092 gm with MB, and in the same time the dry weight of *A.niger* reached to 0.52 gm with CV. There was no significance recorded in dry weights of fungi at the 7 days of incubation.

Keywords: *aquatic life, degradation, dyes, environment, efficiency, fungi*

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1. Introduction

Synthetic textile dyes are one of the most serious water and soil pollutants released into the environment. These pollutants are resistant to ordinary processes of decomposition and therefore persist in the environment for a long time. These dyes effect the life of living organisms in the ecosystem by damaging the health of humans, plants, animals and microorganisms. They also add to the continuously increasing load of environmental water and soil pollutants, which threatens the very existence of life on the earth. With the increasing human population and their needs for such dyes, the variety and quantity of these synthetic chemicals relased into the environment, are increasing day by day. This problem has attracted the attention of many environmental scientists over the last two decades or so. Efforts are in progress to treat wastewaters from textile industries in an economic, biofriendly and environmentally safe manner. Industries generating dye – containing wastewater include pigments manufacture, textile, printing and dyeing [1]. It is estimated that 280.000 tons of textile dyes are discharged in textile industrial effluents every year worldwide [2]. Synthetic textile dyes cause many problems in the environment. They may significantly effect on phosynthetic activity in aquatic life because of reduced light penetration and may be also toxic to some aquatic life due to the presence of aromatics, metals, chloride etc. [3]. Methylene blue is rodex aniline dye and it not regardad as acutely toxic, but it can have various harmful effects [4]. Crystal violet has been classified as a recalcitrant molecule, thereby indicating that is poorly metabolized by microbes and consequently is long lived in avariety of environment [5]. Different physical, chemical and biological methods are used for the treatment of wastewaters from textile industries. The physico chemical methods include adsorption, chemical oxidation, precipitation, coagulation, filtration, electrolysis and photodegradation. The major disadvantage of physicochemical methods has been largely due to the high cost, low efficiency, limited versatility, interference by other wastewater constituents and the handling of the waste[6]. Biodegradation of synthetic textile dyes using different microorganisms is apromising approach. It is now known that several microorganisms including fungi, bacteria, yeast and algae can decolorize and even completely minerlization many azo dyes under certain environmental conditions [7]. The present study is the first work in AI-Nasiriya city- South of Iraq, because no recorded studies by other researchers and the importance of large quantities of waters discharged from the textile industry without treatment to the environment and cause the serious environmental and health hazards. These effluents are being disposed off in water bodies and this water can be used for the agriculture purpose.The aim of this work is to determine the ability of some filamentous fungi to utilize heterocyclic (aromatic) MB and in triphenylmethane CV dyes and for growth thus degrading

in axenic fungi culture, because this method is eco-friendly and with economic low coast than other methods used.

2. Material and Methods

2.1. Organisms and Culture Conditions

A.niger, A.fumigatus, F.solani, P.funigulosum and *R.strolinifer* were used from Marshes Research Center, Thi- Qar University, Environment Laboratory, Iraq. These fungi isolated by Dr. AI- Jawhari from the upper surface of asediments in marshes of AI- Nasiriya city (South of Iraq). Stock cultures were maintained on the Potato Dextrose Agar (PDA) slant subcultured periodically and stored at 4°C.

2.2. Chemicals

The common names of the two dyes have been used for convenience. Methylene Blue (MB), Crystal Violet (CV) were from Merch (Germany). All other chemicals used in the present study produced by Himedia (India).

2.3. Decolorization of MB and CV Dyes in Solid Medium

A disc (5mm) of fungal mycelium was inoculated into the center of petri dishes (85mm) with the previously mentioned culture medium with agar. The medium is containing (2.5 mg /I) of each dye separately in triplicate. The plates were incubated at 25c^{o} for 14 days, after which the mycelia diameter (MD) and decolorization diameter (DD) were determined. The ability of the fungi to decolorize the dye was then expressed as the the decolorization index (DI), which was calculated using the following formula:

$$DI = DD / MD.$$

Each test was replicated 3 times.

2.4. Biomass Production

Three disc (5mm) of *A.niger, A.fumigatus, F.solani, P.funigulosum* and *R.strolinifer* were transferred to 250 mI Erlenmeyer flasks containing 100 mI of autoclaved culture medium contained in g / I : Yest extract 0.3, K_2HPO_4 0.75, KH_2PO_4 0.75, $MgSO_4$. $7H_2O$ 0.05, $CaCI_2$. $2H_2O$ 0.05 and $FeSO_4$. $7H_2O$ 0.02 at pH 7.0 supplemented with 0.5 mg / I of each dye separately, in triplicate. The flask were incubated at 25c^{o} for 7days and shaking manually every day. The biomass was determined by calculated the dry mass of mycelia. Mycelia were harvested from the cultivation liquid medium by filtration using whattman NO. 1 filter paper and dried at 65°C at 30 min and weighted (mg / 10 mI).

2.5. Statistical anaylsis

The present study Conducted an Anova (analsis of Variance) which was performed on all the treatments and done using the SPSS (version 10.0) package to determine whether or not, asignificance difference.

3. Results and Discussion

3.1. Decolorization of MB and CV Dyes in Solid Medium

The dyes evaluated in this study contain aromatic compounds that are degraded by filamentous fungi during secondary metabolisms. The growth and degradation efficiency of the test fungi as determined based on the their decolorization ability in solid medium are shown in Table 1, Figure 1, of the 5 fungi cultured on solid medium with MB.

Table 1. Decolorization of aromatic dyes on solid medium by filamentous Fungi

Name of fungi	Methylene blue			Crystal violet		
	MD*	DD	DI	MD	DD	DI
Rhizopus stolinifer	70	28	0.40	85	-	0.0
Aspergillus fumigates	80	-	0.0	11	0.1	0.009
A.niger	78	34	0.43	74	0.4	0.005
Fusarium solani	77	62	0.80	32	-	0.0
Penicillium funigulosum	50	28	0.56	60	0.2	0.003

* : (mm), (-): Not able to decolorize dys, MD : Mycelial diameter, DD:Decolorization diameter, DI: Decolorization index = DD / MD. The mycelia diameter and decolorization diameter were measured (mm, n = 3) after 14days of incubation

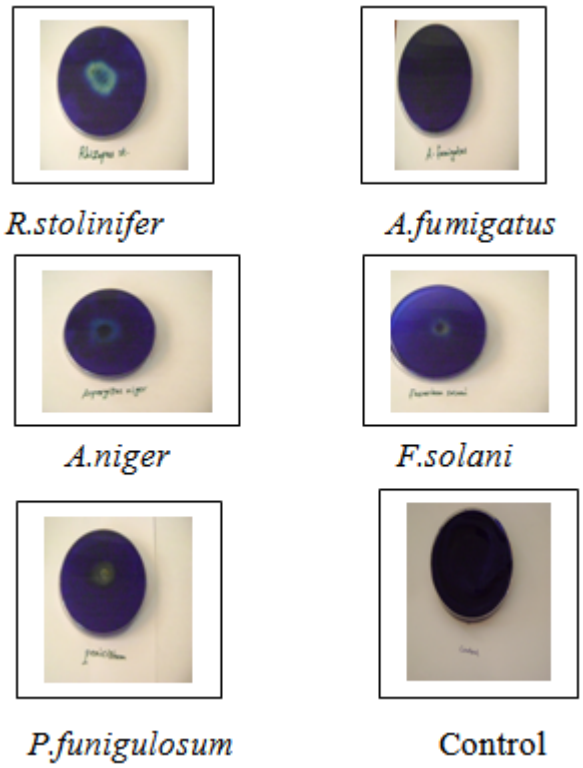

Figure 1. Decolrization of MB by filamentous fungi on solid medium

F.solani and *P.funigulosum* showed that decolorization activity was higher than that of remaining 3 fungi, these ability due to that these fungi have unique systems enzymes for breaking complex organic structures into simple fragments, however the mycelia of the *F.solani* was higher than *P.funigulosum* on solid medium contain MB. Conversely, although MB did not appear to interfere with the mycelia growth of *A.fumigatus*, this fungus was not able to effectively decolorize MB. In addition, *A.niger*

and *R.stolinifer* showed good mycelia growth on solid medium contained MB, but the efficiency of decolorization was low. The value of decolorization of MB on solid medium by the selected fungi was not considerably higher. The same results were obtained by [8], in this study show that when the white rot fungi *Cariolus versicolor* was good mycelia growth on solid media contained MB, but the efficiency of decolorization was very low, and in the same time the decolorization index with this fungus reached to 0.11. [9] reported that when the white rot fungus, *Irpex lacteus*, was grown on different media containing MB it did not show any considerable decolorization. In addition, the results of a study conducted by [10] also showed that the ability of *Pleurotus ostratus* to decolorize MB also increased,so the removal % increased for awide range of concentrations(25-700 mg/L)MB, and in the same time [10] refer that this result due to may attributed to the increasing in production of ligninolytic enzymes as the concentration of MB increased due to their stress on the mycelia cells of *P.ostratus*. However [11] found that *Lentinula edodes* decolorized media that contain 200ppm MB by 60%. Crystal violet belongs to tryphenylmethane aromatic dye group, and all of the fungi evaluated in present study had a poor ability to decolorize CV. However the growth of *A.fumigatus* was lower than other fungi, the growth of this fungus reached to 11mm, but the decolorization index of CV was higher than other fungi and reached to 0.009. Conversely, although CV did not appear to interfere with the mycelia of *R.stolinifer*, this fungus was not able to effectively decolorize CV, Table 1, and there was asiginificance recorded with decolorization. These results due to may be the toxicity of the dye to the growing microbial cells and that in the same time Crystal violet was poorly metabolized by microbes and consequently is long lived in avariety of environment [5]. This results in present study were similarly with the result of [12] have indicated toxicity of a closely related dye, Malachite green to *Kocuria rosea* MTCC 1532 at higher dye concentration, but in the present study, the results were not agreed with the results obtained by [13], In this study showen that *Alternaria solani* is quite tolerante to Crystal violet and decolorize and degrade relatively higher concentrations of the dye. And in the same time the present study was similar with the results obtained by [8], in this study showen that all the 10 fungi evaluated were grow slowly on solid media that contain Malachite green and poor ability to decolorize these dye.

3.2. Biomass Production

Growth study revealed that biomass and dye removal are directly proportional, which may be attributed to the fact, the increase of biomass gave more surface area for sorption of the dye molecules available, and may be due to the shaken of flask, this result was agreement with the results of [14], In this study showen that the most effective fungus in shaken flask experiments was *Bjerkandera adusta*, which was able to decolorize the dye from black-blue to a yellow color in less than 10 days. Table 2 explain that the dry weight of *F.solani* was higher than other fungi, the dry weight of this fungus reached to 0.92gm with MB dye, this extraordinary absorption value may have been due to a reaction of MB with enzymes secreted by the fungal mycelia [8]. And in the same time the dry weight of *A.niger* reached to 0.52gm with CV. The statistical methods obtained that there was no significance in dry weights of fungi at 7days incubation.

Table 2. Mycelial dry weight of fungal strains in liquid medium containing Methylene blue and Crystal violet.

Fungi	MB	CV
R.stolinifer	0.50*	0.23
A.fumigatus	0.12	0.16
A.niger	0.62	0.52
F.solani	0.92	0.28
P.funigulosum	0.60	0.48

* : Mean of triplicate, Dry weight calculated with (gm).

The same results were obtained by [15], in this study shown that the dry weight reached to 0.49gm with *Penicillium citrinum* in liquid media containing CV and the low dry weight reached to 0.22gm with *Mucor racemosus* and *Trichoderma viride*. [16] refer that in liquid culture, rapid dye decolorization by the fungal strain was observed within 24h. It was mainly due to the high adsorption of the dye in the mycelium. In subsequent dyes, dye decolorization may be due to production of extracellular enzymes.

4. Conclusions

The study concluded that, these fungal strains on their own can offer acoast effective, easily applicable and an environmentally sound solution to dye effluents. Rehabilitation of MB and CV dyes contaminated rivers waters by the culture of these fungi were promising as it can reduce the dyes pollution.

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