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Aeration as a factor in textile dye bioremoval by *Aspergillus niger*

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Experiments were done to study the bioremoval/ biosorption of dis-azo dye by *Aspergillus niger* strain 20 in two concentrations using 5 liter bioreactor at five aeration rates. The experimental results are compared for various operating conditions. The dye used was direct brown and the inlet air flow rate was: 1/8, 1/4, 1/2, 1, 2 v/v/min. The aeration rate of 1/2 v/v/min yielded 9.2 g fungal biomass and removed 72 % of the dye. Increasing of the aeration rate to 2 v/v/min increased the removal to 77%, whereas the biomass was decreased markedly at the end of the incubation time. The results also indicate that the fungal biomass obtained at the three other air flow rates was more or less the same after 3 days of incubation. The obtained results indicate that air flow rates 1/8, 1/4 and 1/2 v/v/min gave better dye bioremoval as compared with the high aeration rate (1, 2 v/v/min) and can be recommended for dis-azo dye bioremediation. Isotherm experiments were conducted to determine the sorbents-desorption behavior of examined dye from aqueous solutions using Langmuir and Freundlich equations.

Key words: Textile dyes, removing, dis-azo, batch reactor, modeling, bioremediation.

INTRODUCTION

The textile industry is one of the largest industries in Egypt. The majority of textile plants, which are situated in agricultural areas in the Nile valley and its Delta, discharge their untreated dyes-containing wastewaters. This problem is more complicated with the fact that the overdosing of dye utilization, particularly in old manufactories, which leaves approximately 50% of the dyes in free state as discharged with the factory effluent, and eventually to the irrigation water bodies.

Dyestuffs present in textile industry wastewater causes significant problems in treatment plants since those compounds are hard by biodegraded. Chemical and physical methods including coagulation-flocculation, advanced oxidation and electrochemical methods are efficient in color removal (Kapdan and Kargi, 2002; Daneshvar et al., 2003; Kim et al., 2004; Selcuk, 2005).

Studies on biological decolorization of dyestuff concentrated on utilization of aerobic and anaerobic microorganisms (Banat et al., 1996; Robinson et al., 2001; Ozyurt, 2003; Wafaa et al., 2003; Wafaa and Moawad, 2003). White-rot fungi can also effectively bio-degrade textile

dyestuffs by their extracellular enzyme system (Wesenberg et al., 2003; Mazmanci and Unyayar, 2005). However, it is difficult to keep them in functional form in conventional wastewater treatment systems, because of their special nutritional requirements and environmental conditions. Although most of the dyestuffs are resistant to aerobic biodegradation (Ganesh et al., 1994). Coughlin et al. (1997) reported the aerobic degradation of azo dyes and Ekici et al. (2001) showed that degradation under aerobic conditions proceeds via oxidation of the substituents located on the aromatic ring or on the side chain.

Sampa and Dutta (2004) investigated the effects of process parameters; catalyst loading, initial dye concentration, airflow rate, UV- radiation intensity, and pH on the extent of photocatalytic degradation. Substantial reduction of COD, besides removal of color, was also achieved. They also showed that the oxygen required for scavenging electrons generated by UV-radiation came from the air bubbled through the liquid. The airflow rate was also sufficient to keep the ZnO particles in suspension. It is therefore, pertinent, and useful to study the effect of airflow rate on the rate of photocatalytic degradation (PCD). Therefore, the enhancing textile dis-azo dye bioremoval by aeration was evaluated in this study.

The Freundlich and Langmuir adsorption models were

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used for the mathematical description of the adsorption equilibrium of direct dye by fungal biomass sorbent. The obtained results showed that the adsorption equilibrium data fitted very well to both models. According to Treyball (1980), it has been shown using mathematical calculations that values of n between 1 and 10 represent beneficial adsorption. This work is aiming to study the effect of aeration on textile dye removal by one fungal strain, *A. niger*.

MATERIAL AND METHOD

Inoculum preparation for batch experiments

A. niger strain 20 was obtained previously from a damping site by Wafaa (2000). The 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 propagation was: 10 g sucrose, 0.5 g/l H_2PO_4 , 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.1 g/l NaCl (Wafaa et al., 2003). The inoculum was prepared by using fresh slant which was inoculated using sterile loop full of fungal spores, and incubated at 28°C for 72 h. The slant was used to inoculate 300 ml of sterile media in 500 ml round flask using sterile loop, which was incubated on a controlled environment incubator shaker operated at 150 rpm at 28°C for 72 h. These inoculated flasks were used to inoculate 5 liters bioreactor with working volume of 3.5 liters. The bioreactor together with the media were sterilized by autoclaving at 121°C for 20 min.

Fungal dye removal using a bioreactor

The major textile dyes used in the industry are Azo dyes. Dis-azo dye (direct brown) bioremoval using fungal biomass from an aqueous medium was studied. One strain of fungi, *A. niger* 20 was used grown in liquid medium containing sucrose as carbon source. The sucrose yeast medium 3.5 liters was put into the bioreactor at the start. *A. niger* (0.5g dry weight of mycelium) previously grown on platform shaker was feed to the bioreactor. Fungal strain was individually cultured in a liquid mineral basal medium as mentioned above. A synthetic dye solution was prepared in the lab by dissolving the dye in distilled water. The initial concentrations of the dyes in the media were 300 and 600 ppm. The dye solution was added to the bioreactor with fungal growth after three days of inoculation to give the dye concentration of 300 mg dye l^{-1} . The dye concentration was increased by adding the same dye to the bioreactor after one day of the first addition to reach the total of 600 mg l^{-1} . The aeration level required to give the optimum biomass accumulation was investigated by changing the aeration rates to determine the relation between the aeration rate and the biomass formation. The bioreactor used in this study for optimization of biomass growth was the 5 liter bioreactor (new Brunswick Scientific Co. INC). It consists of a glass jar of 30 cm height and 20 cm internal diameter. The jar is embedded in a double controlled water bath. The bioreactor is provided with a temperature control system to adjust the water bath temperature as required. The aeration rate was adjusted using the air flow-meter.

Samples were withdrawn from the bioreactor at different intervals, 24, 48 and 72 h to determine biomass, remaining sugar and growth media pH changes. The efficiency of bioremoval (decolorization) of the dye was measured in all aeration optimization experiments at the intervals of 2, 4, 6, 24, 48 and 72 h after addition of the dye. If the color change continues after this time the sampling continued to assess the growth and multiplication of the fungus and more color removal. At the end of the experiment the

mycelium was collected by filtration and dried at 105°C to determine the dry weight.

Langmiur isotherm

The Langmiur isotherm is valid for mono layer adsorption on the surface. It is assumed, that all adsorption sites on the surface have the same size, there are no interactions between one adsorbed molecule and another (that is, sites are independent) and only a monolayer can be adsorbed. The Langmiur isotherm is expressed as:

$$q_e = K_L C_e / (1 + a_L C_e) \quad 1$$

where q_e is the equilibrium solid phase concentration a_L and K_L are Langmiur isotherm constants and C_e is the equilibrium liquid phase concentration. In order to determine the constants, a_L & K_L , for a particular system eq. (1) may be rearranged into suitable linear form eq. (2)

$$C_e/q_e = 1/K_L + (a_L/K_L) C_e \quad 2$$

In this case a plot of C_e/q_e versus C_e gives a straight line in which $1/K_L$ equals the intercept and a_L/K_L equals the slope.

Isotherm shape

Weber and Chakravorti (1974) considered the isotherm with a view to predict the favorability of an adsorption system. The essential features of Langmiur isotherm can be expressed in terms of a dimensionless constant separation factor, R which is defined by the following relationship:

$$R = 1 / (1 + a_L C_0) \quad 3$$

The parameter, R , indicates the isotherm shape according to the following:

R value	Type of isotherm
> 1	Unfavorable
= 1	Linear
0 < R < 1	Favorable
R = 0	Irreversible

Freundlich isotherm

The Freundlich isotherm can be derived by modifying the Langmiur assumptions to allow for several kinds of adsorption sites on the solid, each having a different heat of adsorption (Voudrias et al., 2002). The Freundlich isotherm is perhaps the most widely used mathematical description of adsorption in aqueous system.

The Freundlich equation is expressed as:

$$q_e = K_F C_e^{1/n} \quad 4$$

where K_F and n are constants characteristics of the system and can be obtained by linearizing equation (4).

$\log q_e = \log K_F + (1/n) \log C_e$ The magnitude of the exponent n gives an indication of the favorability and capacity of the adsorbent/adsorbate system, a value of $n > 1$ represents favorable adsorption according to Treybal (1980).

Determination of the starter dose of fungal biomass inoculum

The experimental fungus was cultivated in 500 ml round flasks con-

Table 1. Residual sugar (mg/ml) left after growth of fungal strain 20 on sucrose yeast medium at different aeration rates.

Incubation time	Aeration rates (v/v/min)				
	1/8	¼	½	1	2
Zero time	23.36	26.00	15.70	3.7	8
18 h	63.42	78.54	87.35	76.3	88.3
24 h	88.90	91.86	85.78	59.3	56.4
40 h	95.87	101.79	72.65	16.2	40.5
48 h	85.41	83.40	66.38	10.2	22.1
First dye addition (300 ppm)					
66 h	90.02	80.02	17.59	2.0	2.2
68 h	75.64	50.43	15.54	2.0	1.1
69 h	67.31	49.11	10.48	1.1	1.0
70 h	61.47	47.44	9.52	0.2	0.7
Second dye addition (600 ppm)					
72 h	31.55	20.67	6.75	0.1	0.4
76 h	50.32	20.34	5.30	0.0	0.0
89 h	34.95	19.69	0.0	0.0	0.0
91h	29.25	12.33	0.0	0.0	0.0
92 h	25.76	11.35	0.0	0.0	0.0
93 h	15.06	11.35	0.0	0.0	0.0
113 h	6.98	3.17	0.0	0.0	0.0
120 h	3.93	0.74	0.0	0.0	0.0

containing 300 ml of basal mineral medium supplemented with 10g/l sucrose, 0.5 g/l yeast. Flasks were shaken on rotary incubator shaker at 150 rpm and 28°C for 2 - 3 days. Fungal mycelium was separated by centrifugation at 8000 rpm. A volume of 300 ml was dried at 105°C to determine the fungal biomass. A proper volume of fungal biomass suspension containing 0.5 g dry biomass weight was used as inoculum for the batch reactor experiments.

Removing assay

Decolorizing activity was expressed in terms of percentage decolorization and was determined by monitoring the decrease in absorbance at 372 nm of Dis-azo dye (direct brown), against the medium. Decolorization activity (%) was calculated according to the formula:

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

Aliquots (15 ml) of the culture media were collected every 24 h during the operation of the bioreactor to measure the fungal biomass and the remaining sugar. The chemical oxygen demand measurements were obtained by a Hach spectrophotometer test kit (HACH, CO).

Determination of remaining sugar as glucose

The remaining sugar in the culture supernatant was determined by the glucose oxidase enzymatic colorimetric method of Trinder (1969). Kits of Biocon Diagnostik (GmbH, BURBACH, GERMANY, cat. No. 4611) were used. One ml of the glucose reagent was

added to 10 µl of sample containing 70 - 110 µg glucose. The mixture was kept for 10 min. at 37°C. Absorbance of the samples was measured against blank (distilled water) at 500 nm, using spectrophotometer LBK model 4054. The glucose (sugar) content was calculated from a standard curve constructed through identical procedures using standard glucose.

RESULTS

The effect of five rates of aerations 1/8, ¼, ½, 1 and 2 volume air/volume liquid/min (v/v/min) on biomass production and dye bioremoval was investigated. The obtained results are illustrated in Tables 1 - 4 and Figures 1 - 5 which depict the influence of the aeration level on bio-mass accumulation (X) as dry weight g/l, and direct dye removal and also show the pH changes and the remaining sugar concentration (S) as mg/l. The results show that, at all aeration rates, the fungal biomass increases gradually till the end of the experiment. The sugar was approximately totally consumed leaving only 0.1 to 0.4 mg/l after three days of incubation. The unconsumed sugar was exhausted after 89 h of incubation at aeration rates ½, 1 and 2 v/v/min as depicted in Table 1. The pH was continuously decreased at all aeration levels till the end of the experiment as shown in Figure 5. The direct brown dye bioremoval had increased gradually through-out the experimental period using 300 ppm of the dye. The direct dye bioremoval reached the maximum being 84.60, 80, 76.94% at aeration levels of ½, 1 and 2 v/v/min respectively at 24 h incubation (Table 2

Table 2. Effect of aeration on dis-azo dye bioremoval after adding 300 ppm dye.

Incubation time	Removal time after dye addition	Aeration rates (v/v/min)				
		1/8	1/4	1/2	1	2
Zero time	Zero time	0	0	0	0	0
50 h	2 h	34.10	43.07	46.44	52.08	40.57
52 h	4 h	44.48	48.51	62.95	57.73	54.73
54 h	6 h	51.17	53.03	68.80	65.60	53.69
58 h	10 h	60.13	66.48	83.82	84.60	76.90
70 h	24	78.51	79.55	84.60	80.0	76.94

*Figures represent the decolorization % of direct brown dye.

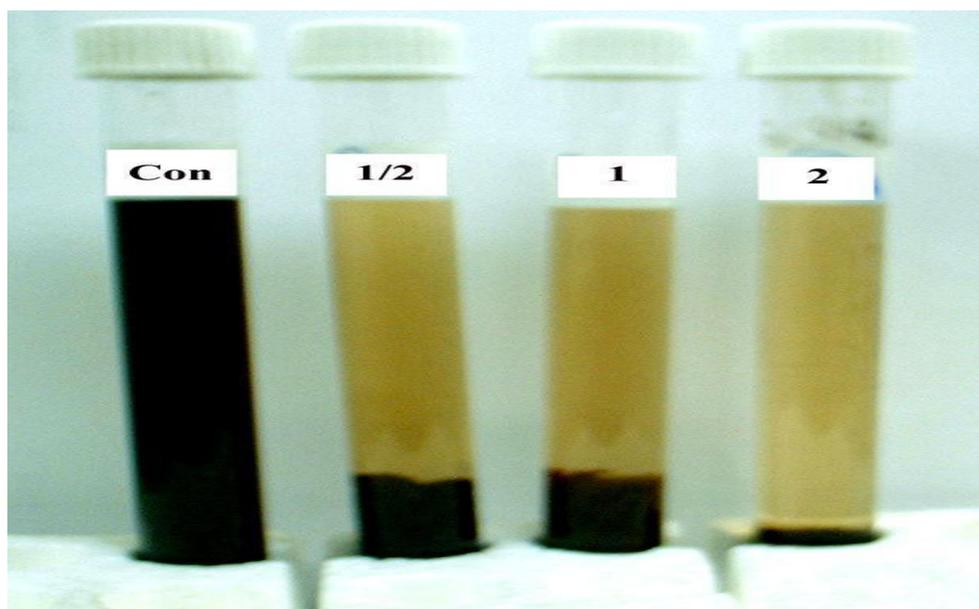


Figure 1. Removal of direct brown dye by *A. niger* strain at 1/2, 1, 2 v/v/min. aeration rate after 10 h incubation.

and Figure 1). The removal rate at this incubation time is too close to the 10 h time of incubation being 83.82, 84.60 and 76.90% for 1/2, 1 and 2 v/v/min respectively (Table 2 and Figure 1). The obtained results with increasing the dye concentration in the bioreactor indicated that the effects of airflow rate are important in bioremoval process.

Figure 2 illustrates the influence of the rate of aeration at 1/2 v/v/min on the dye removal percentages with 300 ppm of dye which was added to the bioreactor two days after incubation, however at lower percentage as compared with the samples taken after the same incubation period in the treatments receiving additional 600 ppm of dyes on the 4th day of incubation as shown in Table 3. This behavior was accompanied with increase in dry weight of biomass. The rate of aeration at 2 v/v/min recorded higher percentage of dye removal (77%) with decrease in biomass dry weight at the end of the incubation time.

The results also indicate that the dry weight of growth with three flow rates of aeration was adequate until three day after incubation time. The use of low flow rates of aeration; 1/8, 1/4, 1/2 v/v/min allowed better biosorption as compared with using high rate of aeration; 1, 2 v/v/min in the case of dis-azo dye bioremoval. Results in Table 3 show that using 1, 2 v/v/min flow rate of aeration for longer incubation time (48 h) results in lowering the decolorization efficiency in the bioreactor. A comparison of biosorbance of dye at higher concentration (600 mg⁻¹ direct brown dye) showed a 25% reduction in the removal of dye with 1 and 2 v/v/min rate of aeration as compared with 300 mg⁻¹ concentration. This decrease is probably due to the sugar limitation in the media with resulted is limited fungal growth after the consumption of the entire carbon source. The dye biosorption is stopped after 48 h incubation at 1/4 v/v/min rate of aeration, which may be attributed to the dye-decolorization effect, which was mainly due to the biological fungal biomass and the

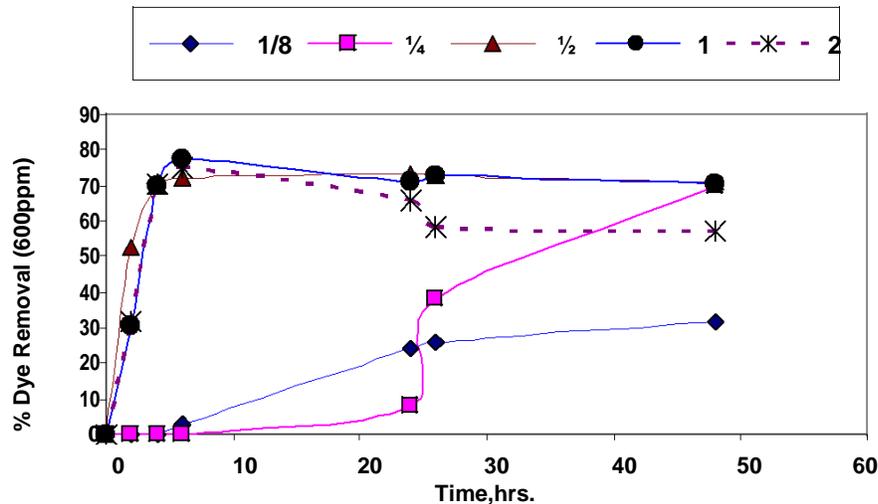


Figure 2. Effect of aeration on removing of dis-azo dye using fungal strain 20 (after addition 600 ppm dye).

Table 3. Effect of aeration on dis-azo dye bioremoval after adding 600 ppm dye.

Incubation time	Removal time after dye addition	Aeration rates (v/v/min)				
		1/8	1/4	1/2	1	2
Zero time	Zero time	0	0	0	0	0
76 h	2 h	0	0	52.48	30.60	31.80
78 h	4 h	0	0	70.00	69.66	70.20
80 h	6 h	2.76	0	72.21	77.45	75.17
100 h	24 h	24.17	8.03	73.26	71.14	65.77
102 h	26 h	26.03	38.34	72.75	72.71	58.05
124 h	48 h	31.86	70.0	71.21	70.67	57.38

*Figures represent the decolorization % of direct brown dye.

aeration rate. The effect of aeration on accumulation of biomass on sucrose-yeast medium is shown in Figure 3. The highest dry weigh of biomass was recorded with 1/8, 1/2 v/v/min aeration rate after five days incubation. The results also show that the aeration rates have no significant on the dry weight after three days incubation as depicted (Figure 3).

At the end of the experiments the COD was measured. The results indicate that the fungal strains reduce the COD value of simulated dyeing effluent by 60 to 70% with most aeration rates (Figure 4).

Figure 5 shows the effect of aeration levels on bio-mass production and on pH changes during the 72 h incubation period. The differences between the five aeration rates in the biomass formation were not marked until 3 days of incubation on the contrary to the changes of pH, which showed marked differences from the beginning of incubation. The efficiency of growth of fungal strain and dye removal activity as affected by aeration rates is illustrated in Table 4. The biomass: sugar conversion shows that the increase in aeration rates did not yield more bio-

mass. This shows water. The biomass was almost two and/or three times since the first dye (48 h) application till the end time of the experiment (120 h), however, the trend of the decrease of biomass with increasing the aeration continued till 48 h this indicates the synthetic dis-azo dye was not toxic to *A. niger* 20 which is tolerant to the dye concentration of both 300, 600 ppm. The fungus is continued to grow and add new biomass. The increase of dye removal efficiency with higher aeration rates in both sampling dates shows certain dye biodegradation capacity by *A. niger* strain 20.

Modeling results

In this study, two types of isotherms have been investigated, namely the Langmuir and Freundlich isotherm. The linear plots of C_e/q_e versus C_e show that the adsorption obeys Langmuir isotherm model (Figure 6). Model's constant K_L and a_L were determined from the Figure 6 and Table 5. It is obvious, that the predicted q_e and calculated value from experimental results are in fair agree-

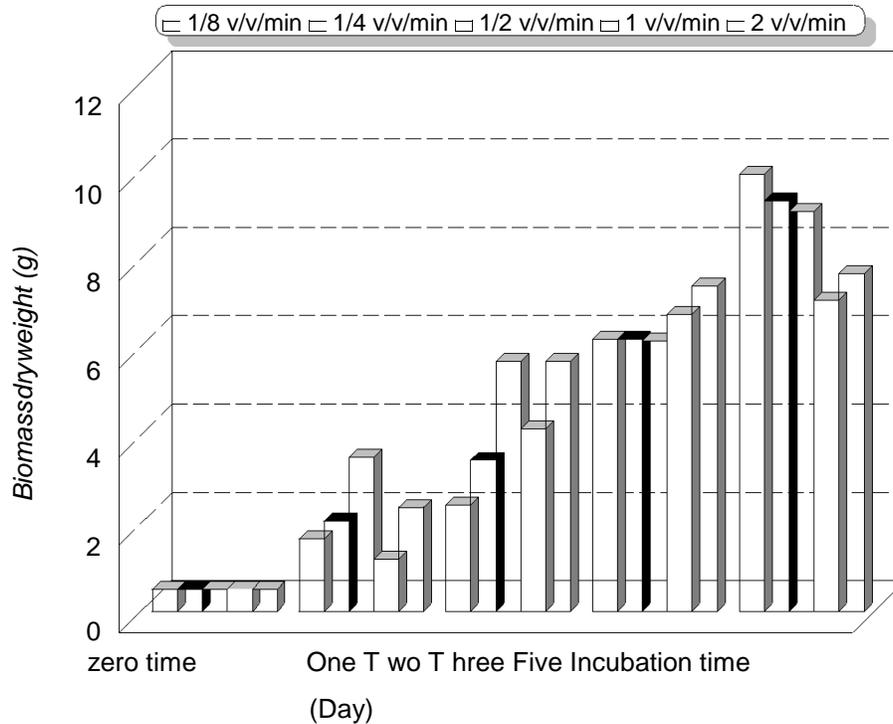


Figure 3. Effect of aeration on accumulation of fungal biomass grown on sucrose-yeast medium.

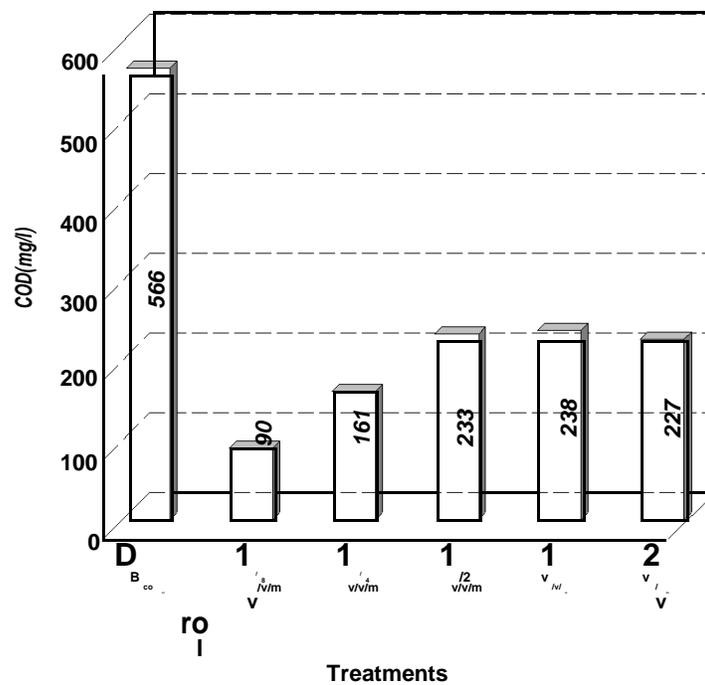


Figure 4. Reducing the COD value of dis-azo brown dye amended with sucrose yeast medium.

ment (Figure 7). The values of R at different concentrations are listed (Table 5). The results indicated that $0 > R$

< 1 at different concentrations it gives favorable adsorption of dye onto fungal biomass (Table 5). The plot of

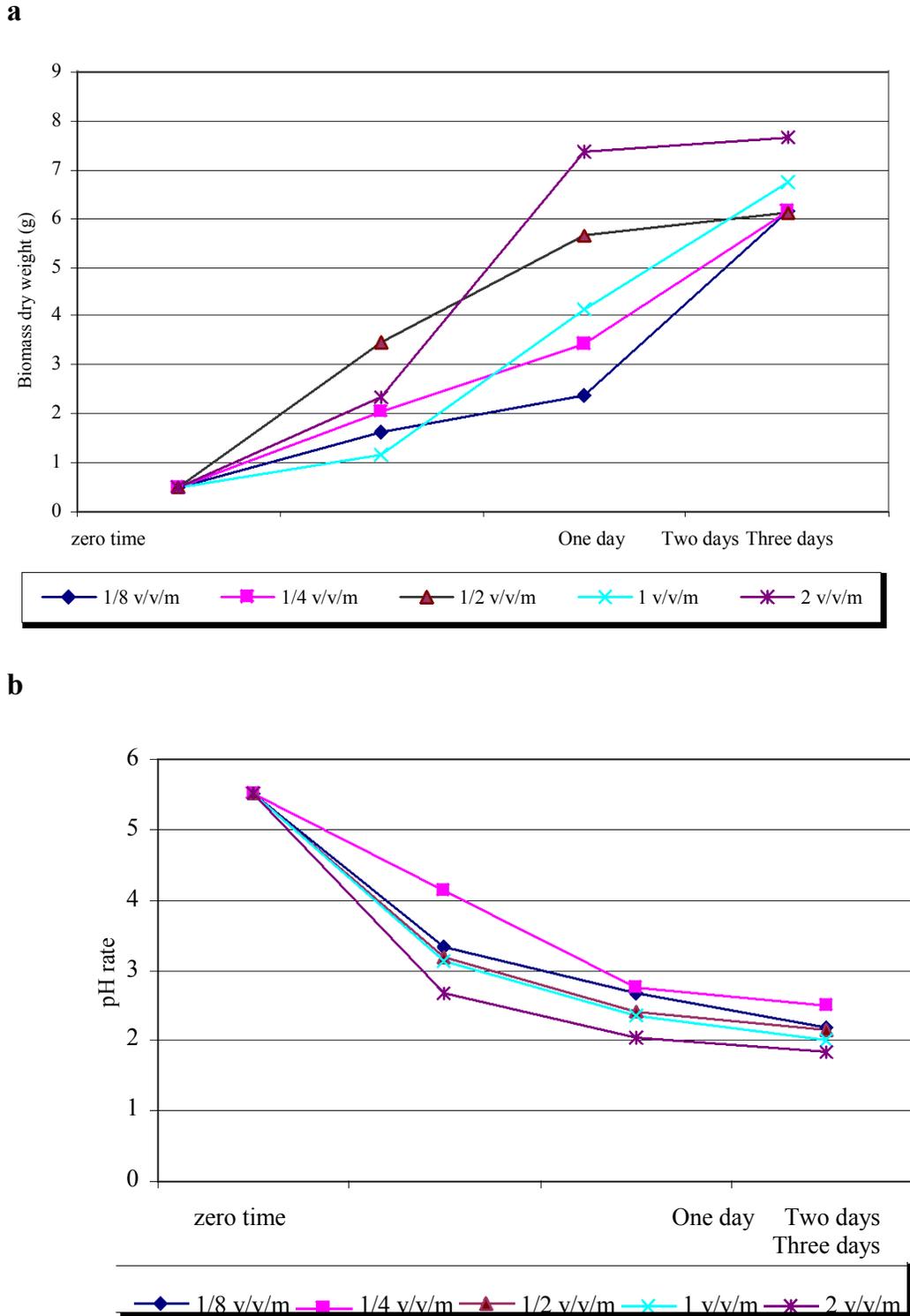


Figure 5. Effect of aeration on accumulation of fungal biomass (a) and pH changes (b) on sucrose-yeast medium.

that the sugar utilization has been converted to CO_2 and plot of $\log q_e$ versus $\log C_e$ shows that the adsorption follows Freundlich isotherm model as well (Figure 8). K_F and n were calculated from this figure were 30.40 and 1.90 mg^{-1} respectively.

The correlation coefficients obtained for direct violet dye from the Langmuir model and Freundlich equation were 0.99 and 0.931, respectively (Figures 6 and 8). The predicted values by Langmuir give good agreement with the experimental data. The agreement of the experimen-

Table 4. Interrelation between aeration, *A. niger* 20 biomass accumulation and dis-azo dye removal in a batch bioreactor.

Aeration rates	Fungal biomass (g/reactor)		Biomass: sugar Conversion c	Dye removal efficiency(%)g biomass	
	48 h	120 h		a	b
1/8 v/v/min.	2.5	9.85	0.33	7.9	3.2
¼ v/v/min.	3.5	9.3	0.31	8.55	7.50
½ v/v/min.	5.6	9.2	0.30	9.2	7.74
1 v/v/min.	4.1	7.1	0.24	11.26	9.95
2 v/v/min.	5.7	7.6	0.20	10.12	7.60

a, after first disazo dye application (300 ppm) 72 h incubation, b, after second dis-azo dye application (600 ppm) 120 h incubation, c, biomass/g utilize sugar at the end of the experiment.

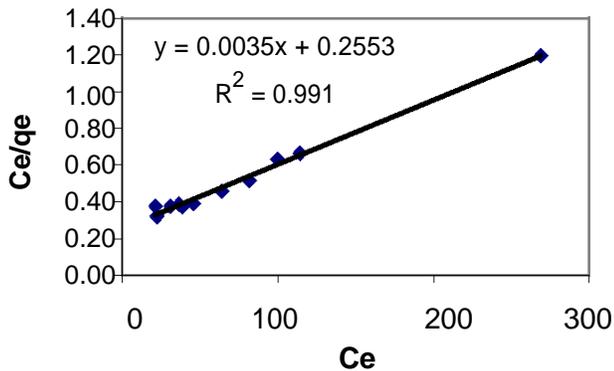


Figure 6. The linearized Langmuir adsorption isotherm of direct dye by fungal biomass at pH 5.5 and T = 28-30°C.

Table 5. Parameters for Langmuir Isotherm.

K_L	a_L (mg g^{-1})	C_o (mg l^{-1})	R
3.9170	0.01371	250	0.225868748
		300	0.195586780
		350	0.172464640
		400	0.154231495
		450	0.139484994
		500	0.127312310
		600	0.108393564
		700	0.094370070
		800	0.083559501
		900	0.074971174
		1000	0.067983738

tal data with the Langmuir model implied that monolayer adsorption existed for the experimental conditions used. Finally the Langmuir model and Freundlich equation were applied to the experimental data and the Langmuir model was found to be in better correlation with the experimen

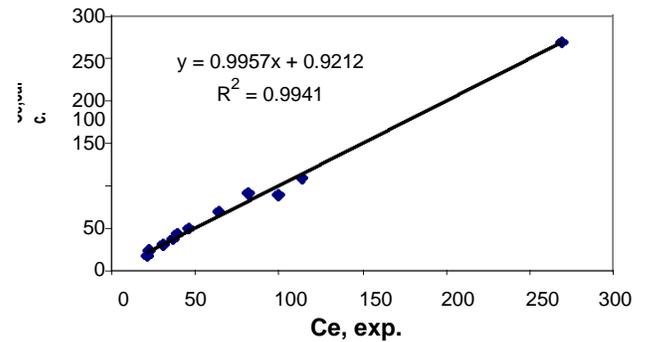


Figure 7. Fitting of experimental and calculated values C_e (mg/l).

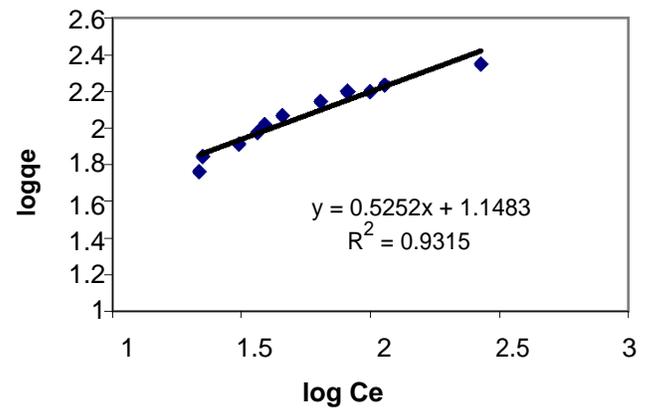


Figure 8. The linearized Freundlich adsorption isotherm of direct dye by fungal biomass at pH 5.5 and T = 28 - 30°C.

tal data.

DISCUSSION

The factors involved in the bioremoval of certain pollutant vary depending of the nature of chemicals and its concentration in the effluent. Among these factors aeration plays a major role. The aim of this work is to study the fungal capacity to remove textile dye under different aera-

tion rates. The effect of five rates of aerations 1/8, 1/4, 1/2, 1 and 2 v/v/min on biomass production and dye bio-removal was investigated. The obtained results with increasing the dye concentration in the bioreactor indicated that the effect of airflow rates is important in bioremoval process. These results agree with those of Sampa and Dutta (2004) who found that the increase in the air flow rate, which leads to the increase of oxygen, is enhancing turbulence, gas holdup, gas-liquid interfacial area, and consequently increase the mass transfer coefficient. Bizani et al. (2006) studied the effect of aeration of the semiconductor suspension and they evaluated the decrease in dye concentration when air is purged through the suspension and without the purging of air. It was obvious that an improvement in the rate of decolorization is achieved in the presence of air.

Similar results were obtained by Sampa and Dutta (2004) who found that the Methylene Blue and Eosin Y dyes degradation (PCD) increased with increase in the airflow rate. Yuan and Bellgardt (1993) indicated that oxygen supply is one of the main determining factors of yeast metabolism and is the limiting parameter for the production process. Sampa and Dutta (2004) studied the fractional-removal of the reaction for Eosin Y dye at various airflow rates. In this study the percentage of dye degradation increased from 39 to 63% as the airflow rate increased from 0 to 11.3 l/min. Corresponding COD-removal in the same study increased from 8.1 to 37.8%. In our work presented in this study the COD of the bio-removed dye was measured at the end of the experiments. The results show the COD has decreased after the fungal treatment from 60 to 70% with most of aeration rates. It is documented that certain fungi are capable in bio-degradation of synthetic dyes (Chao and Lee, 1994; Fu and Viraraghavan, 2001; Koumanova et al., 2002; Bhole et al., 2004). The anaerobic/aerobic cycle of growth and its effect on the dye biodegradation is now a matter of intensive study and the results will be reported later. The optimization of aeration in relation to the dye bioremoval revealed that the rates of aeration using 1/2 v/v/min gave slightly lower removal compared with using the rate of 1, 2 v/v/min. However for economical and practical considerations the 1/2 v/v/min aeration rate would be sufficient for significant portion of dye bioremoval. Two types of isotherms have been investigated in this study, namely the Langmuir and Freundlich isotherm (Voudrias et al., 2002; Choi and Cho, 1996). The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, (Hall et al., 1966) which is defined by $R = 1 / (1 + a_L C_0)$, where, C_0 is the initial dye concentration (mg/l) and a_L is the Langmuir constant. The results presented in this study are in agreement with Nace`ra and Aicha (2005). Also similar results are obtained by Namasivayam and Ranganathan (1994) who found that the R values between 0 and 1 at different concentrations indicate fa-

vorable adsorption of dye onto mixture.

The predicted values by Langmuir give good agreement with the experimental data. The Langmuir model makes several assumptions, such as monolayer adsorption and constant adsorption energy, while the Freundlich equation deals with heterogeneous surface adsorption (Zhou and Ki, 1991). These results obtained in this study are in agreement with Safarikova et al. (2005) who found that the Freundlich adsorption isotherm is generally not suitable for the study of dyes adsorption on magnetically modified yeast. Finally the Langmuir model and Freundlich equation were applied to the experimental Data and the Langmuir model was found to be in better correlation with the experimental data.

In conclusion, the results of the present study indicated that the contribution of aerobic conditions to enhance the textile dis-azo dye bioremoval by increasing in aeration was found to play an important role in dye bio-removal.

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