Testing the Performance of Small Scale Bioremediation Unit Designed for Bioremoval / Enzymatic Biodegradation of Textile Azo Dyes Residues

Wafaa M. Abd El-Rahim and Hassan Moawad

Agriculture Microbiology Department, Agriculture Research Division, National Research Centre,
32 Thrir St. Dokki, Cairo, Egypt 12622
Tel/Fax;+202-37601036
wafaa10m@hotmail.com

Abstract: In a series of our publications concerned with decolorization of textile azo dyes, *Aspergillus niger* strain 20 was identified as efficient bioremediating agent at bench scale level. In this work the evaluation of this bioremediation technology was taken one step forward to upscale the process. For this purpose 20 liter bioreactor (bioremediation unit) was designed specifically to bio-remediate the dye residues in synthetic dye solutions and/or in industrial textile effluents. The extensive work done in this bioremediation unit revealed that this technology at small scale level was successful to remove close to 89 % of the dye within 24 hours. The removal of dyes from synthetic effluent was tested using two approaches. The co-supplementation of the dye with fungal inocula in the bioreactor approach was better than using of fungal biomass build up prior to dye amendment approach. The *A. niger* biomass was capable of bioremoval appreciable amounts of the dye in rather short time. In general, the decolorization capacity of the studied dyes ranged between 31and 90% after one day of incubation. At the end of the experiments the chemical oxygen demand (COD) measurements were determined to assess the removal of dye from the simulated effluents. The results indicate that the fungal strains reduced the COD value of simulated dyeing effluents. The fungal biomass accumulation in the media supplemented with brown, violet and green direct dyes had different trends. The wastewater containing mixture of dyes from a textile dye-producing company and dyehouse were partly decolorized and the COD in the dye containing effluents was reduced by *A. niger* fungal treatment. The induction of laccase, tyrosinase and lignin peroxidase (LiP) enzymes was observed during decolorization. The activities of the three enzymes increased with different degrees with applied the co-supplementation of the dye with fungal inocula in the bioreactor approach. The study shows that the biodegradetive enzymes associated with the removal of certain dyes from single dye solution is not similar to those of their performance in dye mixture and raw wastewater containing mixture of dyes. [New York Science Journal. 2010;3(3):77-92]. (ISSN: 1554-0200)

Key words: Azo dye, decolorization, textile dyes wastewaters, laboratory scale bioremediation unit, enzyme activities.

1. Introduction

The treatment of effluents from dye-based industries can introduce additional water resource for agricultural irrigation purposes, moreover, the maintaining of natural water resources such water channels from pollution by these residues help to preserve the agricultural environment from these hazardous pollutions. In previous studies, *A. niger*, isolated from dye-contaminated wastewaters, decolorize was capable of several direct textile dyes, a toxic azo dyes, under different conditions [Wafaa et al. 2008 a, b]. Recently, many studies have shown that fungi are able to degrade dyes by extracellular, nonspecific and non-stereo selective enzyme systems [Yesilads, 1995; Reddy, 1995; Bezalel et al. 1997]. Until recently researches have focused on either the decolorization of various dyes by a single fungal strain, or the decolorization of a single dye by various fungal strains [Fu and Viraraghavan, 2001; Kapdan et al. 2000; Kapden et al. 2000; Robinsonet al. 2001; Arora et al. 2002]. However, enzyme-based decolorization is an efficient approach and of current interest in industrial effluent treatment. Laccases produced from different cultures were examined by researchers to remediate various xenobiotic compounds including synthetic dyes [Elias et al. 2000 and Torres et al. 2003]. The laccase immobilization procedures and its applications in remediation of industrial pollutants have been reported [Durán et al. 2002]. Laccase-mediated azo dye decolorization has been described using crude and purified enzyme forms from fungi; where laccases required redox mediators [Zille et al. 2003 and Baldrian, 2004]. It was reported that few enzymes can cause cleavage of chromophores, among them oxidative enzymes such as peroxidases and laccases are suitable for enzymatic dye degradation/decolorization [Durán, and Esposito, 2000]. Compared to peroxidases, laccases might be more promising as they do not depend on any co-factors except molecular oxygen [Goszczynski et al. 1994]. These enzymes have been extensively explored for treatment of effluents; containing of phenols, pesticides, and nitro-aromatics in wastewaters either in free or immobilized state [Gianfreda and Rao, 2004].

The aim of this work is to study the bio-remediation of textile dyes either from synthetic single dye solution or mixture of dyes and/or the factory effluent. The values of dye color removal and the changes in enzyme involved in dye biodegradation were recorded as indicators for textile dye bioremediation using *A. niger* in small scale bio-reactor developed for this particular application.

2. Materials and methods

2.1. Dyes and dye removal measurements

Three commonly used textile dyes namely Direct violet, Direct brown, Direct green were tested. These dyes were obtained as pure chemicals from the larges dye manufactory in Egypt (Ixmadye Dyestuffs and Chemicals Co.) and Textile Industries Division at the National Research Center, Egypt. Dye decolorizing activity was expressed in terms of percentage decolorization and was determined by measuring the absorbance at 542, 372, 333 nm for Direct violet (DV), Direct brown (DB) and Direct green (DG) dyes respectively, using spectrophotometer LBK model 4054 (against the original color of the medium). Decolorization activity (%) was calculated according to the formula:

$$\text{Decolorization activity (\%)} = \frac{[(\text{Initial absorbance}) - (\text{observed absorbance})]}{\text{Initial absorbance}} \times 100$$

2.2. Textile industry wastewater characterization

The textile industry wastewater contains various textile dyes: (reactive, sulfur, direct) and NaCl salt. The characteristics of the textile industry wastewaters vary widely. Some properties of the wastewaters used in this experiment are shown in Table (1).

Raw wastewater containing mixture of dyes from a textile dye-producing company and dyehouse were partly decolorized by *A. niger* fungal strain. The decolorization rates were followed throughout five days at room temperature using ten-fold diluted dye-containing effluent using two days pre-grown mycelium. Several trips were done to the textile industrial plants for collecting samples from textile waste effluents. Three samples were obtained from industrial plants (Shams Company and El Mukatem Dyehouse) located near Cairo. Specific absorption spectrum of effluents at different wavelengths was assayed using spectrophotometer LBK model 4054.

2.3. Inoculum Preparation

Efficient fungal strain of *A. niger* previously isolated from dyes damping site was maintained on potatoes dextrose agar slants medium in the refrigerator, and sub-cultured every 4 months. The composition of the culture medium suitable for inoculum preparation and biomass cultivation was: 10 g/l sucrose, 0.5 g/l yeast extract, 0.5 g/l KH_2PO_4, 0.2 g/l $MgSO_4$ $7H_2O$, 0.1 g/l NaCl [Wafaa et al., 2003b]. The procedure for inoculum preparation was as follows: a fresh slant was inoculated using sterile loop full of fungi, and incubated at 28 °C for 72 hrs, This slant was used to inoculate 300 ml of sterile media in 500 ml round flask. The flask was then incubated in incubator shaker operated at 150 rpm at 28°C for 72 hrs. These inoculated flasks were used to inoculate the Laboratory scale bioremediation unit. Laboratory scale bioremediation unit and media were sterilized by autoclaving at 121 °C for 20 minutes.

2.4. Fungal dye removal in aqueous medium containing single dye in lab scale bioreactor

Design and details of the bioreactor developed in our lab for the dye bioremoval is illustrated in Fig 2. The major textile dyes used in the industry are Azo dyes (Direct dyes). Strain of fungi *A. niger* 20 was used. The simulation of dye wastewater was performed in distilled water containing a specific dye. The initial concentrations of the dyes in the media were 600 ppm. Fungal strain was individually cultured in a liquid mineral basal medium as mentioned above. Dye was added to the bioreactor to reach the concentrations of 600 mg/l. The dye addition was either performed after the fungal biomass reached the maximum production in the bioreactor (AG) or the dye was supplemented from the start (BG) in the media inoculated with the fungi. Samples from bioreactor were withdrawn at intervals of 24, 48 and 72 h to determine biomass, remaining sugar and growth media pH. The efficiency of dye bioremoval was tested with every optimization experiment after 2, 4, 6, 24, 48 and 72 hours after addition the dye. The sampling was extended beyond this time to allow growth and multiplication of fungi if necessary. At the end of the experiment the mycelium was collected by filtration and dried at 105^{o}C to determine the dry weight.

2.5. Determination of COD

The COD was measured using a Hatc spectrophotometer test kit (HACH, CO) after digesting of the samples using the kit in the Hatc digester at 150^{o}C for 2h.

2.6. Enzyme analysis

Activities of lignin peroxidase (LiP), laccase and tyrosinase were assayed spectrophotometrically in culture supernatant. LiP activity was determined by monitoring the

formation of propanaldehyde at 300 nm (Shanmugam et al. 1999). Laccase activity was determined by measuring oxidation of ABTS at 420 nm (Hatvani and Mecs, 2001). Tyrosinase activity was determined in a reaction mixture of 2 ml; containing 0.01% catechol in 0.1M phosphate buffer (pH 7.4) by measuring liberated catechol quinone at 410 nm (Zhang and Flurkey, 1997). All enzyme assays were carried out at room temperature and reference blanks contained all components except the enzyme were included in the tests.

Table 1. Some analysis of textile industry wastewater collected from Shams Company and El-Mukatem Dyehouses effluent wastes.

Area	Company/ dyehouse name	Effluent (Efflu) number	Parameters							
			Specific absorption spectrum	K (ppm)	Na (ppm)	Dye type	Color	Total COD	pH	EC (dS/m)
Cairo	El-Mukatem Dyehouse	1	590	50	5800	Reactive	Dark blue	1541	9.90	36.3
	Shams Company	2	555	270	9700	Sulfur	Dark blue	1500	12.4	95.5
	El-Mukatem Dyehouse	3	695	60	3700	Direct	Dark violet	1500	7.85	21.4

3. Results and Discussion

3.1. Bio-removal of three textile dyes and their mixture from synthetic aqueous media

A laboratory scale bioremediation unit was designed (Fig. 1) built and tested for the bioremoval of three direct textile dyes and dyes mixture (simulated effluent) were tested. Ten experiments were carried out to assess the efficiency of bioremediation unit using *A. niger* fungal strain. The commonly used direct textile dyes (brown, violet, green) and their mixture were included in the study. The induction of laccase, tryosinase and LiP enzymes was observed during decolorization. The fungal biomass accumulation in the media supplemented with dyes was investigated. At the end of the experiments the chemical oxygen demand (COD) measurements were determined to assess the removal of dye from the simulated effluents. The results obtained are shown in Figures (2-15).

The sucrose yeast or sucrose peptone media (18 liters) was put into the bio-reactor unit at the start. *A. niger* (2g dry weight of mycelium) previously grown on platform shaker was feed to the bio-reactor unit. The removal of dyes from synthetic effluent was tested using two approaches. The first one was based on the use of fungal biomass build up prior to dye amendment. The second one was based on the co-supplementation of fungal biomass and the dye (600 ppm concentration) at the start of the experiment. In the first approach, the dye solution was added to the bio-reactor three days after fungal inoculation to bring the concentration of dyes in the media to 600 mg/l. Samples were collected from the bioremediation unit for reaction follow up. Samples were withdrawn at different intervals after dye amendment until 72 hrs to determine the dye bioremoval and growth media pH changes.

When used the first approach which was based on the use of fungal biomass short time harvested (AG), the results in Figure 2 (b) show that *A. niger* strain gave good efficiency in the removal of Direct green dye within 72 hours after incubation, where the percentage of decolorization reached 83.56 %. The same fungal strain gave high percentages of decolorization (84%) of direct violet dye after 24 hrs. The same trend was observed with the direct brown dye. This indicates that *A. niger* biomass was capable of bioremoval appreciable amounts of the dye within 24 hrs. The results of using the second approach (BG), where the integration of fungal biomass amendment and the dye took place from the start, Figures 2 (a) show that the strain of *A. niger* recorded high removing efficiency with brown and violet dyes after 72 hours from dyes addition being 63.5, 84.0, with direct brown, violet dyes respectively after 24 hours of incubation. However, these percentages increased reaching to 67.1 and 89.2% after 72 hrs incubation for both dyes respectively (Fig. 2 a).

As regards the performance the *A. niger* strain with dyes mixture, the results in Figure 2 (a&b) show that the fungi was able to remove 39 to 58 % of dyes mixture color after 72 hours incubation using first approach. On the other hand, with mixture of direct green, violet and brown dyes was used second approach, the percentages of removing decreased and being 81.9 to 96.7 through 72 hr incubation. The removal efficiency was dependent on the type of approach was used (Fig. 2 a, b).

The first approach proved to be better than the second one as regards the dye bioremoval. This is much more practical for bioremediation of textile dyes. The growth and harvesting of fungal biomass in appropriate amounts could be in the bioremediation unit prior dyes application. Then the harvested biomass transferred to separate

containers not necessary without need for aseptic conditions.

Figure 3 (a, b) illustrates that the pH is continuously decreased throughout the bioremediation process particularly for dye mixture and direct brown dye (Fig 3 b). This may be attributed to the production of organic acid during the process. This can give evidence that the process is not just related to dye biosorbtion but most likely to the degradation of dye absorbed inside the fungal biomass by intercellular enzyme. The decrease of pH at the end of these experiments may be referred to the excretion of the organic acid by the fungus itself [Abdel-Aal et al. 2001 and Naima et al. 2007]. Dirk et al. [2003] and [Ana et al. 27] observed that the fungus produces organic acids salts such as malonate, oxalate during the initial growth period, which later can be decomposed by specific enzymes such as manganese peroxidase enzyme.

The sugar utilization from the growth media amended with different direct dyes and dyes mixture increased until the fifth day of incubation leaving only between 0.23 to 2.09 mg sugar/dL. The sugar was almost exhausted within 72 hrs after dye addition in both approaches (Fig. 4 a, b). This could be attributed to the use of the dye by fungal strain as easy source of carbon after the main carbon source in the medium was consumed. Mazmanci et al. [2002] studied the decolorization of methylene blue in cultures of the white rot fungus, *Coriolus versicolor and* they found that the maximum decolorization activity at secondary metabolic phase. Decolourization was found higher in media where the C/N ratio was adjusted to 2:1.

Figure (5) illustrates that the accumulation of fungal biomass dry weight on media amended with direct dyes and the dyes mixture after five days of incubation time. The results indicate that the accumulation of biomass with brown, violet and green direct dyes had different trends with using (AG) approach. The highest dry biomass was found with direct green, violet and brown dye after five days of incubation being 42.3, 27.7 and 20.0 g/18 liter, respectively. The results show that the biomass accumulation with tested dyes and their mixture ranged from 6.5 to 9.5 after five days of incubation. This indicates that the mixture of synthetic direct dyes was toxic to *A. niger*. The trend of the decreasing of biomass formation was observed with using the co-supplementation of dyes and their mixture with the fungal inocula in the growth media prior inculcation (BG approach).

The bioremoval of dyes with fungal strain was accompanied by COD decrease. The results indicate that the fungal strains reduce the COD value of simulated dyeing effluent between 384 to 2000 mg/l with all direct dyes and their mixture (Figure 6). Sampa and Dutta, [2004] found that the percentage-removal of Methylene Blue increased from 50 to 58% as the air flow rate increased from 0 to 6.13 l/min. Percentage of COD-removal increased from 20 to 24%. Removal of COD was 94% at static condition. Isik and Sponz [2004 a,b] reported 57% removal of COD at anaerobic condition while 87% and 78% at aerobic and microaerophilic conditions, respectively. The significant reduction in COD showed mineralization of DR5B by *Comamonas* sp. UVS.

The data show that the removed dyes from the mixture were very efficient after 72 hrs. These results indicate that the dyes mixture (simulated effluent) needs prolonged incubation time to be removed by fungal strain. The decreasing of pH values was marked from the beginning of the incubation.

Removal of textile dyes from industrial effluent was performed previously by several methods including alum sludge [Chu, 2001], yeast biomass [Meehan et al. 2000], anaerobic bacteria [Razo et al. 1997], bacterial biomass [Sun-Young et al. 2002], fungi [Chao and Lee 1994, and Juliana and Thuy 2002)] and/or ozonation and electroflocculation [Ciardelli and Ranieri 2001].

In this work the use of *A. niger* to remove direct dyes and textile dyes mixture commonly used in textile industry revealed that this fungal strain was capable to remove dyes in comparatively short time from the media. This indicates that the process involves fast interaction between the fungal mycelium and the dye. This interaction could be based on a biosorption of dyes on the intact fungal biomass. This is in harmony with [Juliana and Thuy, 2002] who studied the decolorization of eight textile dyes by the white rot fungus, *Trametes versicolor*. They found that with the decolorization of the textile dyes by *T. versicolor*, the toxicity of the solution remains unchanged, decreased or even increased. The latter seems to occur with dyes that may be difficult to decolorize or those, which require long decolorization times. These findings also underline the importance of monitoring toxicity changes in any decolorization process as toxicity may increase. The results are in agreement with [Sani et al. 1998] who found that the rate of decolorization of several dyes was dependent on biomass volume (Red HE-8B, Malachite Green, Navy Blue HE-2R, Magenta, Crystal Violet).

So far the fungi were found to be the most efficient bioagent in the bioremedation of textile dyes. The results revealed different patterns of decolorization of growth media amended with different tested dyes. Variations in decolorization intensity (dye removal) differed according to concentration and the dye type. In general, the decolorization capacity of the studied dyes ranged between 31and 90% after one day of incubation. These results are in harmony with [Sani et al. 1998] who demonstrated decolorization of several dyes

(Red HE-8B, Malachite Green, Navy Blue HE-2R, Magenta, Crystal Violet) and an industrial effluent with growing cells of *P. chrysosporium*. All the dyes and the industrial effluent were decolorized to some extent with varying percentages of decolorization (10-66%).

In this study the mixture of dyes was investigated in two experiments, using the aforementioned two approaches. The bioremoval of dyes in the mixture increased with time. This may be due to the physical property of the dyes mixture or to the possible reaction of some reactive groups in dyes. Similar results were obtained by [Jiang and Bishop, 1994] who reported wide differences in dye removal. This study revealed that the fungal strain *A. niger* was capable to remove 50% of Direct tested dyes in 4 - 24 hours after addition of dyes to the fungal biomass.

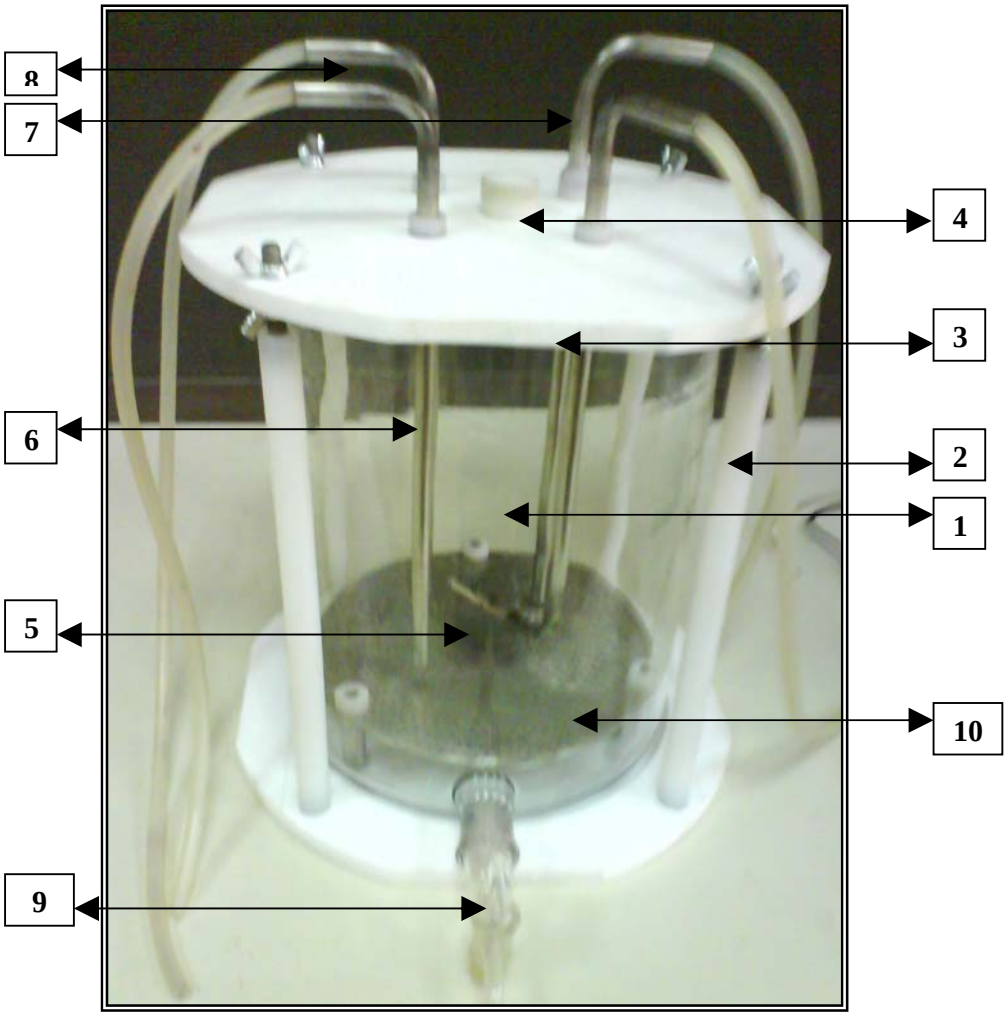


Figure 1. Horizontal overview of Laboratory scale bioremediation unit for textile dyes bioremoval. 1: Glass jar of 20 liters volume (working volume 18 liters): 2, Tephelon\teflon stand, 3: Tephelon\teflon head provided with 4 pores: 4: Pores with screw cap for feeding and inoculation, 5: Aeration tube provided with small pores to distribute the air in the bottom of the jar. (This tube attached with air filter to filtrate the air before passing to the unit and attached with the aeration pump), 6: Sampling pores with silicon rubber tube at different depths, 7: Sampling pores with silicon rubber tube at fixed depth, 8: Exhausting tube for gases exit, 9: Tap for output, 10, Mesh for separation the outputs than cells.

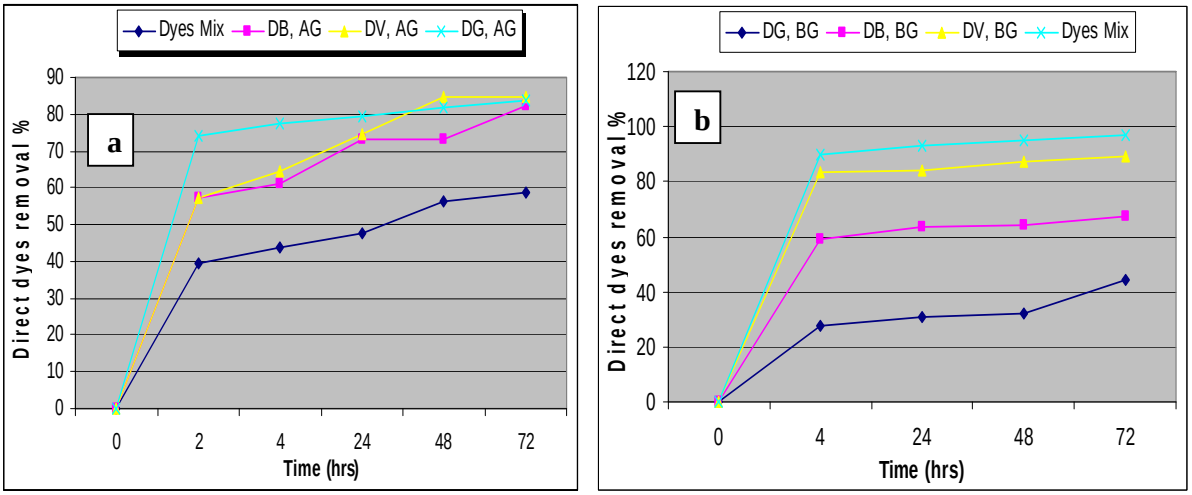
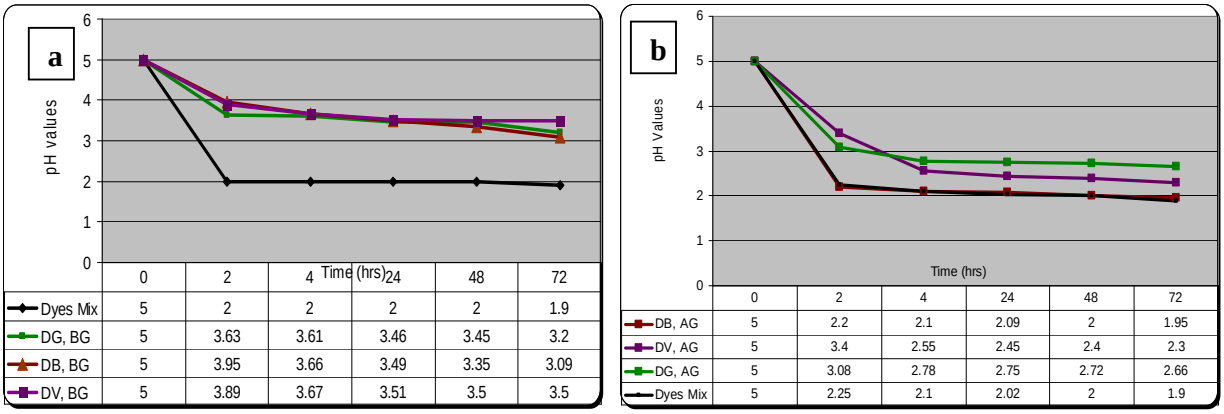


Figure 2. Bioremoval of different textile direct dyes and its mixture in the laboratory scale bioremediation unit using, a) BG approach, b) AG approach.



Time (hrs)	0	2	4	24	48	72
Dyes Mix	5	2	2	2	2	1.9
DG, BG	5	3.63	3.61	3.46	3.45	3.2
DB, BG	5	3.95	3.66	3.49	3.35	3.09
DV, BG	5	3.89	3.67	3.51	3.5	3.5

Time (hrs)	0	2	4	24	48	72
DB, AG	5	2.2	2.1	2.09	2	1.95
DV, AG	5	3.4	2.55	2.45	2.4	2.3
DG, AG	5	3.08	2.78	2.75	2.72	2.66
Dyes Mix	5	2.25	2.1	2.02	2	1.9

Figure 3. Media pH changes with time inculcation throughout bioremoval of several direct textile dyes using, a) BG approach, b) AG approach.

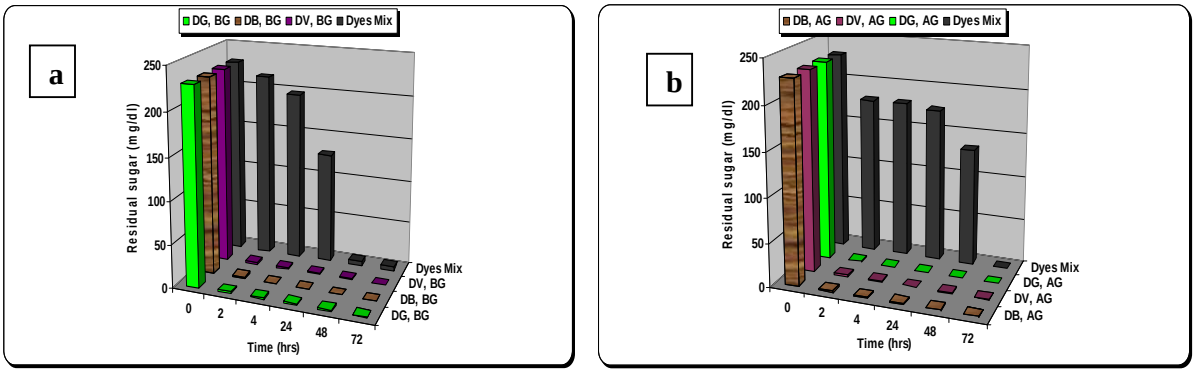


Figure 4. The remaining sugar through removal of several direct dyes by the *A. niger* after five days of incubation using, a) BG approach, b) AG approach.

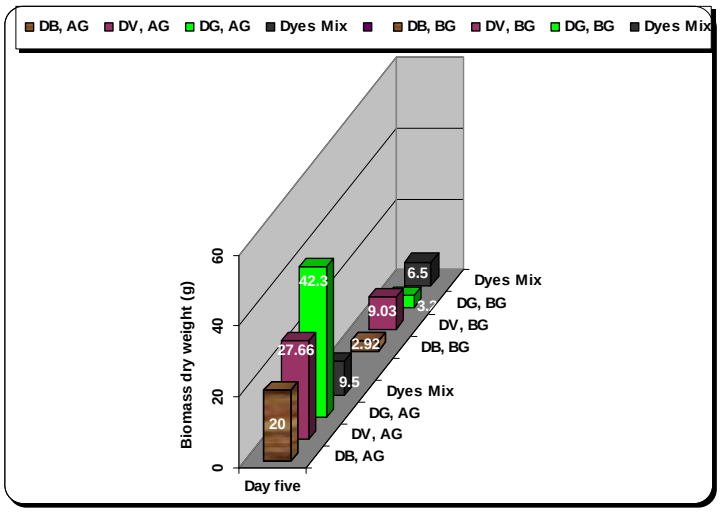


Figure 5. Accumulation of fungal biomass grown on different Direct dyes medium

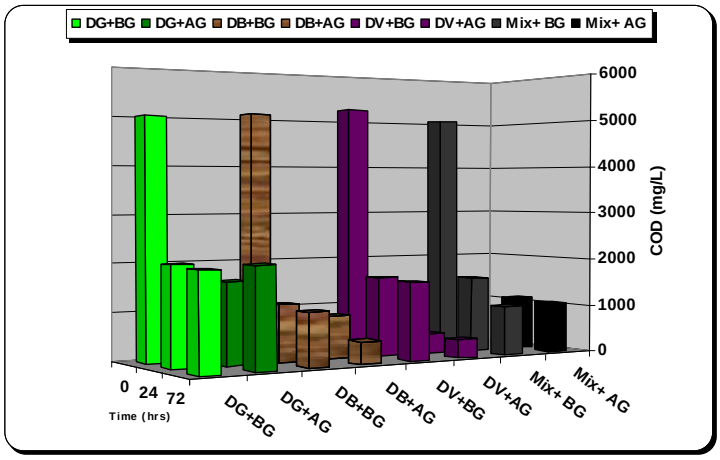


Figure 6. Reducing the COD value of azo Direct dyes and their mixture by fungal strain

3.2. Textile dye removal from industry effluents

The same fungus strain was used as bioremediation agent for decolorization of dye industry effluents. Raw wastewater containing mixture of dyes from a textile dye-producing company and dyehouse were partly decolorized by *A. niger* fungal strain. The decolorization rates were followed for days at room temperature using ten-fold diluted dye-containing effluent after mixing with two days pre-grown fungal biomass.

Effluent samples were analyzed for the chemical oxygen demand (COD), in addition to PH and EC. The analysis of wastewater from textile industrial plants is presented in Table 1. The effluents from textile and dyeing industries/dyehouse have high COD, visible color, pH. Senan and Abraham, [2004] reported that the effluents from textile dyeing industries had high values of COD, BOD, pH and contained metal ions. The pH of wastewater was quite high in three samples; from Shams Company and El Mukatem Dyehouse effluents. Two samples had pH values ranging between 9.90 -12.85 as shown in Tables 1. Another sample from Shams Company had pH of 7.85 as shown in Tables 1.

The electric conductivity (EC) values for three wastewater samples were as follows: El Mukatem Dyehouse EC values were 36.3, 21.4 dS/m for sample 1 and 3 respectively. Shams Company recorded 95.5 ds/m. These elevated values of pH and EC is likely due to the excessive chemicals used in the process of bleaching and sizing where the sodium chloride and sodium hydroxide are used in large amounts.

The decolorization rate in the late dye application to the pre-grown fungal biomass approach was different from that in the treatment were fungal inocula was allowed to grow since the beginning in contact with existing textile dyes in their growth media (Co-supplementation). After 24 h of incubation, the decolorization was more rapid,

which allowed the fungal inocula to grow since the beginning in contact with existing textile dyes in their growth media (Co-supplementation), than in the other treatment where the fungal strain was allowed to grow first and the biomass to accumulate in the media. With the Co-supplementation approach the results revealed that the fungus could not remove the color of effluent sample no. 2, whereas effluents 1 and 3 colors were removed by 39 and 61% respectively after 4 h of incubation. Effluents color was removed by 64, 70% from effluent 1 and effluent 3, respectively. Using the other approach which allowed the fungi to grow first and the biomass to accumulate in the media color was removed by 14, 42, 56% of effluent 1, 2 and effluent 3, respectively after 72 of incubation (Figure 7 a, b). It was speculated that the decolorization mechanism of the dyes effluent by *A. niger* differs in the above fore mentioned fungal application. This could be due to the complexity of the textile industry wastewater and its interaction with fungal biomass applied in the two application approaches. Chulhwan et al. [2007] investigated the biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*. They suggested that it is possible to decolorize a high concentration of commercial dyes, which would be a great advance in the treatment of dye containing wastewater. The above two methods may have a potential application for dye decolorization and for textile effluent treatment.

In Co-supplementation treatment, the decolorization of 63.1 % was achieved after one day whereas other treatment the decolorization of 44.4% was obtained on day one in effluent 3. The color removal by the basidiomycete fungus might be due to adsorption of the dyes to the mycelial surface and metabolic breakdown. Selvam1 et al. [2003] tested the microbial decolorization of azo dyes and dye industry effluent by *Fomes lividus*. Their results suggested that the batch mode treatment of *Fomes lividus* is one of the most efficient ways for color removal in dye industry effluents.

Throughout the experimental period a changes in pH values were recorded. Clear variations in pH changes during the growth and process of decolorization were observed. The final pH was maintained at 1.89-2.2 after five days of incubation (Figure 8 a & b), even though the initial pH was 5.0. The decrease in initial pH was observed with decolorization [Knapp et al. 1995].

The accumulation of fungal biomass on three collected textile wastewater effluents using the pre-grown fungal biomass and Co-supplementation approaches ranged between 3.2 - 70.0 g/ 18 liter working volume of the bioremediation unit (Figure 10). The maximum biomass dry weight was recorded with effluent 2 (Data not show). The results obtained in these experiments (Figure 11) show that the sugar was consumed almost totally after three days of incubation in both approaches.

Chemical oxygen demand values related the total concentration of organics in solution. The changes of COD in the dye containing effluents are showing the degree of mineralization as a function of dye removal. The original effluents solution at zero time gave reading of COD value of 1541 and 1500 mgl^{-1}. These values were reduced to 972, 994 mgl^{-1} and to 1097, 1031 of organic residue of effluent 1 and 2 respectively within 72 h in both applied approaches (BG and AG) under this study (see Figure 12). The wide variation of COD value (27- 37%) could be attributed to recalcitrant nature of certain dye effluents. It is reported that the textile dye effluents are more resistant to oxidation and degradation. These results are in agreement with those obtained by [He Fang et al. 2004] who found that the microorganisms continued to consume the obtained small organics until near-complete removal of COD value of culture. Only 60–70% of COD and 50-60% of color were removed prior to any enrichment procedure.

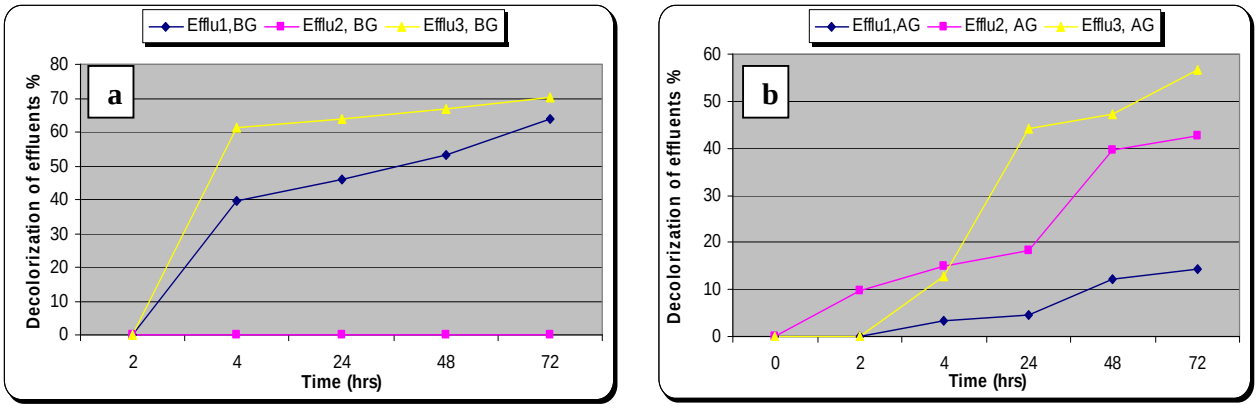


Figure 7 (a, b). Decolorization of textile industry wastewater by *A. niger* grown in mineral medium supplemented with three effluents depending on the time of additional effluent (a, BG, b, AG).

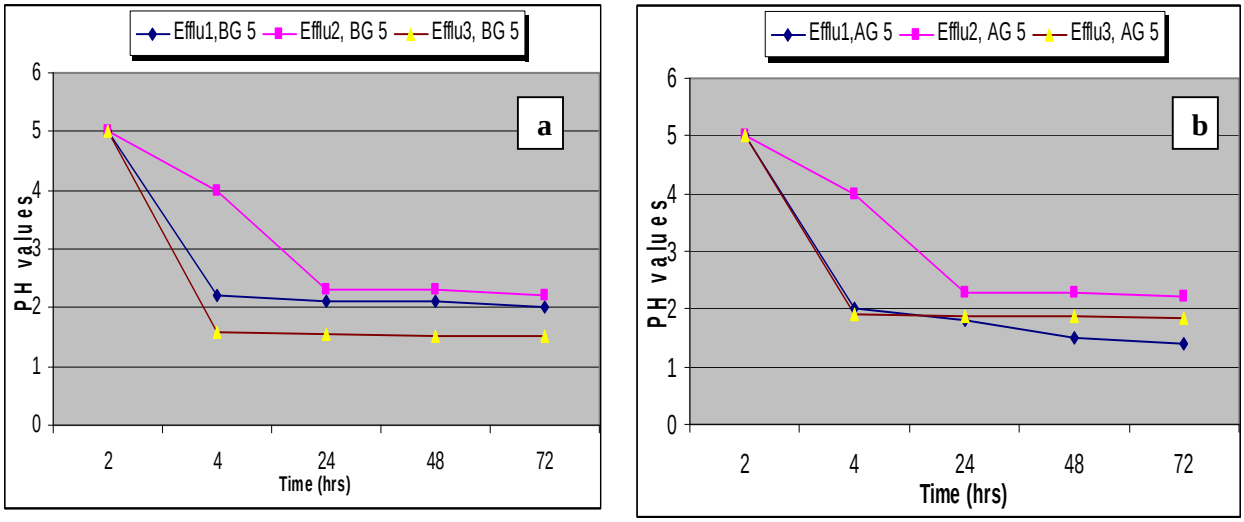


Figure 8 (a, b). Media pH changes with time inculcation throughout decolorization of several effluents using, a) BG approach, b) AG approach.

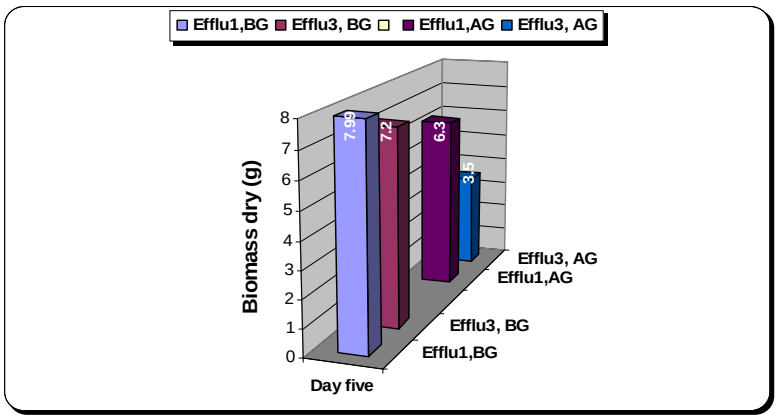


Figure 9. Fungal biomass accumulation grown on different effluents

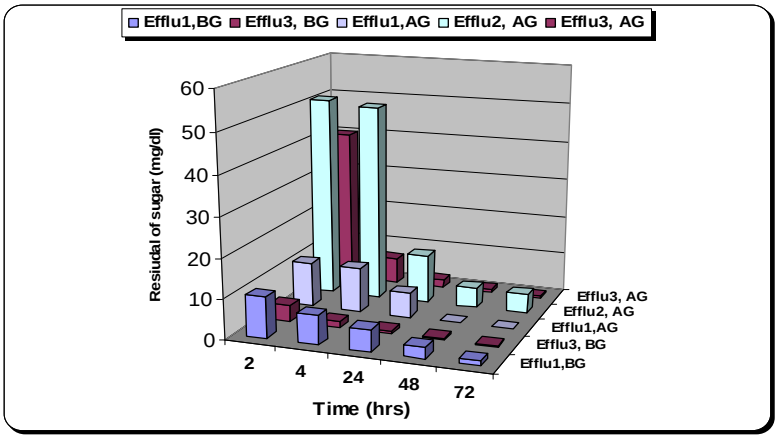


Figure 10. The remaining sugar through removal of several effluents by the *A. niger* after five days of incubation using, a) BG approach, b) AG approach.

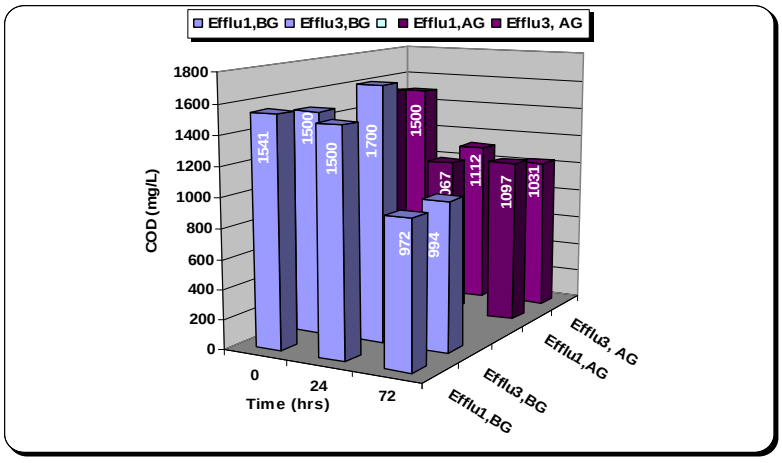


Figure 11. Reducing the COD value of different textile dye effluents by fungal strain

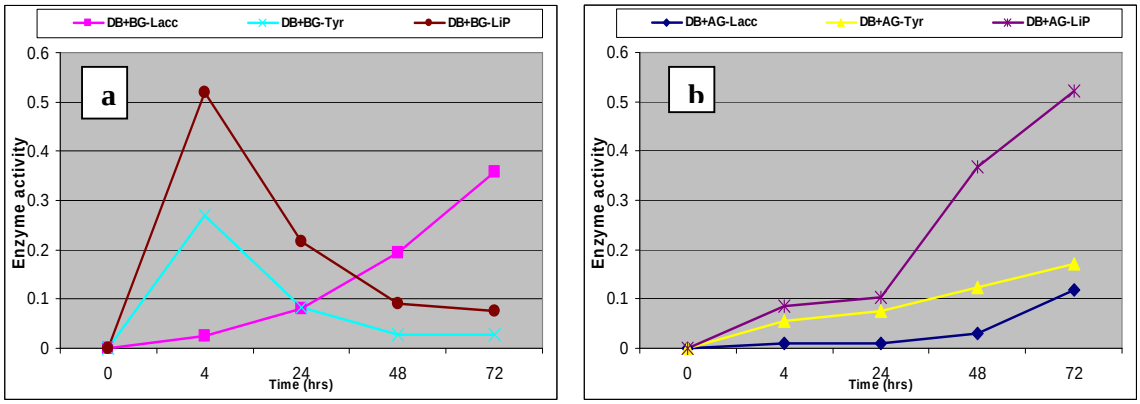
3.3. Enzyme activities associated with decolorization of synthetic textile dye solutions and factory effluents

The bio-activity of any organism is usually linked to complex phenomena mostly related to the activity of specific enzymes either constitutive or induced. Three enzymes namely; laccase (Lacc), tyrosinase (Try) and lignin peroxidase (LiP) are reported to be among the important enzymes affecting the biodegradation of heterocyclic compounds such as textile synthetic heterocyclic azo dyes. The fungal bioremoval of three textile dyes used in this study and their mixture were evaluated in the light of specific activity of the three aforementioned enzymes. The enzyme activity was evaluated by the maximum absorbance wavelength of the enzymatic reaction products for each enzyme present. The same two approaches were applied. In the first approach allowed the fungal inocula to grow (Co-supplementation) from the beginning with dyes, where as the second one allowed the fungi to grow first and the biomass to accumulate in the media, then the textile dyes were added to the media with accumulated fungal biomass after 48 hours (Late dye application).

The co-supplementation of direct brown (DB) dye with the fungal inocula in the growth media prior inculcation has resulted in the induction of the three enzymes as shown in Figure 12a. The activities of the three enzymes increased with different degrees. The lignin peroxidase (LiP) enzyme activity was always higher than the other two enzymes. The maximum activity of this enzyme was obtained after 24 hours following the incubation, and then the activity started to drop. Similar trends were observed with tyrosinase enzyme, however at much lower enzyme activity levels. The laccase enzyme activity showed gradual increases with incubation time tell the end of the experiment (72h of incubation). The enzyme activity in late DB dye application to pre-grown fungal biomass and DB dyes showed slow gradual increase of the activities of the three enzymes until 24 hours of mixture incubation with LiP being the highest and the laccase the lowest (Figure 12b). The increase in the activity of the three enzymes continued till the end of the experiment (72 hours incubation after the dye addition to the pre-grown fungal biomass).

The results presented in Figure 13a show that, of the direct violet dye (DV), gave threefold enhancement in LiP activity when co-supplementated with the fungal inocula in the growth media prior inculcation compared to the activity of tyrosinase enzyme. On other hand the results showed sharp decrease in laccase activity. With the late DV dye application to the pre-grown fungal biomass (Figure 13b) gradual increase of three enzyme activities were observed however the LiP enzyme was much high in all sampling times as comperad with the laccase and tyrosinase enzyme. The co-supplementation of direct green (DG) dye with the fungal inocula in the growth media prior inculcation, the activities of the LiP, Laccase and tyrosinase enzymes increased with different degrees (Figure 14a). The lignin peroxidase (LiP) enzyme activity was always higher than the other two enzymes. The highest activity of this enzyme was obtained after 4 hours following the incubation, and then the activity started to decrease with different degrees. However, the enzyme activity reported with the late DG dye application to the pre-grown fungal biomass showed gradual increase of the activities of the LiP and laccase enzymes until 72 hours of mixture incubation with LiP being the maximum and the laccase the lowest (Figure 14b). On the other hand the decreased activity of tyrosinase continued until the end of the experiment.

The industrial effluents often contain several dyes which create difficulty to evaluate enzyme performance. Therefore in this work enzyme activities were evaluated in synthetic three direct textile dyes mixture subjected to dye bioremoval. The activity of three enzymes (laccase, tyrosinase and lignin peroxidase) related to biodegradation of dyes in the industrial effluent was also assessed after the bio-treatment of the effluents. These results are presented in Figure 15 and show that the activity of lignin peroxidase and laccase was high than the tyrosinase enzyme activity. The maximum activity of lignin peroxidase and laccase were recorded at 24 h. These results were reported with late dye mixture application to the pre-grown fungal biomass approach. On the other hand the co-supplementation of dye mixture with the fungal inocula in the growth media prior incubation approach showed different trends of activity of three studied enzymes. This study shows that the biodegradetive enzymes associated with the removal of certain dyes from single dye solution is not similar to those of their performance in dye mixture. The degradation of simulated effluents was already tested by other methods, such as ozonation [Sarayu et al. 2007], flocculation [Kang et al., 2007] and photodegradation [Zainal et al. 2006]. Degradation of dye mixture Laccase-mediator systems (LMS) were already described in some works for degradation processes of single reactive textile dyes [Peralta-Zamora et al. 2003; Mechichi et al. 2006; Murugesan et al. 2007].



Figures 12 (a, b). The highest ligninolytic, laccase and tyrosinase enzymes activities (expressed in OD) obtained by *A. niger* grown in mineral medium supplemented with direct brown dye depending on the time of additional dye (BG& AG).

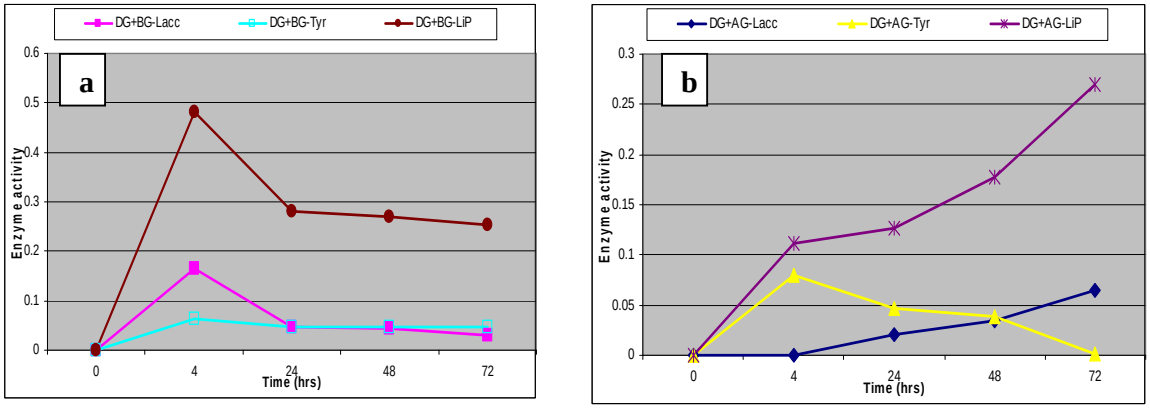


Figure 13 (a, b). Time course of lignin peroxidase, laccase and tyrosinase activities during decolorization of direct green dye.

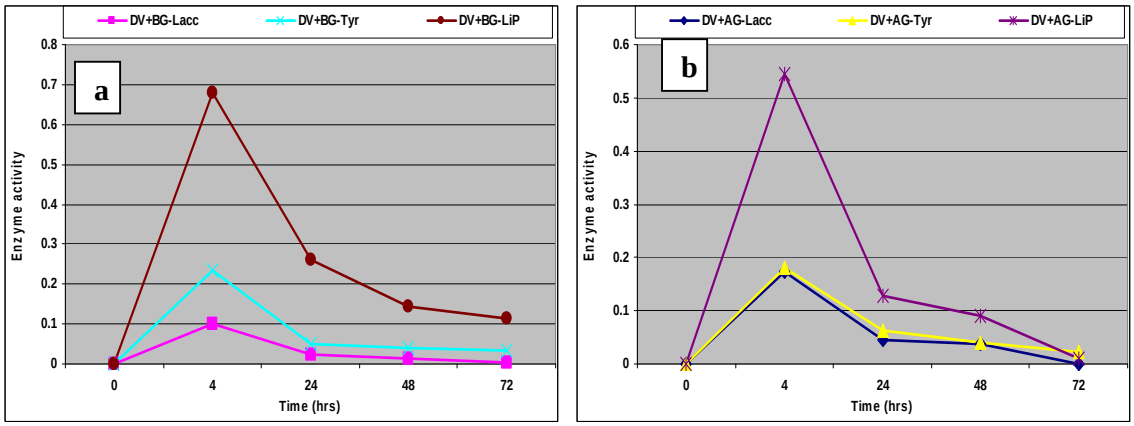


Figure 14 (a, b). Time course of lignin peroxidase, laccase and tyrosinase activities during decolorization of direct violet dye.

The activities of laccase, LiP and tyrosinase in textile dyes effluents treated with *A. niger* bio-removing agent were studied. In factory effluent (1 and 3) obtained from El Mukatem Dyehouse containing reactive and direct dyes treated with dye co-supplementation approach and effluent addition showed that the activity of LiP was the highest among all other enzyme throughout all incubation period. The laccase and tyrosinase were at much lower rates till the end of the experiment (Figure 16). On the other hand raw effluent wastewater from a textile dye-producing dyehouse was partly decolorized by the *A. niger* fungus (Figure 16). The fungus had higher lignin peroxidase (LiP), tyrosinase and laccase activities when grown with dye effluent than in control cultures. Maximal enzyme activity of three enzymes was achieved on 48 h at 28 ^{O}C (Figure 17) using the late El Mukatem Dyehouse effluent application to the pre-grown *A. niger* fungal biomass approach. The decolorization of dyes by microorganisms was reported to have relation with biotransformation enzymes like lignin peroxidase, laccase which take part in mineralizion of the synthetic dyes [Raghukumar et al. 1997]. The role of lignin peroxidase, laccase in the decolorization of dyes likely is different for each microorganism [Pointing and Vrijmoed, 2000]. Khandelbauer et al. [2004] reported the role of fungal peroxidase and laccases in the oxidation of sulfonated azo dyes. Azo dye degrading bacteria were also able to produce peroxidase enzyme [Cao et al. 1993]. The time course of LiP, laccase as well as tyrosinase was studied for *Comamonas* sp. UVS during decolorization of DR5B. Cell lysate of *Comamonas* sp. UVS has shown presence of LiP and laccase activity, during decolorization. In this study, the induction of LiP and laccase, tyrosinase enzyme was started after 4, and continued at 24, 48 and 72 h of addition of the dye. The decrease of enzymes activities within incubation time may be attributed to the decrease in dye concentration after the removal of the dyes which reduced the induction of the enzyme. The presence of very low concentration of dye within 48-72h could be the reason for decrease in the enzyme activities. Kalme et al. [2007] reported similar induction of extracellular LiP, intracellular laccase and tyrosinase. The activity of tyrosinase was low. No lignin peroxidase, laccase and tyrosinase activity were observed in culture supernatants.

4- Conclusion

The designed bioremoval unit proved to be efficient in the bioremoval of the three dyes. The biodegradation of the three dyes in the bioreactor is evidenced by the induction of the enzymes associated with biodegradation of heterocyclic compounds. The results of bioremoval of textile dyes either from single dye solution or mixture of dyes as well as the factory effluent as indicated by the values of dye color removal and changes in enzyme activities shows reliable approach for textile dye bio remediation using *A. niger* and the bio-reactor-unit developed in this study.

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Correspondence Author:
Wafaa M. Abd El-Rahim, Ph.D.
Agriculture Microbiology Department,
Agriculture Research Division
National Research Centre (NRC)
32 Thrir St. Dokki, Cairo, Egypt 12622
E-mail: wafaa10m@hotmail.com

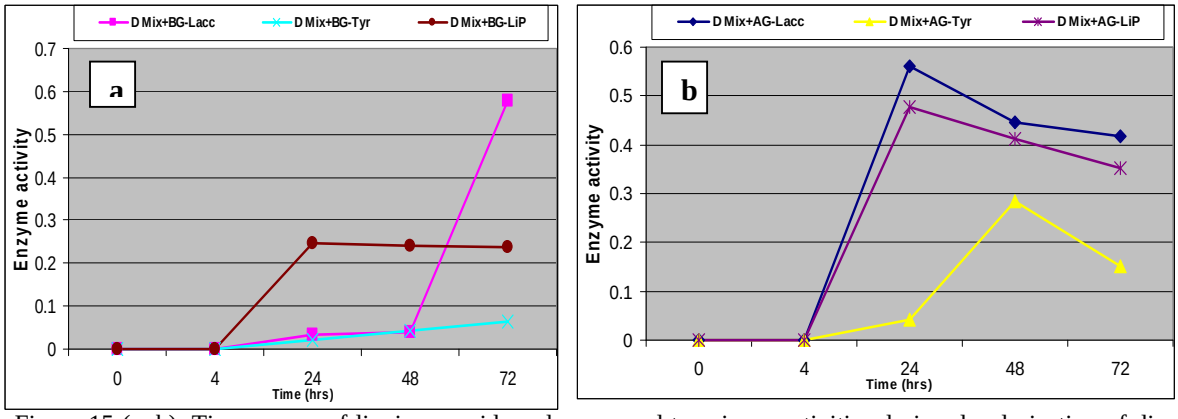

Figure 15 (a, b). Time course of lignin peroxidase, laccase and tyrosinase activities during decolorization of direct mixture dye.

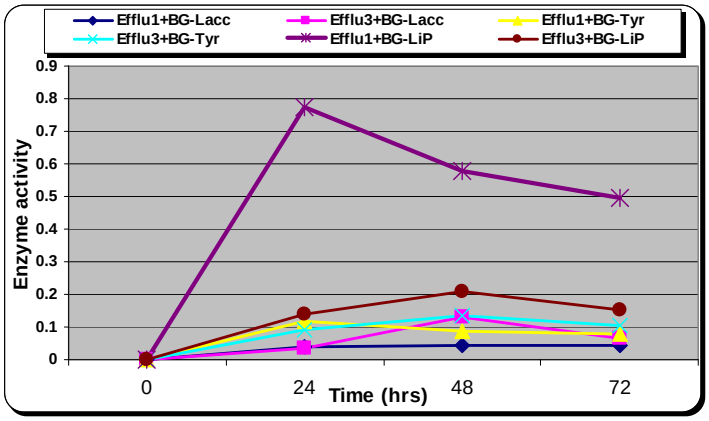

Figure 16. Peroxidase, laccase and tyrosinase activities of co-supplementation approach (BG) of *A. niger* grown in textile industry wastewater effluents from El Mukatem Dyehouse.

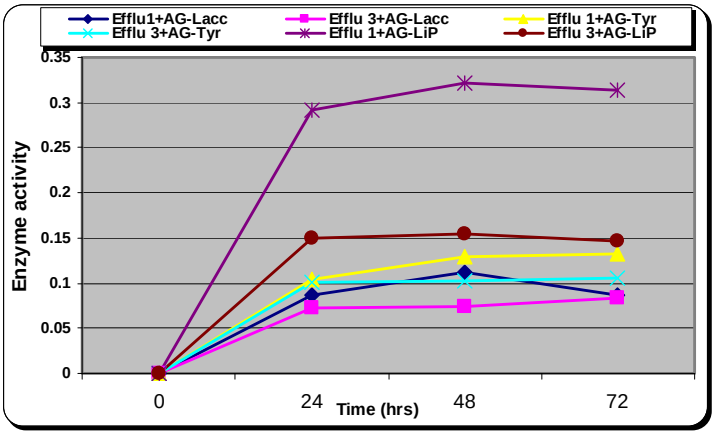

Figure 17. Peroxidase, laccase and tyrosinase activities of late effluent application to the pre-growth fungal biomass approach (AG) of *A. niger* grown in textile industry wastewater effluents from El Mukatem Dyehouse.

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