IN this study, fungal isolate D2-1 was isolated from contaminated soil collected from textile industry companies and showed high potentiality for decolorization of two different azo dyes. This isolate was identified as *Aspergillus niger* D2-1 using morphological and cultural characteristic as well ITS gene sequencing. Decolorization process under different optimized condition of azo dye concentration, pH, incubation periods, inoculum size and different carbon and nitrogen sources were investigated. The maximum decolorization efficiency against 100 ppm dye concentration of reactive yellow (4GL) and reactive red (4BL) dyes of 98.62 % and 92.42 %, respectively, were recorded for *Aspergillus niger* D2-1 at pH 9.0, in presence of 2 % glucose and 0.5% yeast extract as carbon and nitrogen sources respectively at room temperature after 7 days on shaking conditions. The decolorization percentage was confirmed by ultraviolet-visible (UV-Vis) spectrum analysis of untreated/treated reactive yellow (4GL) and reactive red (4BL) dyes, which showed complete disappearance of peaks at ~ 425 nm and at ~ 520 nm, indicates the degradation of dyes due to fungal activity. Treatment of textile wastewater effluent by *Aspergillus niger* D2-1 showed high decolorization percentage (59%) for effluent, also physico-chemical characteristics of textile effluent such as chemical oxygen demand (COD), total dissolved solids(TDS) and total suspended solids (TSS) were decreased from 756 mg/L, 1597 mg/L and 821 mg/L to 391 mg/L, 845 mg/L and 362 mg/L respectively. Moreover, Gas chromatography - mass spectroscopy (GC-MS) of textile effluent before and after treatment were recorded and confirmed the potentiality of *Aspergillus niger* D2-1 in dyes wastewater treatment.

**Keywords:** Decolorization, Azo dyes, Reactive yellow (4GL), Reactive red (4BL), Textile industry, *Aspergillus niger*, GC-MS.
The current study aims to explore and compare the decolorization efficiency of two different textile azo dyes by *Aspergillus* spp. isolated from the effluent of textile wastes dyeing industry. In supplement, the effect of many parameters such as pH, dye concentration, inoculum sizes and different nitrogen and carbon sources on dyes decolorization was assessed. The augmentation of dyes decolorization was estimated by the optimization of these microbial and chemical parameters (one parameter at a time course).

**Materials and Methods**

Azo dyes and samples collection

Wastewater and soil samples (30° 58’ 46.8876” N, 31° 10’ 59.5488” E and 30° 58’ 10.8156” N, 31° 10’ 5.0988” E respectively) used for fungal isolations were collected from Ghannam for textile Industry Company, El Mahala El Kubra, Gharbia Governorate, Egypt. Two different azo dyes, Reactive yellow 4GL (150%) and Reactive red 4BL (100%) were obtained as a gift from the same textile industry.

Fungal isolations

The collected samples were screened for isolation of the most potent dye-decolorizing fungi. One ml or one gram of either wastewater or soil respectively were transferred to 9 ml distilled water and serially diluted up to 10^(-7). About 0.1 ml of dilution was inoculated onto Sabouraud dextrose agar (SDA) plates supplemented with 100 mg/L chloramphenicol to suppress the bacterial growth. The plates were incubated at 28°C for 5-7 days. The fungal growth was checked for purity and inoculated in culture slant for preservation at 4°C further study. Twenty-one purified fungal isolates were growing on mineral salt media[26] supplemented with different concentrations of azo dye Reactive yellow 4GL (150%) and Reactive red 4BL (100%) as a sole carbon source to select most potent fungal isolates on the basis of their ability to tolerate high dyes concentration.

Molecular identification of azo dye-degrading fungi

Molecular identification was conducted based on amplification and sequencing of internal transcribed spacer (ITS) region. Genomic DNA was extracted using the protocol of Gene Jet Plant genomic DNA purification Kit (Thermo). The ITS region was amplified in polymerase chain reaction (PCR) using the genomic DNA.
as template and ITS primers of ITS1 (5’ - TCCGTAAGGTGAACCTGCGG -3’) and ITS4 (5’ - TCCTCCGCTTATTGATATGC-3’). The PCR mixture (50μL) contained Maxima Hot Start PCR Master Mix (Thermo), 0.5μM of each primer, and 1μL of extracted fungal genomic DNA. The PCR was performed in a DNA Engine Thermal Cycler by Sigma Scientific Services Company (Cairo, Egypt) with a hot starting performed at 94°C for 3 min, followed by 30 cycles of 94°C for 0.5 min, 55°C for 0.5 min, and 72°C for 1 min, followed by a final extension performed at 72°C for 10 min. The commercial sequencing was conducted using ABI 3730x1 DNA sequencer at GATC Company (Germany). The ITS sequence was compared against the GenBank database using the NCBI BLAST program. Sequences were then compared with ITS sequences in the GenBank database using BLASTN.

Detection λ max for each dye

The absorption maxima (λ max) of Reactive yellow 4GL and Reactive red 4BL dyes were determined by using a Spectrophotometer UV (Shimadzu 1800). Optical density of each dye solution in water was observed at different wavelength between visible regions (300-800 nm).

Measurement of azo dye decolorization % using most potent fungal isolate

About 2ml of growing mineral salt media (supplemented with 100 ppm of separately each dye and inoculated with fungal isolate) were withdrawn aseptically and centrifuged at 10,000rpm for 10 minutes. The clear supernatant was used for measuring the absorbance by UV-Vis spectrophotometer (Shimadzu 1800) at λmax for two dyes individually. The uninoculated mineral salt media supplemented with the same dye concentration was used as control. Distilled water was used as blank. All dyes were prepared in triplicate and compared with control. The percent decolorization was determined using the following formula according to[27].

\[ D = \frac{[\text{Dye (i)} - \text{dye (1)}]}{\text{dye (i)}} \times 100 \]

where, D, decolorization percentage %; dye (i), initial absorbance; dye (1), final absorbance.

Optimization the decolorizing ability of the most potent fungal isolate

The effects of different culture conditions such as incubation periods, dye concentrations, pH, inoculum size and different carbon and nitrogen sources on shaking conditions (150 rpm) of decolorization of Reactive yellow (4GL) and Reactive red (4BL) by most potent fungal isolate was investigated.

Effect of different dye concentrations on decolorization percent (%)

This experiment was carried out to investigate the effect of different dyes concentrations on dye decolorization process. The fungal isolate D2-1 was growing on mineral salt media supplemented with different concentrations of dye Reactive yellow (4GL), Reactive red (4BL) (viz. 50, 100, 150, 200, 250, 350, 500, and 650 ppm) as a sole carbon source. The decolorization percent (%) was recorded at maximum λ max for each dye after 6 days of incubation.

Effect of different incubation periods, incubation pH values and inoculum size on the decolorization percent (%)

In order to investigate the effect of different pH values on the decolorization process, fungal isolate D2-1 was inoculated in mineral salt media adjusted at different pH values (viz. 6, 7, 8, 9, 10 and 11) and supplemented with optimum dye concentration. The decolorization percent was detected at different incubation times (3, 4, 5, 6, 7, 8 and 9 days). Similarly, the decolorization percent for each azo dye were estimated under different inocula size. Different inocula size (viz. 1, 2, 3 and 4 discs, each disc has a diameter 0.7 mm) of heavy spore fungal growth was inoculated in 100 ml mineral salt media supplemented with 100 ppm for each individual dye. At the end of each incubation period, dye decolorizations (%) were assayed after 7 days for Reactive yellow (4GL) and Reactive red (4BL).

Effect of different carbon and nitrogen sources on the dye decolorization by fungal isolate D2-1

In order to evaluate the effect of different carbon and nitrogen sources on dye decolorization, different carbon sources were added to mineral salt broth media containing 100 ppm of each dye at 2% concentration with equimolecular level for each sugar. The media without carbon source was used as a control (containing only dye concentration of 100 ppm as carbon source). The carbon sources were represented by glucose, sucrose, starch, bagasse and cellulose. Similarly, with the equivalent amount of nitrogen level located at 0.5 %, the effect of different organic and inorganic nitrogen source such as sodium nitrate, sodium nitrite, ammonium sulphate, urea, peptone and yeast extract on dye decolorization.

were evaluated in media supplemented with 100 ppm. In each case, all previously mentioned optimal conditions of pH, temperature and inoculation size were taken into the consideration.

UV-Visible study (λ max) of each dye before and after all optimized condition

Untreated stock solution of each dye (control) and treated (with fungal isolate D2-1 after all previously mentioned optimal conditions) were centrifuged at 10,000 rpm for 10 min. UV-Visible absorption spectra of the supernatants of untreated and treated dye effluents were recorded using a UV-Visible spectrophotometer (Shimadzu 1800) at room temperature.

Textile wastewater treatment by azo dye-degrading fungi D2-1

Textile wastewater sample was collected from Ghannam for textile Industry Company, El Mahala El Kubra, Gharbia Governorate, Egypt. Plastic bottles were used for water samples collection [28]. The plastic bottles were sterilized by ethylene oxide at low temperature for microbiological analysis.

Analysis of textile wastewater

Optical density of the effluent was observed at different wavelength between visible regions (300-800 nm) to determine λ max for textile wastewater. Physicochemical characteristics of textile wastewater analysis before and after the treatment processes were estimated for: pH, conductivity, color, TDS, TSS, COD and BOD. These were carried out according to the standard methods recommended by American public health association. [29]which are recommended by Egyptian Law 93/1962 Dec. 44/2000 to measure reduction in these parameters.

GC-MS Spectroscopy for untreated/treated wastewater

The samples were analyzed at Chemical War Department, Ministry of Defense, Cairo, Egypt. These tests were evaluated by using The Agilent 5975C Series GC/MSD and The Agilent 7890A Gas Chromatograph. Gas chromatography (GC) and mass spectroscopy (MS) are an effective combination for chemical analysis. Gas chromatography analysis separates compounds in complex mixtures, and mass spectroscopy analysis determines the molecular weight and ionic fragments of individual components, aiding in the identification of those compounds. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector temperature was maintained at 280°C, while the oven conditions were 80°C for 2 min, followed by an increase to 200°C based on a rate of 10°C/min, followed by a further increase to 280°C based on a rate of 20°C/min. The compounds were identified on the basis of their mass spectra and using the National Institute of Standards and Technology (NIST) library[30, 31]. Numerous American Society for Testing and Materials (ASTM) standards that cover GC/MS are also utilized for routine determinations.

Statistical analysis

Data were statistically analyzed by Sigma plot v, was used for multiple sample comparison, when normality and homogeneity of variance were satisfied.

Results and Discussion

Fungal isolation and identification

At the current study, twenty-one fungal isolates were isolated from soil and water samples of the textile industry. Amongst, twenty-one purified fungal isolates, isolate coded D2-1 showed highest decolorization percentage for two different azo dyes. The taxonomic status of fungal isolate was defined by sequencing of ITS genes. The morphological, culture characteristic as well molecular identification based on ITS sequencing analysis for isolate D2-1 was similar to Aspergillus niger as showed in Fig 1.

Determination of absorption maxima (λmax) of Reactive yellow (4GL) and Reactive red (4BL) dyes

The absorption maximum for used azo dyes were determined using spectrophotometer UV (Shimadzu 1800). Optical density of each dye dissolved separately in water was observed at different wavelength between visible regions (300-800 nm). Reactive yellow (4GL) and Reactive red (4BL) dyes showed maximum absorbance at 425.0 nm and 520.0 nm respectively. Therefore, the detected λ max was used in the following optimization process to detect decolorization percent.

Effect of dye concentrations on fungal decolorization

In this study, it was noted that, when dye concentration increased from 50 ppm to 650 ppm, the capacity of Aspergillus niger D2-1 for dyes removal was decreased. The dye concentrations between 150 ppm to 650 ppm were more incomprehensible to be decolorized than the low dye concentrations. Data reported in Table 1 showed that, dye concentration of 100 ppm was
sub-lethal dose which hinders the dye removal capacity of the two azo dyes. In addition to the toxicity of high dye concentration on fungal growth, also, dye decolorization was hardly to be achieved and insufficiently data is known about the fungal dye decolorization at that concentration. Therefore, dyes concentration of 100 ppm was chosen for further examination in this study (Table 1). There is a report in which decolorization was reduced due to intensify in initial dye concentration [32]. Certain reports proved the high toxicity of azo dyes to microbes involved in bio-degradation. Toxicity is interacted to dye type, dye concentration and the active sites of azo reductase enzymes blockage by dye particle[16, 33]. The toxicity of the dyes to fungal cells, especially at higher concentrations may be attributed to high molecular quantity, structural complication and the presence of inhibitory groups in the dye such as sulfonic acid[34]. Fetyan et al., [35]reported that, Saccharomyces cerevisiae showed high efficiency to decolorize direct blue 71 to maximum activity (100%) and this decolorization percentage decreased at higher concentration above 200 ppm which may be appropriate to the toxic effect of these higher concentrations.

Fig. 1. Phylogenetic tree of ITS sequences of the fungal isolate D2-1 with the sequences from NCBI and designated as Aspergillus niger D2-1.

TABLE 1. Effect of different azo dye concentrations on bio-decolorization process by Aspergillus niger D2-1.

<table>
<thead>
<tr>
<th></th>
<th>50 ppm</th>
<th>100 ppm</th>
<th>150 ppm</th>
<th>200 ppm</th>
<th>250 ppm</th>
<th>350 ppm</th>
<th>500 ppm</th>
<th>650 ppm</th>
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<tr>
<td>Ctrl</td>
<td>0.14±0.00</td>
<td>0.12±0.00</td>
<td>0.02±0.00</td>
<td>0.02±0.01</td>
<td>0.08±0.00</td>
<td>0.03±0.01</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>D2-1</td>
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<td>10.19±0.02</td>
<td>4.08±0.04</td>
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<td>0.01±0.01</td>
<td>0.01±0.00</td>
<td>0.38±0.06</td>
</tr>
</tbody>
</table>

<table>
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<tr>
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<th>650 ppm</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.25±0.02</td>
<td>0.01±0.00</td>
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<td>0.00±0.00</td>
</tr>
<tr>
<td>D2-1</td>
<td>54.84±0.17</td>
<td>29.74±0.65</td>
<td>8.69±0.06</td>
<td>4.95±0.21</td>
<td>0.03±0.00</td>
<td>0.00±0.00</td>
<td>0.01±0.00</td>
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</tr>
</tbody>
</table>

Ctrl, control without fungal inoculation; D2-1: inoculated with Aspergillus niger D2-1. Data are expressed as mean values of three replicates ± SE of the mean.
concentrations for microbial cell. Also, Congo red and Bromophenol blue added to culture media (PDA) separately were inhibited the growth of *Aspergillus sp.*, at high concentration when contrasted to their respective controls [36].

**Effect of different incubation periods and pH values on dye decolorization percentages**

The maximum decolorization of two azo dyes by *Aspergillus niger* D2-1 was recorded at pH 9, and any increase or decrease in pH value from the optimal pH are reduced the decolorization efficiency (Fig. 2 A and B). Our finding supports that, the decolorization of azo dyes reaches the greatest value at alkaline pH range, but acidic or highly alkalinity was inhibit dye decolorization by fungal strain. Our results showed that, maximum decolorization percentage of Reactive yellow (4GL) and Reactive red (4BL) by *Aspergillus niger* D2-1 was reached to 48.01% and 32.40% respectively at pH 9.0. It is achievable that pH change affects the transport of dye particles crossway the cell membrane of microbes, which is reflected as the rate-limiting stage for the decolorization [6]. The results in present study was slightly similar with the results obtained by [33], reported that, the maximum decolorization of Remazol black was attained at pH 9. Although greatest azo-reductase enzyme optimum pH is 7, some alkali-thermostable azo-reductase showed maximum decolorization and best pH within variety from pH 8 to 9 [33, 37]. Reactive azo dyes under alkaline condition lose hydrogen ions, which leads to the ionization of the dye, affecting its constancy and facilitating its elimination from solutions [33]. In contrast, [34] reported that decolorization of dyes at higher concentration was carried out an acidic condition, which further facilitates their better removal enzymatic or by cell wall adsorption by the fungi. Finally, our results are more applicable in decolorization process at large scale because most textile wastewater were alkaline pH values and decolorization under alkaline states has been usually preferred in industrial treatments, as procedures using reactive azo dyes are functioned under alkaline conditions.

Data represented in Fig. 2 A and B revealed that, the maximum decolorization of Reactive yellow (4GL) and Reactive red (4BL) dyes were 48.01% and 32.40% respectively at 7 days of incubation period. After 7 days, the decolorization decreased gradually which may be attributed to the efflux mechanism of dye from the fungal cells to reduce dye toxicity. The result in present study was slightly similar with the results obtained by [36], showed that, the maximum decolorization of Direct blue dye treated by *Aspergillus flavus* was obtained at 7 days of incubation period and the decolorization decreased gradually after 7 days of incubation due to enzymatic biodegradation activity along with physical binding of dye on fungal biomass and accumulation of dyes results that might have hindered growth and metabolizing latent of fungi. Ali et al., [38] reported that, decolorization rate by brown-rot fungi for azo dyes was generally high at 96 h. Besides, it maintained on declining.

![Fig. 2. Effect of different incubation periods and pH values on the decolorization percentages treated by *Aspergillus niger* D2-1. A) Reactive yellow (4GL), B) Reactive red (4BL). Data are expressed as mean values of three replicates ± S.E.](image_url)
with transitory time in stirred fungal cultures. El-Sayeh, [39] also found that, the maximum decolorization of Direct Violet dye treated by *Aspergillus fumagatus* was observed in the 4th day of incubation but decreased after that as well as the level of enzymes production.

**Effect of different inocula size (disc) on the decolorization rate**

The data presented graphically in Fig. 3 A and B showed the relation between inocula sizes and decolorization percentages. The optimal inoculum size needed for the maximum decolorization were three heavy growth discs of *Aspergillus niger* D2-1 inoculated in 100 ml of broth media. The decolorization percentages were reached to 57.62% and 39.86% for Reactive yellow (4GL) and Reactive red (4BL) dyes respectively.

**Effect of different carbon and nitrogen sources on the dye decolorization**

In this study, it was found that highest dye decolorization by *Aspergillus niger* D2-1 was reached 89.5% and 84.41% for Reactive yellow dye (4GL) and Reactive red dye (4BL) respectively in glucose supplemented medium (Fig. 4 A and B). With regard to the influence of different carbon sources on decolorization process, it was found that the tested fungus was able to grow well and decolorize dyes with all of the tested carbon sources. Importantly, *Aspergillus niger* not only use glucose and sucrose, as carbon source, but also utilize other agro-industrial wastes such as baggas. The results in present study was similar with the results obtained by Namdhari et al., [34], showed that the highest decolorization of Reactive Blue MR by *Aspergillus niger* was achieved with glucose supplementation. Addition of second carbon source beside dye was enhance fungal growth and hence increase adsorption/degradation rate [40]. Besides, the dyes removal rate can be linked with the accessible co-substrates [41], and with the exponential growth phase [42]. Glucose were used to investigate their influence on the decolorization efficacy of the fungal isolates because of it is rapid utilization as a simple sugar by organisms before act of complex structure like dye [43, 44].

Data analysis showed that, the best nitrogen sources utilized by *Aspergillus niger* D2-1 to maximum decolorization of reactive yellow dye (4GL) and reactive red dye (4BL) was yeast extract. After adding yeast extract as nitrogen source, the decolorization percentages were reached to 98 and 92 for reactive yellow dye (4GL) and reactive red dye (4BL). The results in the present study was agreement with Pourbabaee et al., [45], showed that, Synthetic and real effluents lost 60% of the initial color intensity within 30h of incubation in the presence of glucose and yeast extract whereas only 10% decolorization occurred in their absence. Fukushima and Kirk, [46]reported
Fig. 4. Effect of different carbon source on dye decolorization process. A) Reactive yellow (4GL), B) Reactive red (4BL). Data are expressed as mean values of three replicates ± SE of the mean.

Fig. 5. Effect of different nitrogen source on decolorization process. A) Reactive yellow (4GL), B) Reactive red (4BL). Data are expressed as mean values of three replicates ± SE of the mean.
that, five different organic and inorganic nitrogen sources approximating peptone, yeast extract, beef extract, sodium nitrate and ammonium chloride were tested for extracellular laccase and other protein production in *Ganoderma* sp. Yeast extract supported the maximum decolorization activity and maximum extracellular protein content of 140 μg/mL.

**UV-Visible spectrum for two azo dye before and after optimization**

The full UV-Visible spectrum of the decolorized samples could reveal the mode of decolorization. A decrease in the visible region (200-800 nm) indicates a decrease in dye color, while complete disappearance of the peaks indicated that, color removal occurs through breakdown of azo bond which is responsible for dye color. The absence of the peak at visible region are probably due to color removal and aromatic amines formation from cleavage of azo bond[47]. In the present study, complete disappearance of the peaks at ~ 425 nm and at ~ 520 nm in the corresponding UV-Visible spectrum (Fig. 8) of the treated yellow and red dyes respectively, strongly indicates the degradation of dye effluent due to fungal action. Decolorization of dyes may occur by adsorption[48] or degradation[49]. The adsorption of dyes receipts location only on the surface of living cells, while creation of new compounds is the result of dye degradation by fungal enzymes. In case of dye removal through biodegradation, the chief visible light absorption peak completely disappears, or a new peak performs [50]. Dye adsorption can also be easily observed by the presence of clearly colored cell pellets, whereas those retaining their prototype colors are accompanied by the occurrence of biodegradation[51]. In the present investigation, 98% decolorization was achieved in 60 h and the cell pellets were not pigmented. These results brightly support the evidence of biodegradation of textile effluent by *Aspergillus niger* D2-1 in the decolorization process.

**Textile wastewater treatment**

The effluent released by industries of textile has leads to hazardous problem of soils and groundwater pollution. The physio-chemical analysis of tested textile effluent helped us to estimate the pollution level. Thus, the physio-

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**Fig. 6.** UV-Visible spectrum for azo dye before and after optimization using a Spectrophotometer UV (Shimadzu 1800). A) Reactive yellow dye (4GL). B) Reactive red dye (4BL).
The maximum absorption of textile wastewater by using a Spectrophotometer UV (Shimadzu 1800).

Fig. 7. The maximum absorption of textile wastewater by using a Spectrophotometer UV (Shimadzu 1800).

Chemical parameters test for effluents were examined and conducted. The absorption maximum (λ max) of textile wastewater was determined, and the effluent showed maximum absorbance at 427.0 nm.

Physico-chemical characterization of textile effluent

The physicochemical parameters of the raw textile wastewater (effluent) before and after treatment by Aspergillus niger D2-1 was conducted. The results showed that effluents have dark yellowish color with a conductivity of 1340 μs/cm, temperature 29°C (measured by a laboratory thermometer), pH 9.6, BOD, COD, TDS and TSS were 451, 756, 1597 and 821 mg/l respectively, which indicates a broad range of chemical contaminants (Table 2). Effluents color is dark due to the mixture of chemicals and various dyes used in the dyeing procedure [52]. The pH of the effluent modifies the physio-chemical properties of water which in turn badly affects the biodiversity. High pH is mainly due to the use of bicarbonate, carbonate, NaOH and H₂O₂ during bleaching process in the textile industry [53]. Soil permeability gets affected, which results in polluting the underground resources of water [54]. Elevated temperature leans to decrease the gases solubility in water, which is eventually expressed as high BOD/COD. The high COD (756 mg/L) and BOD (451 mg/L) level in the textile effluent, indicates the high toxicity of the effluent and this is very harmful for the whole ecology and aquatic system of the obtaining waterbodies. High TDS value reduces the sun light diffusion into the water and eventually reductions the photosynthesis in aquatic flora. This cause reduction in dissolved oxygen value of water bodies, which results for extremely depleted purification of wastewater by microbes [34]. Data recorded in Table 2 illustrated that, treatment using A. niger was decolorized 59% of textile wastewater (effluent) after 7 days. The results in present study was similar with the results obtained by Saroj et al., [55], who showed that, fungal consortium including three strains Penicillium oxalicum, Aspergillus niger and Aspergillus flavus has been developed and this consortium exhibited remarkably great potential to decay azo dyes (Acid Red 183, Direct Blue 15 and Direct Red 75) at different initial concentrations. The reduction in BOD and COD level after treatment with fungal isolate could be as a result of removal of organic load from the effluent and ultimately the toxicity decreased as stated by [56]. Reduction in the concentration of TDS after treatment may be attributed to the

<table>
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<tr>
<th>Sample parameters</th>
<th>Decolorization (%)</th>
<th>TDS (mg/L)</th>
<th>TSS (mg/L)</th>
<th>Conductivity (μs/cm)</th>
<th>pH</th>
<th>BOD (mg/L)</th>
<th>COD (mg/L)</th>
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<td>Raw effluent</td>
<td>0</td>
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<td>821</td>
<td>1340</td>
<td>9.6</td>
<td>451</td>
<td>756</td>
</tr>
<tr>
<td>Aspergillus niger D2-1</td>
<td>59</td>
<td>845</td>
<td>362</td>
<td>1235</td>
<td>9.1</td>
<td>169</td>
<td>391</td>
</tr>
</tbody>
</table>
ability of tested *A. niger* to desalinate the dye residues as previously reported by Ali et al.,[41] and Kumar et al., [57].

**GC –MS of untreated and treated textile effluent by Aspergillus niger D2-1**

This analysis was used for confirming the biodegradation of textile wastewater effluent after treatment by *Aspergillus niger* D2-1 as compared controls (wastewater effluent without any treatments). The intermediates obtained during the biodegradation of textile wastewater were compared to the NIST library [30, 31]. The GC-MS spectra revealed four major compounds of the wastewater before treatment at retention time 12.5 (1,2,3,4,5-pentamethylcyclopentane) with peak area 39.13%, 12.6 (1,2,3,4-pentamethylcyclopentene) with peak area 11.97%, 14.031 (3,3,4-trimethyldecane) with peak area 10.75% and 14.13(1,4-dimethylcyclohexane) with peak area 20.10% as shown in Fig. 8 A.

Our data represented in Fig. 8 B showed that, the mass spectra of the treated textile effluent by *Aspergillus niger* D2-1. When compared to the mass spectra of the untreated effluent it is seen that, the values of peaks are diminished upon biotreatment and become 6.37 % (1,2,3,4,5-pentamethylcyclopentane) at retention time 12.5, 1.86% (1,2,3,4-pentamethylcyclopentene) at retention time 12.6, 1.91 % and 0.71 % (3,3,4-trimethyldecane) at retention time 14.02 and 14.3 respectively and finally 0.21 % (1-methyl,4-methyl,cis, cyclohexane) at retention time 12.8. Low peak area values in GC-MS analysis indicated cleaving of azo dyes and further mineralization of aromatic amines into simpler aliphatic compounds due to *A. niger* treatment as reported previously by [58, 59].

The biodegradation pathway of dyes is supported by other reports which appeared that the first stage of the biodegradation of some dyes was either an oxidation reaction or a direct demethylation process followed by a stepwise demethylation and deamination process [31, 60, 61]. Generally, the mechanism of fungal dye decolorization and degradation is either biodegradation and biosorption by living cells or biosorption by dead cells which implicates physico-chemical interaction such as deposition, adsorption, and ion-exchange [36].

**Conclusions**

At this study, *Aspergillus niger* D2-1 isolated from the textile effluent contaminated soil has high potentiality in azo dye degradation. The optimum conditions for dye decolorization efficiency of *Aspergillus niger* was achieved *in vitro* with glucose, yeast extract supplementation, and 3 discs (7 mm) of fungal inoculum size, at pH9 and incubation time 7days. Significant reduction in BOD, COD, TSS and TDS was detected after treatment of textile wastewater industry by *Aspergillus niger*, thus suggesting safe for discharge. GC–MS and UV spectroscopy
analysis confirmed biodegradation. Finally, this study concluded that, biotreatment of azo dye and textile wastewater effluent by fungal cell was provided a cost-effective, easily applicable and eco-friendly.

Acknowledgements

Authors are greatly thankful to Dr. Saad El-Din Hassan for helping in molecular identification of fungal isolate.

References


BIOLOGICAL DECOLORIZATION AND DEGRADATION OF AZO DYES FROM TEXTILE...


In this study, a strain of the fungus Aspergillus niger D2-1 was isolated from contaminated soil samples from textile industries. The strain was used to decolorize and degrade azo dyes. The results showed that the strain could effectively decolorize and degrade two different azo dyes with efficiencies ranging from 92.42% to 98.62%.

The study also investigated the effect of different conditions such as pH, fermentation time, and carbon and nitrogen sources on the decolorization process. The results indicated that the optimal conditions for decolorization were a pH of 6.5, a fermentation time of 7 days, and a carbon source of 2% untreated yeast extract.

The study also included an analysis of the treated dye solutions using UV-Vis spectrophotometry, gas chromatography-mass spectrometry (GC-MS), and other analytical techniques. The results confirmed the decolorization and degradation of the azo dyes and the potential of the fungus Aspergillus niger D2-1 as a biocatalyst for dye removal.