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Direct-dyes bioremoval using *Aspergillus niger* in pilot scale bioreactor

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ABSTRACT

Treatment of effluents from dye-based industries represents one of the most important approaches to protect the surrounding agricultural environment from heavy pollutions particularly in developing countries. In this work, decolorization of two structurally different direct textile dyes; Direct Violet and Direct Green, were studied to determine the optimum conditions for dye removal, particularly in relation to agitation rate and temperature. Experiments on the decolorization of the dyes were carried out in a batch mode bioreactor using *Aspergillus niger* strain 20. The effect of various temperatures (30°C, 35°C, 40°C, 45°C, 50°C) and agitation rates (0, 100, 200, 300 and 400 rpm) on the dye removal were investigated. The maximum removal of Direct Green dye was recorded at temperature 40°C, where 75.75% of this dye was removed in 2 h. The data revealed that the agitation has a significant effect on the percentage of the dye decolorization by fungal biomass. The removal ranged from 72.3 to 96.6 after 24 h. The static treatment without agitation gave higher percentage of Direct Violet dye removal reaching 92.3% after 4 h of incubation. The kinetics of the dye bioremoval process in relation to temperature and agitation changes was studied. The equations generated from kinetics studies can be applied to predict the dye removal at temperature in ranges of 30–40°C and agitation between 0–200 rpm.

Keywords: Kinetics; Direct-dye bioremoval; Fungal strain; Pilot scale bioreactor

1. Introduction

The textile industry azo dyes residues constitute a major threat to the surrounding environment due to their toxic nature [1,2]. Some azo dyes have been identified as the most problematic compounds in the textile effluents as they are difficult to remove due to their high water solubility and low exhaustion [3]. The removal of azo dye from wastewater has become a matter of great concern in many countries. Microbial bioremediation is among the successful approaches for dye bioremoval either by adsorbtion or biodegradation processes. Currently an extensive research is being performed in many laboratories to obtain microbial biomass that can be used to separate contaminating dyes from the large volumes of polluted wastewater [4]. The physico-chemical factors play a significant role in textile dyes decolorization and the cell biomasses accumulation. Yesilada et al. [5] investigated the effect of various conditions such as initial pH, dye concentrations, amount of pellet, temperature and agitation on decolorizing activity by *Funalia trogii*. Kinetics of Direct Fast Scarlet decolorization was also examined by He and co-authors [6]. The effect of temperature on the dye decolorization was studied by several authors [6–8]. Their results demonstrated that the optimal decolorization activity was observed with the temperature

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range between 20–40°C. In many systems, the rate of color removal increased with increasing of temperature within a defined range that depends on the system. The temperature required to enhance the rate of color removal tends to correspond to the optimum growth temperature of 35 and 45°C. The decrease in color removal activity at higher temperatures was reported and can be attributed to the loss of cell viability or to the denaturation of the azoreductase enzyme [9].

The effect of agitation on the dye decolorization was investigated by Ge et al. [10]. The rotational speed in the bioreactor was considered as an important factor affecting the system performance. Agitation also was reported to have an adverse effect on the stability of the lignolytic enzymes. Asma et al. [11] investigated the effect of agitation on the decolorization process by microbial biomass under both static and agitated conditions. He et al. found that the highest decolorization was at the agitation frequency 2.5 Hz. Knapp et al. [12] showed that the decolorization of Orange II dye was 45% after 23-h incubation in static conditions and 98% in agitated conditions. The kinetic studies are rarely reported with respect to dyes decolorized by fungal strains. In this study, the kinetics of bioremoval of Direct Violet and Green dyes by a strain of *Aspergillus niger* in relation to the agitation rate and temperature were studied.

2. Material and methods

2.1. Dyes and their structures

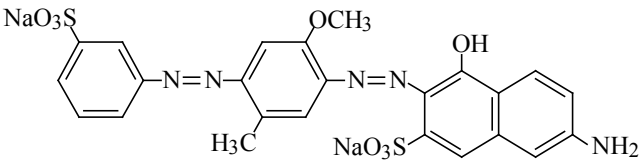
Direct Violet and Direct Green were obtained as pure chemicals from Ixmadye Company, Egypt (Dyestuffs and Chemicals Co.). The chemical structures of the two direct dyes are shown in Fig. 1.

2.2. Fungal inoculum preparation

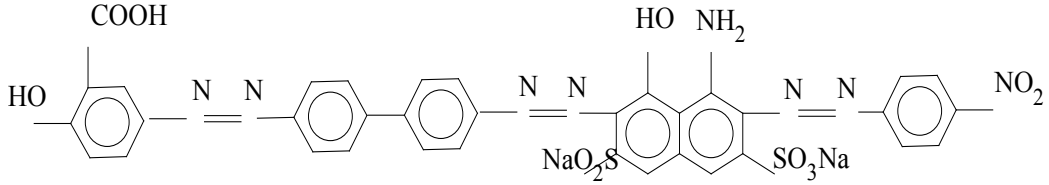
Fungal strain, *Aspergillus niger* 20 from cultural collection of Agricultural Microbiology Department at the National Research Centre, Egypt was used in this study. The fungal strain was maintained on potatoes agar slants medium in the refrigerator, and sub-cultured every 4 months. The composition of the culture medium (sucrose medium) for inoculum preparation and biomass cultivation was: 10 g/l sucrose, 0.5 g/l H_2PO_4, 0.2 g/l $MgSO_4\,7H_2O$, 0.1 g/l NaCl [13]. The procedure for inoculum preparation was the following: a fresh slant was inoculated using loopful of *A. niger* and incubated at 28°C for 72 h. A loopful of fungal growth was used to inoculate 300 ml of sterile media in 500 ml round flask, and the flask was incubated in an incubator shaker operated at 150 rpm at 28°C for 72 h.

2.3. Experimental work

Decolorization of direct textile dyes, namely, Violet and Green was conducted in a stirred tank bioreactor (New Brunswic Scientific Co. Inc.) with a 10-l working volume. The sucrose yeast medium (10 l) was put into the bioreactor vessel and autoclaved at 121°C for 20 min. The *Aspergillus niger* (2 g dry weight of mycelium) previously grown on a platform shaker was fed to the bioreactor. The dye solution was added to the bioreactor with fungal growth after three days of inoculation to give the concentration of 500 mg Direct Violet or Direct Green dyes l^{-1}. Growth and dye removal were investigated at five different agitation rates (0, 100, 200, 300 and 400 rpm) for Direct Violet dye, at 30°C incubation. The bioremoval of Direct Green dye was studied at 30°C, 35°C, 40°C, 45°C and 50°C. Both studies were done at the optimum aeration (0.5 vvm) and pH 3. Using agitation with one dye and temperatures with another dye, we used this as the model to understand a possible difference in the performance of fungal strain with the Direct class of dye under different values of temperatures and agitation. Samples were collected daily for analysis. The aliquots



Direct Violet RN (C.I. Direct Violet 31)



Direct Green G (C.I. Direct Green 8)

Fig. 1. Chemical structure of Direct Violet and Direct Green dyes used in this study.

were filtered through filter paper to determine biomass and growth media pH. The samples were withdrawn at different intervals for 24, 48 and 72 h. The efficiency of bioremoval of the dye was tested with every experiment after 2, 4, 6, 24, 48 and 72 h after addition of the dye. If the color change was observed, the sampling was extended beyond this time to allow growth and multiplication of fungi. At the end of the experiment the mycelium was collected by filtration and dried at 105°C to determine the dry weight.

2.4. Color removal assay

Dye decolorizing removal was expressed as percentage decolorization that was determined by measuring the absorbance of the supernatant at wavelength 542 and 304 nm for Direct Violet and Direct Green dyes respectively. The values were divided by the absorbance by the original media containing dye. The absorbance was measured by spectrophotometer LBK model 4054. The decolorization activity (%) was calculated according to the formula:

Decolorization activity (%) = ([(Initial absorbance of dye) – (Absorbance of sample)] / Initial absorbance of dye) × 100)

2.5. Determination of the chemical oxygen demand (COD)

The COD was measured using a Hatch spectrophotometer using test kit obtained from Hach, Co. The samples were digested in the aforementioned kit in Hatch digester at 150°C for 2 h.

3. Results

The stirred tank bioreactor was employed in the following experiments for the optimization of the biomass growth and the removal of Direct Green and Direct Violet dyes using different temperatures and different agitation rates.

3.1. Optimization of fungal growth and dye-bioremoval at different temperatures

In this work, the effect of different temperatures (30, 35, 40, 45 and 50°C) on growth and Direct Green removal efficiency by *Aspergillus niger* strain was studied (Fig. 2). The fungal biomass showed a high decolorization percentage particularly at 40°C (Fig. 4), where the color of Direct Green dye was removed after 2, 4 and 24 h by 75, 75 and 76% respectively. The increase in fungal biomass was observed after 5 days of incubation (Fig. 4). The accumulation of fungal biomass at different temperatures was 20.40, 24.33, 26.05, 9.16 and 7.41 g/10 l after 5 days of incubation respectively. The maximum biomass dry weight was recorded at 40°C. A decrease in biomass accumulation was noted with the rise of the temperature over 40°C (Fig. 4). The results show that the maximum decolorization of Direct Green dye was at 40°C, in which the percentage of decolorization reached 75% after 2 h and 77.7% after 72 h. At 35°C the maximum Direct Green decolorization was 77% after 72 h. The decolorization of this dye at 35°C did not exceed 43% after 2 h (Fig. 2).

At a higher temperature (50°C), the fungal decolorization of Direct Green dye was zero, this may be due to either retarded growth of fungi (Fig. 4) or to the decrease and denaturation of extracellular (azoreductase) enzymes known to initiate the degradation process of dye or these enzymes tend to denature by the effect of high temperature. All further experiments on Direct Green dye bioremoval were carried out at 35–40°C based on the optimum temperature identified in this experiment.

3.2. Optimization of fungal growth and dye-bioremoval at different agitation rates

The optimum agitation rates for fungal biomass build up in the bioreactor and removal of Direct Violet dye were tested. The effect of five agitation rates 0, 100, 200, 300 and 400 rpm on dye bioremoval was investigated. The

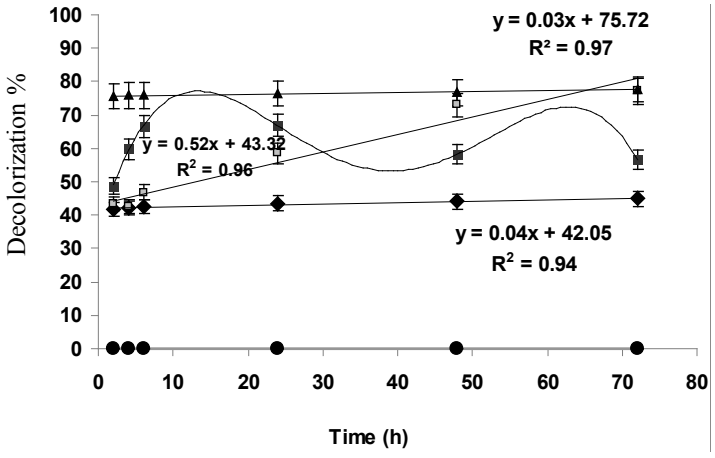


Fig. 2. Correlation between bioremoval of Direct Green dye by *Aspergillus niger* 20 and different temperatures. Incubation temperatures in °C: 30 (◆), 35 (■), 40 (▲), 45 (■) and 50 (●) at 100 rpm.

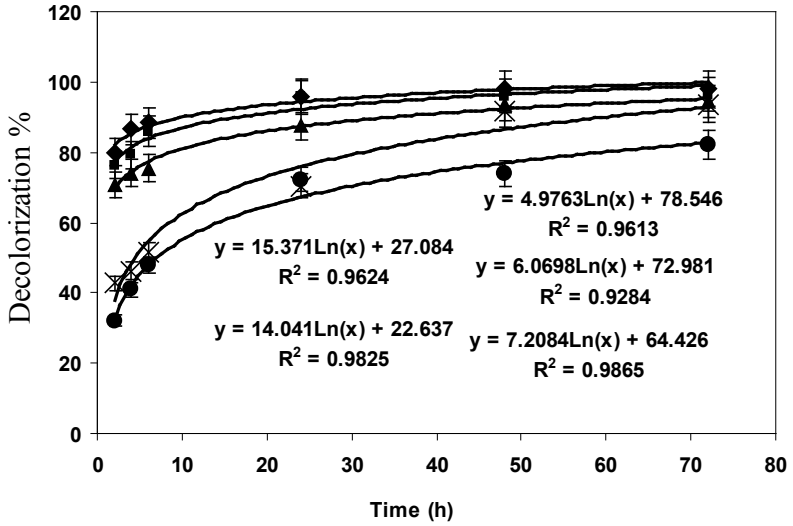


Fig. 3. Correlation between bioremoval of Direct Violet dye by *Aspergillus niger* 20 and different agitation rates. Agitation rates in rpm: 0 (◆), 100 (■), 200 (▲), 300 (×) and 400 (●) at 30°C.

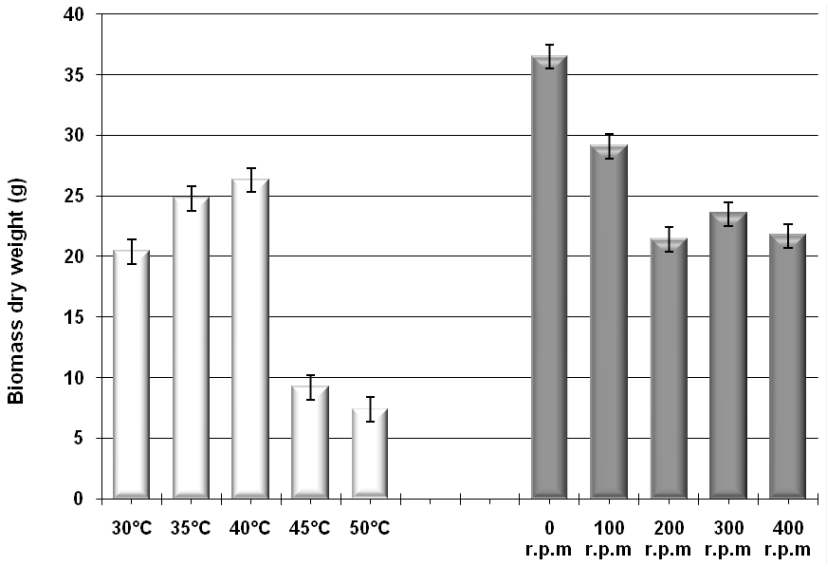


Fig. 4. Accumulation of fungal biomass in media amended with Direct Green and Violet dyes at different temperature degrees and agitation rates, respectively (after 5 days).

obtained results are illustrated in Fig. 3, where it is clear that the mechanical agitation in the bioreactor decreased the bioremoval process at early growth of fungi. The optimum bioremoval was obtained in the static media without agitation (Fig. 3). The non-agitated treatment gave higher dye removal that reached 86.6% after 4 h of Direct Violet dye addition. This treatment had increase in biomass at the end of the incubation time (72 h). The bioreactor agitation with mechanical stirrer can result in cell dispersion which might affect the bioremoval of dissolved dye. The results show that the dry weight of fungal biomass with three rates of agitation (200, 300 and 400 rpm) were almost the same after 5 days of incubation (Fig. 4). The results in Fig. 3 show that the high rates of agitation (300 and 400 rpm) for longer incubation time (72 h after addition of dye) resulted in increasing the decolorization efficiency that reached 93% and 82% respectively. The bioremoval of the Violet dye at high agitation rates (200, 300 and 400 rpm) showed 23% reduction after 24 h from the dye addition compared with the bioremoval at agitation rates 0 and 100 rpm (Fig. 3). The bioremoval of dye increased after 48 h at agitation rates of 200, 300 and 400 rpm.

3.3. Changes of pH and COD

Throughout the experimental period a follow up of changes in pH values was recorded. Clear variations of pH during the growth and process of decolorization were observed. The final pH was 2.05–4.14 after 5 days of incubation (Fig. 5a). Furthermore, Fig. 5b shows the effect of agitation on pH changes during 72 h of incubation period.

The chemical oxygen demand values are related to the total concentration of organics in solution and the changes of COD are showing the degree of mineralization as a function of dye bioremoval. The COD was measured at the end of the experiments (5 days). The results show that the fungal biomass could reduce the COD value of synthetic dyeing effluent (Fig. 6). The original dye solution at zero time had COD value of 887 mg l^{-1}. This COD value reduced to 62 mg l^{-1} within 72 h of incubation at 40°C (Fig. 6). The changes of COD values in Direct Violet dye media were not sharp. This may be due to the destruction of fungal biomass pellets due to the aggressive agitation.

3.4. Study of decolorization step

The changes in decolorization percentages were plotted with time at different temperature rates (30, 35, 40, 45 and 50°C). Fig. 2 shows that the decolorization percentage at 40°C with time is a step function, where it was the maximum decolorization % (>75%) from the beginning of the treatment (2 h) and continued to be constant to the end (77%). At 35°C the decolorization percentage reached the same values after 48 h. While at 45°C the decolorization percentage showed fluctuations. At 30°C decolorization percentage did not exceed 45% after 72 h. Thus we can conclude that the decolorization percentage at 30, 35 and 40°C behave as a step function.

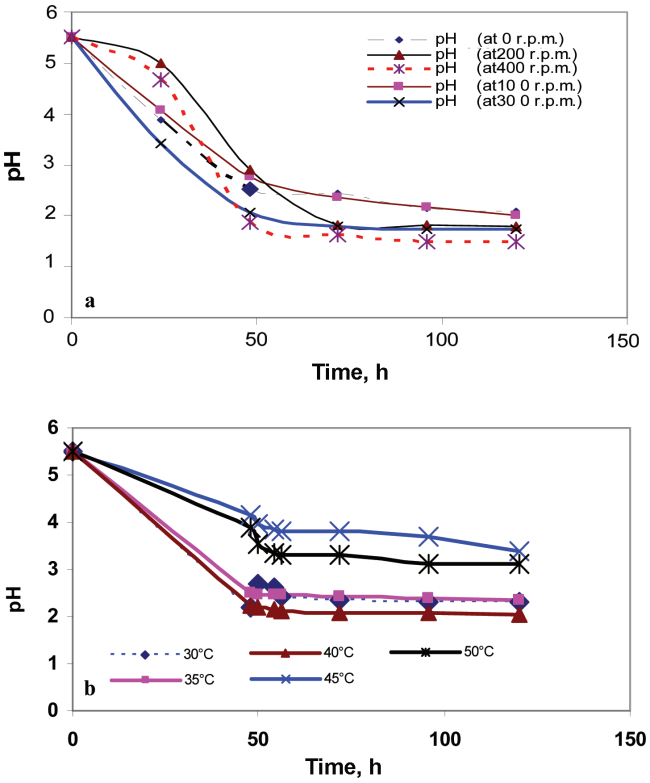


Fig. 5. Changes of media pH associated with fungal strain bioremoval of Direct Violet (a) and Direct Green (b) dyes by *Aspergillus niger* at different agitation rates and temperature degrees.

Decolorization % = 0.3 t + 75.7 (at 40°C, time range from 0 to 72 h) valid in the range of 30–40°C (Fig. 7).

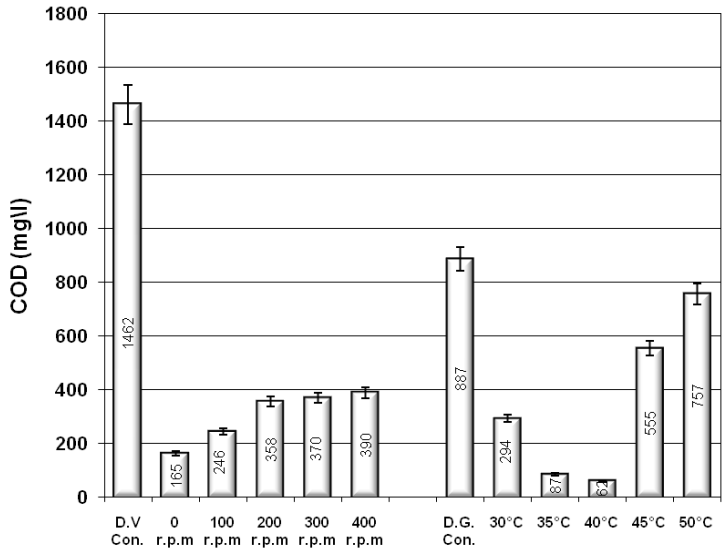


Fig. 6. Changes of COD of water based Direct Violet (D.V) and Green dyes (D.G) as affected by *Aspergillus niger* growth at different agitation rates and temperature degrees (readings were recorded after 5 days of incubation).

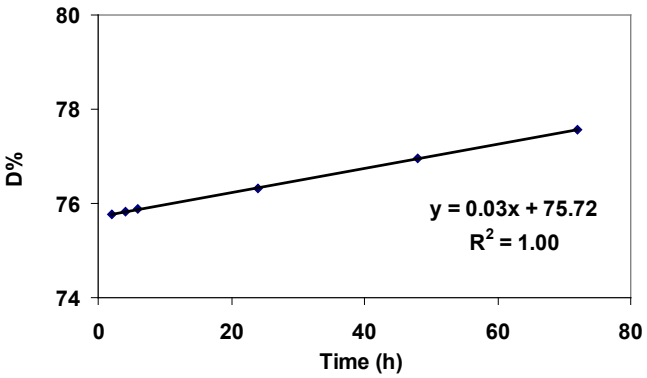


Fig. 7. Kinetics of bioremoval of Direct Green dye by *Aspergillus niger* 20 as average of bioremoval at different temperatures: 30, 35 and 40°C at 100 rpm.

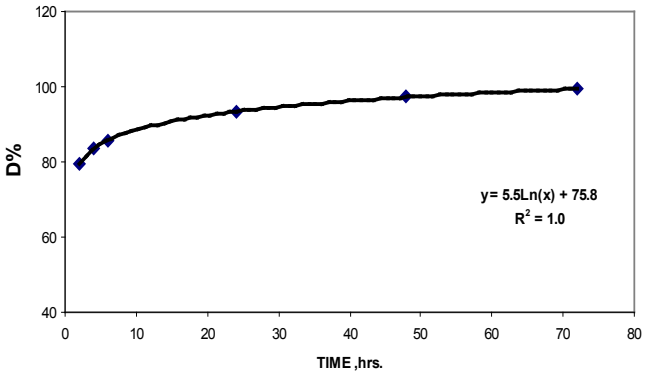


Fig. 8. Kinetics of bioremoval of Direct Violet dye by *Aspergillus niger* 20 as average of bioremoval at agitation rates 0, 100 and 200 rpm at 30°C.

The obtained data in Fig. 3 was plotted as the percentages of decolorization with different agitation rates (0, 100, 200, 300 and 400 rpm.) at different intervals. Fig. 8 depicts the changing in the percentage of decolorization with time. It is obvious from the plotted data that the decolorization percentages at the different agitation rates (0, 100 and 200) are too close to each other. Therefore, one might take an average of their constant to give the following expression:

$D\% = y = 5.5 \ln (t) + 75.8.$

where D is the decolorization, t is the time of decolorization. $R^2 = 1.0$. This correlation was valid for agitation rates (0, 100 and 200) up 72 h, by verification of this equation at 2, 4, 6, 24, 48 and 72 h as shown in Fig. 8. The other agitation rates (300, 400) give a marked deviation from such relation.

4. Discussion

In this work, the bioremoval process of two direct textile dyes (Violet and Green) has been tested using *Aspergillus niger* biomass. It was shown that (bioremoval) activity was enhanced by raising the temperature up to 40°C. This may be due to the relative increase in the tendency of dye molecules to escape from the liquid phase to the solid phase (fungal biomass) with increasing temperature of the solution [14,15]. The temperature affects the rate of removal of dyes by altering the molecular interactions and the solubility of dyes [16]. Besides, the highest dye removal efficiency was found at 40°C and agitation rate (100 rpm) which might correspond to the rate of dissociation of the studied dye with maximum ionisation of the molecule. Similar results are mentioned by other researchers [17–19]. The obtained results revealed that the *Aspergillus niger* biomass could be considered as a promising bio-agent to remove direct textile dyes at the proper agitation rates and temperatures. These results are in agreement with Radha [7] who found that the maximum percentage (more than 75%) decolorization for direct dye was found at the temperature 35°C. In addition, Chang and Kuo [8] stated that an E. coli strain was able to effectively decolorize high concentration of azo dyes at temperature range between 20 and 45°C.

Using life biomass, Chang et al. [15] found that the temperature required producing the maximum rate of color removal tended to correspond to the optimum cell culture growth at temperatures of 35–45°C. The decline in color removal activity at higher temperatures can be attributed to the loss of cell viability or to the denaturation of the azoreductase enzyme. The results showed no thermal deactivation of the decolorization activity under realistic operational temperatures.

Many authors studied the bioremoval of textile dyes at different growth conditions including different pH and temperature. He et al. [6] demonstrated that the optimal decolorization activity was observed at pH range between 4 and 9, and the temperature range between 20 and 40°C. Yu et al. [21] also found that the decolorization activity increased considerably when the temperature was raised from 10 to 35°C, and then declined quickly over 40°C. In relation to the effect of agitation rates on dye decolorization the results indicate that using low rate of agitation (100 rpm) allowed better bioremoval as compared with using higher rates. These results are in agreement with Ge et al. [10] who reported that vigorous agitation had adverse effect on the stability of the lignolytic enzymes. Similar results are mentioned by other researchers [18] who found the optimum values of agitation rate on bioaccumulation of azo dyes 150 rpm. In addition, the high mixing rate was reported to decrease fungal growth and lignolytic enzyme activity [21]. Ge et al. [10] also studied the biological decolorization of textile reactive dyestuff by the white-rot fungus *Phanerochaete sordida* in a rotating biological contactor (RBC). They found that the highest decolorization efficiency was reported at a low speed (40

rpm). The results of this work show that lower agitation rates permitted superior dye bioremoval. These results are not in harmony with those obtained by Asma et al. [11] and Knapp et al. [12] who achieved up to 95% and 98% decolorization within 1 and 2 days respectively with agitation. Malachite Green was degraded equally efficiently in the stationary and the shaken cultures of Phanerochaete chrysosporium [22]. However, the degradation of the structurally similar dye Crystal Violet by *P. chrysosporium* was five times more efficient in the shaken culture than in the stationary culture [22]. Generally, the increase of agitation was accompanied by decrease in bioremoval of both dyes studied. This may be due to the destruction of fungal beads responsible for bioremoval and/or to the reverse desorption of the dye sorbed on the surface of fungal biomass. Thus, agitation rate in the vicinity of 0 < rpm < 100 would be a suitable rate for optimum decolorization. It would be more relevant to select 4 h treatment for dye bioremoval to be more competitive to the physico-chemical treatment requiring more time and being more expensive and causing additional pollution. Furthermore, the increase in the time of decolorization to 24 h and using higher agitation rates would only slightly improve the decolorization efficiency (3–4%). From the practical point of view, omitting of agitation step will be techno-economically more feasible for reducing the cost of this bioremediation technology. The decrease in initial pH did not affect the decolorization. Wesenberg et al. [23] and Conesa et al. [24] observed that the fungus produces organic acid salts such as malonate, oxalate during the early growth, which are later decomposed by the enzyme manganese peroxidase. The decrease of COD value to reach 14.6–93% with time could be attributed to recalcitrant nature of Direct Green dye. These results are in agreement with those obtained by He et al. [6] who found that the microorganisms continued to consume the organics until near-complete removal of COD. Only 60–70% of COD and 50–60% of color were removed prior to any enrichment procedure. The study of the kinetics of the bioremoval at various temperatures and agitation rates showed that the bioremoval percentages were in direct proportion to the increase in temperature at the range 30–40°C and agitation rate of 0–200 rpm. These results can help to predict the bioremoval capacity of dye at certain temperatures and agitation rates that can help to design more efficient and less time consuming bioremediation technology for dye bioremoval.

5. Conclusion

Based on the above results, the agitation rates and temperature range are considered to be important factors in the color bioremoval by *Aspergillus niger* strain 20. The fungal biomass particularly showed the highest activity of dye decolorization at 40°C. The results indicate that the use of no agitation or low rate of agitation (100 rpm) allowed better biosorption as compared with higher rates of agitation. The results showed that the fungus used for bioremediation could reduce the COD value of synthetic dyeing effluent. The developed correlation between the incubation time and the decolorization percentage, at different agitation and temperature rates can help to predict the decolorization efficiency at any time in the studied range of 0–80 h.

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