CATRINA (2006), 1 (2): 119-126
PROCEEDINGS OF THE FIRST INTERNATIONAL CONFERENCE ON CONSERVATION AND MANAGEMENT OF NATURAL RESOURCES, ISMAILIA, EGYPT, JUNE 18-19, 2006

Accelerating Textile Dye Bioremoval by Aeration

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ABSTRACT

A series of batch and bioreactors experiments were carried out for absorption of dis-azo dyes present in textile mill effluents under different aeration conditions. One fungal strain with five rates of air was used to absorb direct brown dye. Five liters bioreactors were applied to study the removal performance. The experimental results are compared for various operating conditions. The effects of airflow rate (1/8, ¼, ½, 1, 2 v/v min) inlet on the dye removing were assessed. It was found that the rate of aeration of ½ v/v min induced increase in dye removal percentages (72%) and fungal biomass (9.2 g); at the rate of aeration of 2 v/v min, high dye removal percentage (77%) was recorded with a decrease in biomass dry weight at the end of the incubation time. The results also indicated that the biomass dry weight obtained at three flow rates of aeration was more or less similar until the end of the growth stage (after incubation for three days). The results obtained indicate that using low rate of aeration (1/8, ¼, ½ v/v min) was better for dye biosorption than high rate (1, 2 v/v min), and therefore it is recommended for dis-azo dye removing.

Key words: Aeration, batch fermenter, fungal strain, removing of dis-azo, textile dyes.

INTRODUCTION

The textile industry is among the largest industries in Egypt. The majority of textile industrial plants, which are situated in agricultural areas in the Nile valley and its delta, dispose dyes-containing wastewaters untreated. This problem is further compounded with the fact that the overdosing of dye utilization, particularly in old manufactories, leaves approximately 50% of the dyes in free state discharging in the factory effluent and eventually into the irrigation water bodies.

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

Most of the studies on biological decolorization of dyestuff concentrate on utilization of aerobic and anaerobic microorganisms (Banat *et al.*, 1996; Robinson *et al.*, 2001; Ozyurt and Atacag, 2003; Wafaa *et al.*, 2003; Wafaa and Moawad, 2003). White-rot fungi can also effectively biodegrade textile dyestuffs by their extracellular enzyme system (Mazmanci and Unyayar, 2005; Wesenberg *et al.*, 2003). 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) and Coughlin *et al.* (1997) reported the aerobic degradation of azo dyes. 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, such as catalyst loading, initial dye concentration, airflow rate, UV-radiation intensity, and pH, on the extent of photo 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. This work aimed to study the capacity of the fungal strain for removing textile dye.

MATERIALS AND METHODS

Inoculum preparation for batch experiments

Aspergillus niger 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^{-1} H_2PO_4, 0.2 g l^{-1} $MgSO_4$ $7H_2O$, 0.1 g l^{-1} NaCl (Wafaa *et al.*, 2003). The procedure for inoculum preparation was as follows: a fresh slant was inoculated using sterile loop full of fungal spores and incubated at 28°C for 72 hrs. This slant was used to inoculate 300 ml of sterile media in 500 ml round flask using sterile loop. The flask was incubated on a controlled environment incubator shaker operated at 150 rpm at 28°C for 72 hrs. These inoculated flasks were used to inoculate 5 liters fermenter. The working volume of the fermenter was 3.5 liters. The fermenter and media were sterilized by autoclaving at 121°C for 20 minutes.

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Fungal dye removal in bioreactor with aqueous medium containing a single dye

Fungal dye decolorization in an aqueous medium was studied using Dis-azo dye (direct brown). One strain of fungi, *A. niger* 20, was used. One carbon source (sucrose) was tested for fungal growth. The imitation dye wastewater contained distilled water and a single dye was used in this study. The initial concentrations of the dyes in the media were 300 and 600 ppm. The major textile dyes used in the industry are Azo dye. Fungal strain was individually cultured in a liquid mineral basal medium as mentioned above. Dye was added to 5 liters fermenter to reach the concentrations of 300 and 600 mg l^{-1} after the fungal biomass reached the maximum. In this study, we used 5 liter fermenter (New Brunswic Scientific Co., INC). It consists of a glass jar of about 30 cm height and 20 cm internal diameter. The Jar is embedded in a double controlled water bath. The fermenter is provided with a temperature control system to adjust the water bath temperature as required. The aeration rate, required to give the optimum biomass, was adjusted by changing the agitation speeds and correlated to biomass formation. In the primary experiments, samples were withdrawn at different intervals, 24, 48, and 72 hrs to determine biomass, remaining sugar, and growth media pH. The efficiency of biosorption (decolorization) of the dye was measured with every optimization experiment 2, 4, 6, 24, 48, and 72 hours after addition of the dye. If the color change is observed the sampling was extended beyond this time to allow growth and multiplication of the fungus. At the end of the experiment the mycelium was collected by filtration and dried at 105^{o}C to determine the dry weight.

Fungal biomass development in Batch Model

Biomass growth rate of fungal strain in a batch process can be described by the following kinetic model:

$$dx/ds = u \times x$$

Where **X** is biomass growth (gl^{-1}) and **S** is the sugar used. The specific growth rate was determined according the above equation.

Determination of the starter dose of fungal biomass inoculum

The experimental fungus was cultivated in 500 ml round flasks containing 300 ml of the basal mineral medium supplemented with 10 g l^{-1} sucrose, and 0.5 g l^{-1} yeast. Flasks were shaken on rotary incubator shaker at 150 rpm at 28^{o}C for 2-3 days. Fungal mycelium was separated by centrifugation at 8000 rpm. A volume of 300 ml was dried at 105^{o}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 hrs during the operation of the bioreactor to measure the fungal biomass and the remaining sugar. The chemical oxygen demand measurements were obtained by a Hatc 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. Absorbances of the samples were 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 optimization of fungal growth and dye-bioremoval in 5 L fermenter using different aeration rates were studied. The glass bioreactor was used for optimization of biomass growth. 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 fed to the bioreactor. The dye solution was added to the bioreactor with fungal growth after three days of inoculation with the concentration of 300 mg dye l^{-1}. The dye concentration was increased by adding 600 mg l^{-1} of the same dye to the bioreactor after one day of the first addition. Samples were collected from the bioreactor for reaction follow up. Samples were withdrawn from the bioreactor at different intervals for 48 hrs to determine biomass, remaining sugar, and growth media pH changes. The aeration level, required to give the optimum biomass, was tested by changing the aeration rate and was correlated to the biomass formation.

Effect of five levels of aerations, 1/8, ¼, ½, 1, and 2 vol. air/vol. liquid/min, on biomass production and

dye bioremoval was investigated. The results obtained are illustrated in Tables (1-4) and Figures (1-5), which show the influence of the aeration level on biomass accumulation (X), as dry weight g l^{-1}, and Direct dye removal. The results also show the pH changes and the remaining sugar concentration (S) as mg l^{-1}. The medium, with fungal growth in bioreactor, was agitated at 200 rpm speed at 30°C incubation.

The data show that at all the five aeration rates, the fungal biomass increases gradually till it reaches its maximum at 76 hours of incubation then no further increase was noticed. The sugar was consumed vigorously leaving only 0.1 to 0.4 mg l^{-1} after three days of incubation. The rest of the sugar was exhausted after 89 hrs of incubation at aeration rates ½, 1, and 2 vol. air/vol. liquid/min (Table 1).

The Direct brown dye bioremoval had increased gradually throughout the experimental period using 300 ppm of the dye. The direct dye bioremoval reached the maximum being 84.60%, 80%, and 76.94% at aeration levels of ½, 1, and 2 vol. air/vol. liquid/min, respectively, after 24 hrs incubation (Table 2 and Fig. 1a). The removal rate after 10 hrs incubation (83.82%, 84.60%, and 76.90% for ½, 1, and 2 vol. air/vol. liquid/min, respectively) was very close to that after 24 hrs incubation (Table 2 and Fig. 1b). Results obtained with increasing dye concentration in the bioreactor indicated that the effects of airflow rate (1/8, ¼, ½, 1, 2 vol. air/vol. liquid/min) are important in bioremoval process. These results are in agreement with those of Sampa and Dutta (2004), who found that the increase in the airflow rate increased the supply of oxygen by enhancing turbulence, gas holdup, gas–liquid interfacial area, and the mass transfer coefficient. Therefore, the number of hydroxyl and superoxide radicals produced are also increased. Hence, the degree of photodegradation increased with the increase in the airflow rate. Bizani *et al.* (2006) studied the effect of aeration of the semiconductor suspension and evaluated the decrease in dye concentration when air is purged through the suspension and without purging. It was obvious that an improvement in the rate of decolorization is achieved in the presence of air.

Table (1): Residual sugar (mg l^{-1}) left after growth of fungal strain 20 on sucrose-yeast medium at different aeration rates.

Incubation time	Aeration rates (vol. air/vol. liquid/min)				
	1/8	¼	½	1	2
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

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

Incubation time	Removal time after dye addition	Aeration rates (vol. air/vol. liquid/min)				
		1/8	¼	½	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

Biomass inoculum | **Dis-azo dye concentrated 300 ppm** | **Dis-azo dye after 85% bioremoval**

Figure (1a): Decolorization of direct brown dye by *A. niger* strain at ½ v/v/min aeration rate after 24 hrs incubation.

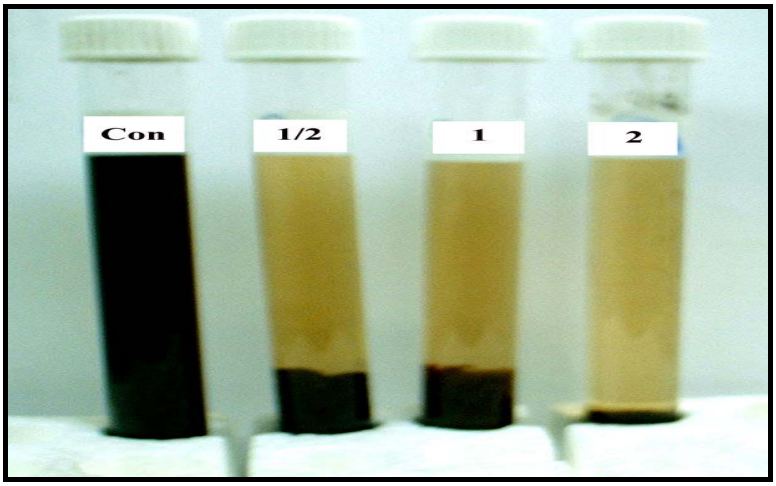

Figure (1b): Removal of direct brown dye by *A. niger* strain at ½, 1, 2 v/v/min aeration rate after 10 hrs incubation, con = control.

It was found that the rate of aeration at ½ vol. air/vol. liquid/min increased the dye removal percentages (Fig. 2) when 300 ppm dye concentration was added to the bioreactor two days after incubation. This, however, took place at lower percentage compared to samples taken after the same incubation period in treatments receiving additional 600 ppm of dyes in the fourth day of incubation (Table 3). This was accompanied by an increase in dry weight biomass. The rate of aeration at 2 vol. air/vol. liquid/min recorded higher percentage of dye removal (77%) with a decrease in biomass dry weight at the end of the incubation time. Similar results were obtained by Sampa and Dutta (2004), they found that the Methylene Blue and Eosin Y dyes photocatalytic degradation (PCD) increased with the increase in the airflow rate.

The results also indicate that the dry weight of growth with three flow rates of aeration was adequate until three days after the incubation time. The use of low rates of aeration, 1/8, ¼, and ½ vol. air/vol. liquid/min allowed better biosorption as compared with using high rate of aeration (1 and 2 vol. air/vol. liquid/min) in the case of Dis-azo dye bioremoval. Results in Table (3) show that using 1 and 2 vol. air/vol. liquid/min flow rate of aeration for longer incubation time (48 hours) resulted 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 vol. air/vol. liquid/min rate of aeration as compared with 300 mg^{-1} concentration. This decrease is probably due to the sugar limitation in the media which resulted in limited fungal growth after the consumption of the entire carbon source. The dye biosorption stopped after 48 hrs incubation at ¼ vol. air/vol. liquid/min rate of aeration, suggesting that dye-decolorization was mainly due to the biological fungal biomass, which depended on the aeration rate. Yuan and Bellgardt (1993) indicated that oxygen supply is one

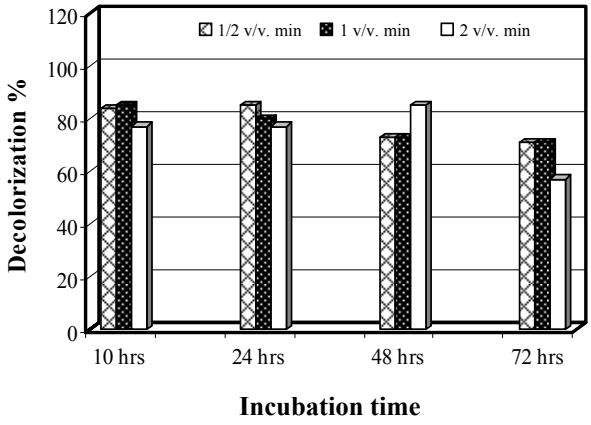

Figure (2): Effect of aeration on removing of dis-azo dye using fungal strain 20 (after addition 300 ppm dye (10, 24 hrs), and another 600 ppm (48, 72 hrs).

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

Incubation time	Removal time after dye addition	Aeration rates (vol. air/vol. liquid/min)				
		1/8	¼	½	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

of the main determining factors of yeast metabolism and is the limiting parameter for the production process. The effect of aeration on accumulation of fungal biomass on sucrose-yeast medium is shown in Figure (3). The highest dry weight of biomass was recorded with 1/8, ½ vol. air/vol. liquid/min aeration rate after five days incubation. The results also show that the values of dry weight for all aeration rates under investigation were close to each other after three days of incubation (Fig. 3).

Sampa and Dutta (2004) showed the fractional-removal of the reaction for Eosin Y dye at various airflow rates. 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 increased from 8.1 to 37.8%. At the end of the experiments, the COD was measured using a Hatc spectrophotometer test kit (HACH, CO). The results indicate that the fungal strains reduce the COD value of simulated dyeing effluent by 60 to 70 % with most aeration rates (Fig. 4). The percentage of degradation increased from 39 to 63% as the airflow rate increased from 0 to 11.3 l/min. The COD increased from 8.1 to 37.8%. For Methylene Blue, the percentage-removal increased from 50 to 58% as the airflow rate increased from 0 to 6.13 l/min. Percentage of COD-removal increased from 20 to 24% (Sampa and Dutta 2004).

Figure (5) shows the effect of aeration levels on biomass production and on pH changes during the 72 hrs incubation period. The pH showed continuous decrease with each sampling at all aeration levels until the end of the experiment (Fig. 5). The differences between the five aeration rates in the biomass formation were not marked until three days of incubation on the contrary to the changes in pH, which showed marked differences from the beginning of incubation

Growth efficiency of the fungal strain and dye removal activity, as affected by aeration rates, is illustrated in Table (4) based on the equation, $dx/ds = u \times x$ relating the biomass accumulation to sugar utilization under each aeration condition. Table (4) shows that the increase of aeration rates enhances the total biodegradation of sugar to CO_2 and water and does not support the biomass accumulation. The biomass was almost two and/or three times since the first dye application (48 hrs) till the end of the experiment (120 hrs). The observed trend of biomass decline with increasing the aeration, however, continued till 48 hrs. This indicates the synthetic dis-azo dye was not toxic to *A. niger* 20, which is tolerant to dye concentrations of 300 and 600 ppm. This fungus continued to grow and add new biomass. The increase of dye removal efficiency with higher aeration rates in both sampling dates indicates a certain dye biodegradation capacity by *A. niger* strain 20.

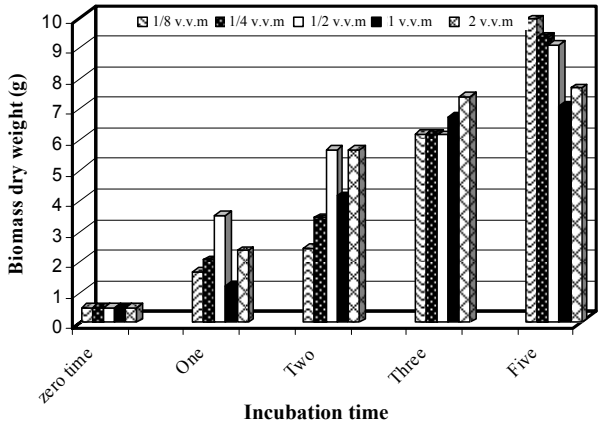

Figure (3): Effect of aeration on accumulation of fungal biomass grown on sucrose-yeast medium.

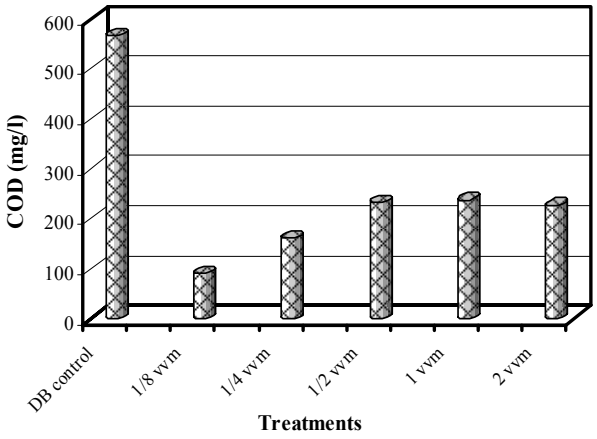

Figure (4): Reducing the COD value of dis-azo brown dye (DB) amended with sucrose yeast medium

It is documented that certain fungi are capable of biodegrading synthetic dye (Chao and Lee 1994; Fu and Viraraghavan 2001; Koumanova *et al.*, 2002; Bhole *et al.*, 2004). The anaerobic/aerobic cycle of growth media and its effect on dye biodegradation is now the subject of an intensive study and the results will be reported later. The optimization of aeration for dye bioremoval revealed that the rates of aeration using ½ vol. air/vol. liquid/min gave slightly higher removal compared with using a rate of 1, 2 vol. air/vol. liquid/min. For economical and practical considerations, however, the ½ vol. air/vol. liquid/min aeration rate would be

Table (4): Interrelation between aeration, *A. niger* biomass accumulation and dis-azodye removal in a batch bioreactor.

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

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

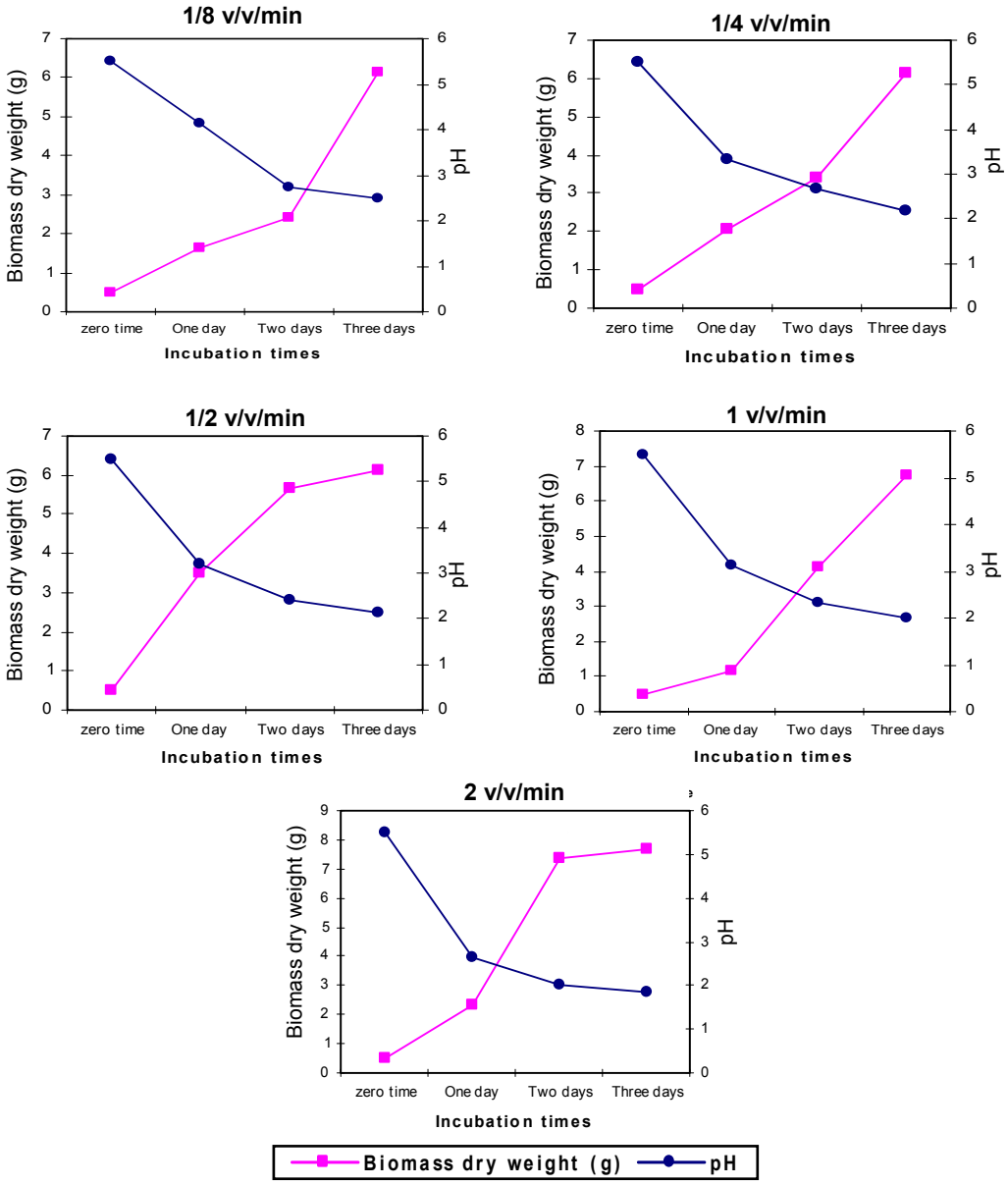

Figure (5): Effect of aeration on accumulation of fungal biomass and pH changes on sucrose-yeast medium.

sufficient for significant portion of dye bioremoval. The contribution of aerobic conditions to enhance the textile dis-azo dye bioremoval by increasing aeration was found to play an important role in dye bio-removal.

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Received August 31, 2006
Accepted February 3, 2007

CATRINA (2006), 1 (2): 119-126
PROCEEDINGS OF THE FIRST INTERNATIONAL CONFERENCE ON CONSERVATION AND MANAGEMENT OF NATURAL RESOURCES, ISMAILIA, EGYPT, JUNE 18-19, 2006

تفعيل إزالة الصبغات النسجية بالتهوية

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الملخص العربـــى

أجريت سلسلة من التجارب فى مفاعل حيوى لتفعيل أحد الصبغات النسجية البنية المباشرة باستخدام معدلات تهوية مختلفة. وقد استخدمت فى هذه الدراسة سلالة فطرية واحدة مع خمسة مستويات من التهوية. ونفذت سلسلة التجارب فى المفاعل الحيوى سعة خمسة لتر لمتابعة أداء إزالة الصبغة البنية بواسطة الفطر. وبمقارنة نتائج الإزالة لصبغة تحت ظروف تهوية مختلفة (8/1، ¼، ½، 1، 2 حجم هواء/حجم محلول صبغة/ دقيقة) وجد أن معدل التهوية ½ حجم/حجم/ دقيقة أحدث زيادة عالية فى الإزالة بنسبة وصلت إلى 72% وزيادة فى الوزن الجاف للكتلة الحية وصلت إلى 9.2 جرام فى نهاية فترة التجربة. وعند معدل تهوية 2 حجم/حجم/ دقيقة سجلت السلالة الفطرية نسبة معالجة وصلت إلى 77% مع نقص فى الوزن الجاف للكتلة الحية فى نهاية فترة التجربة. وأوضحت النتائج أيضا أن ثلاث معدلات تهوية سجلوا وزن جاف للكتلة الحية متقارب بعد ثلاث أيام من التلقيح. وتشير النتائج إلى أن معدلات التهوية المنخفضة (8/1، ¼، ½ حجم/حجم/ دقيقة) كانت افضل فى إزالة الصبغة بالمقارنة المرتفعة (1، 2 حجم/حجم/ دقيقة) ويمكن التوصية بإستخدام المعدلات المنخفضة لتفعيل إزالة الصبغة.