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Application of Bioluminescence for Appraisal of Fungal Bioremoval and Detoxification of a Textile Dye

Wafaa M. Abd El-Rahim[*], D. Abd El-Haleem[**] and H. Moawad[*]

[*]Agricultural Microbiology Department, National Research Centre, Cairo, and [**]Environmental Biotechnology & Bioprocess Development, Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Scientific Research and Technology Applications, New Burg-Elarab City, Alexandria, Egypt.

TEXTILE DYES are among the recalcitrant organic compounds in industrial wastes. The effect of these residues on the ecosystem depends on the way they are handled. In developing countries residues of coloring materials are usually discharged without treatment into the terrestrial and/or aquatic ecosystem. Bioremediation technologies appear to be promising to treat these residues prior to its disposal. Among these technologies, use of fungal biomass proved to be effective in removing dye residues. In this study, experiments were curried out to gain the highest production of fungal biomass to use in the bioremoval studies.

The results indicate that *Aspergillus niger* strain 20 was among the best biomass accumulators based on biomass yield and specific growth rate. In this study the ability of this strain to remove direct violet textile dye in concentrations ranging between 100 to 1000 mg/l was assessed. The removal capacity was analyzed using decolorization of dye solution and the toxicity of the degradation products.

The results show that the fungus biomass was able to remove 70 % of dye color in two hours from a range of concentration up to 800 ppm. The color removal decreased in higher concentration (above 800 mg/l), however, it was still relatively high. In the higher concentration of dye the removal has increased with time reaching close to 73% after 72 hr of incubation.

The bioreporter *Acinetobacter* DF4/PUTK2 carrying luciferase genes luxCDABE was employed to determine the amount of dye toxicity. The strongest bioluminescence inhibitory effect of the dye was recorded with the highest three dye concentrations. However, the rest of tested concentrations showed decrease in bioluminescence inhibition values, indicating a reduction of dye toxicity level. Chemical analyses indicate that fungal treatments reduced the COD (chemical oxygen demand) value of the dye solutions. This is

evidence that the fungal treatment was successful in dye bioremediation. The luminescence biosensor was proved to be fast technique for tracing dye removal and toxicity.

Keywords: Bioremediation, Textile dye, Fungi, Toxicity, Luminescence biosensor

Despite technological advances in the textile and dyeing industry, they also caused new and significant environmental concerns, as dye residues represent a major threat to the environment. Not all dyes currently used could be degraded and/or removed by physical and chemical processes, and there is a possibility that the degradation products become more toxic than the dye itself. These pollutants not only add color to water but they also may cause extensive toxicity to the aquatic and other forms of life. Nearly 10–15% of the total dyes from various textile and other industries are discharged in wastewater causing extensive pollution (Robinson *et al.*, 2001 and Keharia & Madamvar, 2003). Therefore, the treatment of industrial effluents containing dyes (aromatic compounds) becomes necessary prior to their final discharge. However, most of the conventional methods for the effective removal of phenols, aromatic amines and dyes are outdated due to their certain inherent limitations (Robinson *et al.*, 2001, Abadulla *et al.*, 2000, Sumathi & Manju, 2000, Knapp *et al.*, 1997 and Kapdan *et al.*, 2000) reported the ability of fungal strains to decolorize two dyes; Orange II and Everzol Turquoise Blue G. What are the current methods for renovation of industrial waste water from textile dye companies are still limited particularly in developing countries due to the lack of appropriate cost effective techniques.

Several toxicity and mutagenicity bioassays have been described and different methods using microorganisms have been used to assess the acute toxicity of industrial wastewaters. Among these bioassays are Ames test (Ames *et al.*, 1973) tests based on algae growth (Wei *et al.*, 2006) and bioluminescence tests (Marinella and Damia, 2003). The last test is based on the inhibition of the bioluminescence of luminescent bacteria *Vibrio fischeri* or *Photobacterium phoshoreum* as a factor of toxin removal (Bitton, 1983).

The present work aims at assessing the use of fungi as a bioremediation for certain textile dye residues using bioluminescence biosensor technique.

Material and Methods

Dye

One of the most commonly used textile dye namely direct violet RN (direct violet 31) was used. The dye was obtained from Ixmadye Dyestuffs and Chemicals Co. Removal of dye by the fungal culture was monitored using spectrophotometer LBK (model 4054). The λ_{max} value of the RN (direct violet 31) dye was 542 nm and the reduction in the absorbance value at this wavelength

was taken as an indication of color removal from the medium. The dye structure continues four cycling rings with two azo bounds and two sodium sulphonate groups.

Source of fungal strains used for biomass growth

Experiments were done in 500ml Erlenmeyer flasks with 300ml sucrose/yeast medium as described in Wafaa and Moawad (2003) to obtain the highest production of fungal biomass. The flasks were inoculated with a loop full of fresh fungal growth from slant and incubated on shaker (150 rpm) at 28°C for a period of 4 days. Inoculated flasks were visually examined at intervals 1, 2, 3 and 4 days for checking the fungal growth. For each fungal strain 5 flasks are used. Mycelia from the flasks were collected daily by filtration followed by drying at 105°C to determine the dry weight.

Dye bio-removal

A fresh slant was inoculated with a full loop of three days old *Aspergillus niger* strain, and incubated at 28 °C for 72 hr. The new fungal growth was used to inoculate 300 ml of sterile media (sucrose medium) containing: 10 sucrose, 0.5 g/l H_2PO_4, 0.2 g/l $MgSO_4$ $7H_2O$, 0.1 g/l NaCl (Wafaa *et al.*, 2003) in 500 ml round flask using sterile loop, and the flask was incubated on incubator shaker operated at 150 rpm at 28°C for 72 hr. The old *Aspergillus* biomass corresponding to 200 mg dry weigh was added to the direct violet synthetic dye in aqueous solution containing 0.5 g/l H_2PO_4, 0.2 g/l $MgSO_4$ $7H_2O$, 0.1 g/l NaCl in 250 ml flasks containing aliquots of 50 ml dye solutions. Different concentrations of dyes (100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 mg/l) were tested. The flasks were shaken on incubator (28°C) at 150 round per minute (rpm) after adjusting pH to 5.5. At the end of incubation intervals (2, 4, 24, 48 and 72 hr), fungal pellets were removed by centrifugation at 10000 rpm for 10 minutes and supernatant was analyzed for the residual dye using spectrophotometer at 542 nm wavelength. The experiments were carried out in triplicates and means were presented. Decolorization activity (%) was calculated according to the formula:

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

Measuring of chemical oxygen demand (COD)

The COD of the treated direct violet dye broth samples free biomass of fungi was measured at different concentrations at the end of the incubation period using a Hatc spectrophotometer test kit (HACH, CO). Increasing dye concentration in solution has resulted in increase of COD value which shows correlation between dye concentration and COD. Two ml of samples were added to the kit vial. Using the deionized water 2 ml as blank. Then digest them for 2 hr at 150 °C (HACH digester), wait for twenty minutes to allow the vials to cool. This test was done using a Hatc spectrophotometer.

Bioluminescent bioreporter and toxicity assay

The bioluminescence toxicity assays were performed using the bioreporter *Acinetobacter* DF4/PUTK2 that had been genetically modified by conjugation (Abd-El-Haleem *et al.*, 2006) to contain the plasmid PUTK2 (Burlage *et al.*, 1990), with the Tn4431 *lux* transposon downstream of a putative plasmid maintenance promoter to produce continual visible light so-called bioluminescence. Bioluminescence was measured at 5 min regular time intervals from zero to 480 min by a luminometer (LumiStar, Galaxy, BMG, Germany). For the toxicity assay, a culture of the bioreporter strain DF4/PUTK2 was grown in LB medium (10 Bacto-Tryptone, 5 Bacto-yeast extract and 10 NaCl g/L) on an orbital shaker at 200 rpm at 30°C for 16 hr. Subsequently, the cells were diluted 1:10 in LB medium and incubated on an orbital shaker at 30°C to achieve an optical density 0.6 measured with a spectrophotometer (Spectronic Genesys 5; Spectronic Instruments, Rochester, NY) at 600 nm wavelength. After two washes in a sterile LB medium, the pellet was resuspended in 1 ml sterile LB.

Subsequently aliquots 100 µl of each sample mixed with 100 µl of a $PUTK_2/DF_4$ cell suspension were transferred to 96-well microtiter plates (Nunc) to produce 4 replications of each sample. Along with each treatment two controls were incorporated into each plate, the first contained 100 µl from the bioreporter DF4/PUTK2 mixed with 100 µl MilliQ water (no toxicity control), while the second contained the bioreporter mixed with the original samples (non diluted toxic substances). Wells were then covered with transparent plate sealer and placed in the luminometer for luminescence detection at room temperature. In all measures, the percent of bioluminescence inhibition (BI %) was determined by comparing the response given by a control (no addition) to that corresponding with the sample (Farré and Barceló, 2001).

Results and Discussion

Screening of Aspergillus niger strains for biomass accumulation

Ten fungal strains previously isolated, screened for textile dyes removal (Wafaa, 2000) were assayed for biomass production to select the highest biomass producer. The fungal strains were grown on sucrose/ yeast medium since many researchers identified sucrose as the optimal substrate for fungal biomass production (Eva *et al.*, 1990, Cui *et al.*, 1998, and Thorsten *et al.*, 2000).

The biomass accumulation of the fungal strains was examined at intervals of 1, 2, 3 and 4 days from inoculation. Data in Table 1 show that the highest strains in biomass accumulation after three days of incubation were strains number 20, 31 and 21. The dry weight of biomass reached up to 270.4, 245.1 and 223 mg/300ml of media respectively. The specific growth rates in Fig. 1 for five strains (1, 2, 20, 31 and 53) were in the range of 0.041 - 0.042 h^{-1} which indicate the fast nature of growth and biomass accumulation compared to the rest of strains. Youn *et al.* (2002) studied the characteristics of *Paecilomyces sinclairii*

grown on starch and on sucrose media and found that the specific growth rate in sucrose medium being 0.04 h^{-1} was higher than that in starch medium.

After four days of incubation the biomass accumulation reached 283.2, 252.9, 249.3, 243.4 and 240.3 mg/300ml on sucrose/yeast medium for strains 20, 2, 39, 5, 1 and 37 respectively. Richard (2006) studied the optimal substrates for the *in vitro* growth of *Morchella elata* using sucrose, mannose and lactose as carbon sources. He found that the rapid growth was most reliably achieved in a composite medium containing sucrose + mannose (1:1). Results in Table 1 show that the biomass yield obtained in strain number 24 was the lowest throughout the experimental time. On the other hand, the strain *A. niger* 20 was among the best biomass accumulators based on biomass yield in Table 1 and specific growth rate in Fig. 1. therefore it was selected for dye bioremoval study.

TABLE 1. Growth and accumulation of fungal biomass on sucrose/yeast medium.

Strains	Time of incubation/ days			
	One	Two	Three	Four
Aspergillus flavus No.1	0	128.3	208.1	243.4
Aspergillus flavus No. 2	0	118.2	213.7	252.9
Aspergillus ochrocous No.5	10.3	147.0	176.9	194.6
Aspergillus niger No. 20	0	117.6	270.4	283.2
Aspergillus terres No.21	61.5	189.9	204.2	222.9
Botryodiplodia spp No. 24	41.8	86.4	96.6	152.6
Aspergillus niger No.31	0	117.6	245.1	245.5
Aspergillus niger No.37	35.9	158.0	183.3	240.3
Aspergillus niger No.39	119.5	162.3	203.4	249.3
Aspergillus parasiticus No.53	20.0	146.2	210.0	213.2

*Figures in above table represent the dry weight of biomass (mg/ 300 ml medium).

Dye bioremoval with time using different initial dye concentration

As previously pointed out based on the capacity of the fungal strains to accumulate biomass in short time, *A. niger* strain 20 was identified as promising strain for decolorization studies. The relation between initial direct violet dye concentrations and color removal efficiency using this strain was tested. It is agreed that the reliability of any bioremediation system depends on the capacity of such system in tolerating the increased concentrations of the targeted pollutant in the media. The synthetic dye containing solutions were prepared to assess the effect of ten concentrations of fungal biomass on dye removal capacity. The concentrations used ranged between 100 mg l^{-1}dye to 1000 mg l^{-1} dye. At least half of the tested concentrations (over 500 mg l^{-1}) are considered high enough than any possible dye discharge in the industrial plants effluents at one time (Peter, 1995).

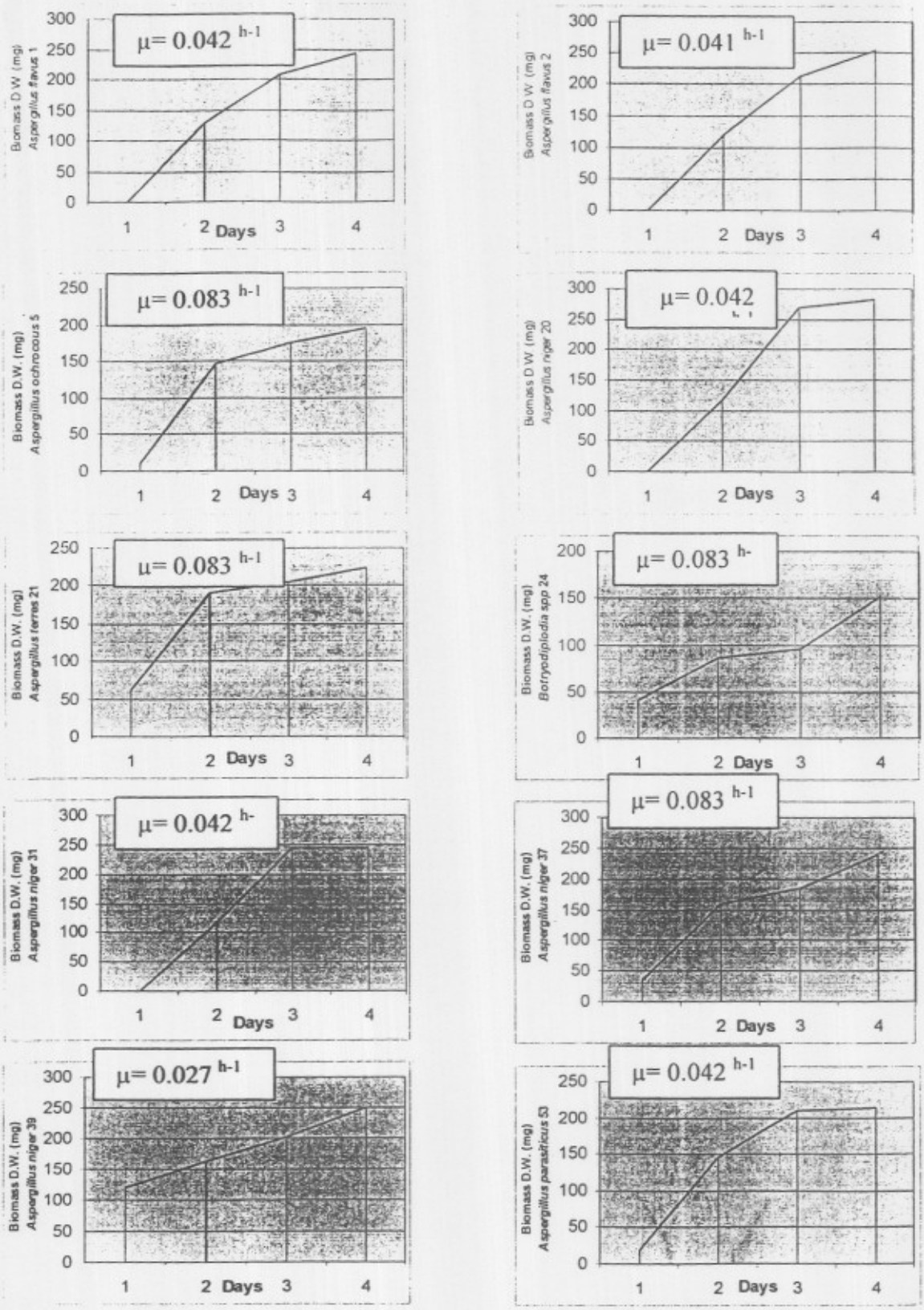

Fig. 1. Growth curve and growth rate value of the ten tested fungal strains on sucrose-yeast medium.

The results of color bioremoval changes with time in Table 2 clearly show that the identified bioremoval system using *A. niger* 20 biomass is capable to remove more than 70% of the dye in two hours retention time at wide range of dye concentrations in the solution (100-800 mg l^{-1} dye). This indicates that the tested bioremediation system meets the requirements of biotreatment of dye residues in terms of tolerance to elevated dye concentrations and quick removal of the dye from aqueous solutions. With the exception of the highest two concentrations 900 and 1000 mg l^{-1}, dye incremental doses were removed until 72 hrretention time. However, the increases were modest as compared with the high removal rate during the first two hours. At the highest two concentrations of dye namely 900 and 1000 mg l^{-1} the marked increase in bioremoval took place later, being 70, 88 and 89% at 24, 48 and 72 hr respectively in the 900 mg l^{-1} concentration; whereas, for the concentration of 1000 l^{-1} the maximum bioremoval was achieved after 72 hr retention time and amounted 73% only. The late increase in dye bioremoval in the highest two concentrations may be due to the time required by fungal biomass to adapt to the high doses of dye and possibly to the required time for the fungal biomass surface to handle high dye concentration. The upper limit of the dye concentration (900 and 1000 mg l^{-1} dye) exceeds twice the recommended dye concentration used in the industry for coloring textiles (Moawad *et al.*, 2003). The *Funalia trogii* cultures tested with an initial concentration of biomass of 370 mg/50 ml and 13 mg/l dye concentration retained decolorization activity for 5 days, 370 mg/ 50 ml pellet and 264 mg/l dye containing cultures showed good decolorization performance for only 2 days (Ozfer *et al.*, 2003). In addition, the results are in agreement with those obtained by Jo-Shu & Tai-Shin (2000) and Kobya & can (2003) who studied the decolorization of reactive and azo dyes solutions. They found that the decolorization rate was in high azo dye concentrations accelerated, and the maximal rate occurred at 2000 mg/l of dye. None of the dye treatments were totally cleared from the dye color after fungal bioremoval. This may be due to certain equilibrium between bio-sorption and bio- desorption of dyes in the solution (Fine *et al.*, 1999, and Victor & Flavia, 2004).

The most important finding in this experiment is the capacity of this bioremoval system to tolerate high doses of dye pollutants discharged into the media. Adosinda *et al.* (2001) while studying the azo dyes as important chemical pollutants of industrial origin found that the removal of dyes through biodegradation depended on the dye structure and concentration. The biosorption of dye on fungal biomass has been reported. The isotherm data of biosorption was reported to fit into Freundlich model suggesting the heterogeneous nature of this process (Rachna and Sumathi, 2008).

TABLE 2. Changes in bioremoval of direct violet dye by *A. niger* 20 with time (h) at ten initial concentrations (%).

Initial dye concentration (mg/l)	2h	4h	24h	48h	72h
100	75.5	78.7	78.9	79.5	86.9
200	72.0	72.9	86.4	86.5	93.3
300	88.0	88.0	92.4	92.5	95.5
400	80.0	78.3	94.3	94.5	96.6
500	73.6	78.4	93.8	94.2	96.2
600	76.9	79.4	92.4	93.5	97.6
700	74.5	77.2	89.6	90.8	97.7
800	73.2	77.4	79.3	81.2	85.7
900	46.9	53.2	70.4	87.6	88.9
1000	49.8	54.9	57.9	61.0	73.1

Tracing dye toxicity

The environmental protection against the harmful industrial wastes requires fast and reliable techniques to trace the wastes in order to apply the necessary bioremediation measures in the appropriate time. In addition, the proper dye removal management should be based on accurate and reliable measurements of dye residues in the aqueous solutions. The ten concentrations of direct violet dye used in the above section after their bio-treatment was evaluated using two techniques. These two techniques were tested for tracing the textile dye residues. One is based on bioluminescence bioreporter and the other on the chemical oxygen demand (COD). Both techniques are gaining wide acceptance in environmental studies.

For the first time, Kahru *et al.* (2000), proposed bioluminescent bacteria as whole-cell biosensors for the toxicity monitoring. The advantages of a bioluminescent bacteria-based toxicity assay include; a very conveniently measured signal, a short exposure time, and cost effectiveness (Ren e*t al.* 2003). The most thoroughly studied luminescent bacteria-based bioassay for aquatic toxicity testing is the Microtox assay marketed by Azur Environmental (Carlsbad, CA). The bacterium used in the Microtox assay is *Photobacterium phosphoreum*, a marine bacterial strain which is excessively sensitive to many toxicants compared to other methods (Ren *et al.*, 2003).

However, in the present work a recently constructed *Acinetobacter* bioluminescent bacterium strain designated DF4/PUTK2 (Abd-El-Haleem *et al.*, 2006) was employed to monitor dye toxicity. Toxicity assaying using bioreporter DF4/PUTK2 might serves as a realistic indicator of the environmental toxicity. Species of *Acinetobacter* have been attracting increasing attention in environmental applications. Some strains of this genus are known to be involved

in biodegradation of a number of different pollutants such as biphenyl and chlorinated biphenyl, amino acids, phenol, benzoate, crude oil, acetonitrile, and in the removal of phosphate or heavy metals (Abd-El- Haleem, 2003).

The bioluminescence assay can in shortest time (5 min only), show the response of the bioreporter strain $DF_4/PUTK_2$ to the toxic effect of certain pollutants. Similar bioassay methods were based on microbial bioassays and biosensors (Tothill and Turner, 1996, Ren and Frymier (2003). To improve the efficiency of the assays Stanislaw *et al.* (2001) determined the toxicity of the dye in terms of effective concentration EC_{50} (concentration of a toxic material that induces a response halfway between the baseline and maximum) using mixed cultures of activated sludge as well as pure culture of luminescent bacteria *Vibrio fischerii* NRRLB-11177.

In dye toxic studies, the percent of the bioluminescence inhibition (BI %) that reflects the changes in the level of toxicity was determined by comparing dye containing media and media treated for dye removal. In other words, the presence of high luminescent activity in dye treated medium indicates no toxic effect of the dye on the growth of bacterial culture, whereas the decreases in bioluminescence show that the bacteria were adversely affected by the toxic waste. Increase in bioluminescence inhibition (BI %) means that the toxicity is high (Abd-El-Haleem, 2003). In this study, Fig. 2 shows that the bioluminescence of the bioreporter strain DF4/PUTK2 was increased over the control after dye bioremoval by fungal biomass within the initial dye concentrations range from 100 to 800 ppm. Dyes exhibit low toxicity to mammals and aquatic organisms (Churchley, 1998). Only 2% of about 300 colorants tested by Clarke and Anliker (1984) had an LC_{50} for fish lower than 1.0 mg l^{-1}, whereas around 96% of compounds had values above 10 mg l^{-1}. (LC_{50} refers to dose required to kill half the members of a tested population). Also Wafaa *et al.* (2008) investigated the genotoxicity of the azo dye 'Direct Violet' and the removal of this dye by *Aspergillus niger* strain at different conditions in male rats. They suggested that *Aspergillus niger* was able to prevent the genotoxic effect of the direct violet-induced genotoxicity in male rats through its ability to remove the excess of the toxic elements of direct violet.

The results show that the dye without fungal biomass treatment induced severe bioluminescence inhibition (BI%) which reached 61% as compared with the bioluminescence in water without any dye. Moawad *et al.* (2003) evaluated the toxicity of eight textile dyes using Ames test bioassay and six concentrations of each dye. They found that, most of the dyes were mutagenic for the test *Salmonella typhimurium* strains used in the study. In addition, they found that the high concentrations of dyes eliminated microbial colonies due to the high-frequency of mutation causing lethal death of the cells. Few studies have evaluated toxicity changes after biological dye decolorization. Some studies have shown that decolorization could lead to less toxic end-products (Abadulla *et al.*,

2000). Wafaa and Moawad (2003) tested the possible toxicity of the remaining supernatant after dye removal with fungal biomass using Ames test to assess the residual mutagenic effect remaining after dye removal. Three strains of *Salmonella typhimurium* (TA 1535, TA 1537, TA 1538) were included in this assay. They found that the toxicity of the dyes measured by Ames test could be removed by the dye sorbed on the fungal biomass.

As shown in Fig. 2, it was interesting to observe that bioluminescence of the bioreporter strain DF4/PUTK2 increased (indicating no toxicity) over the entire exposure period from zero to 480 min with concentration ranging between 100-800 ppm. Wang *et al.* (2002) studied the toxicity of 11 reactive dyestuffs from a textile dyeing mill in Ayazaga, Istanbul, Turkey. They found that the inhibition effects of numerous dyestuffs to luminescent bacteria differed considerably with the concentration. In addition, Rehorek *et al.* (2004) studied the application of ultrasound treatment on azo dye degradation and they found that the ultrasound was able to mineralize azo dyes to non-toxic end products, which was confirmed by respiratory inhibition of luminescent bacteria test of *Pseudomonas putida*.

Among the widely used techniques in environmental studies is the measuring of chemical oxygen demand (COD) Abu El-Reish (2005). The increase of COD indicates the presence of heavy organic load in the media. The results indicate that the treatments with fungal biomass could greatly reduce the COD value of the treated dye solutions (Fig. 3) . The treatment of ten direct violet dye initial concentrations with fungal biomass reduced COD values markedly at different rates depending on initial dye concentration. The largest reduction in COD in fungal treated dye solutions was correspondent to the highest dye removal (Table 2). When the color was removed by 86.9 to 97.7% in the dye solution after 72 hr (Table 2) following the treatment with fungal biomass COD was also decrease from 27 to 330 mg l^{-1} (Fig. 3). The *A. niger* biomass reduced the COD values by 58 to 99% at initial dye concentration 100 and 900 mg l^{-1}, respectively. This shows that the concentration of dye is important factor in dye removal from the dye containing media.

The results also show that both bioluminescence and COD techniques are reliable for evaluating the toxic effect of textile dyes. These results confirmed that the fungal treatment was successful in dye bioremoval and the bioluminescence toxicity assay could be used as fast technique for tracing dye removal and toxicity. This suggests the appropriateness of using these techniques in environmental management studies particularly when these studies are dealing with textile dye residues. The main focus of this work was the removal of dyes by fungal biomass. The disposal of the fungal biomass-dye complex must be taken into consideration either by desorption of the dyes from the biomass which can be used again or by developing powerful biodegradation systems to decompose the complex to release its elemental components.

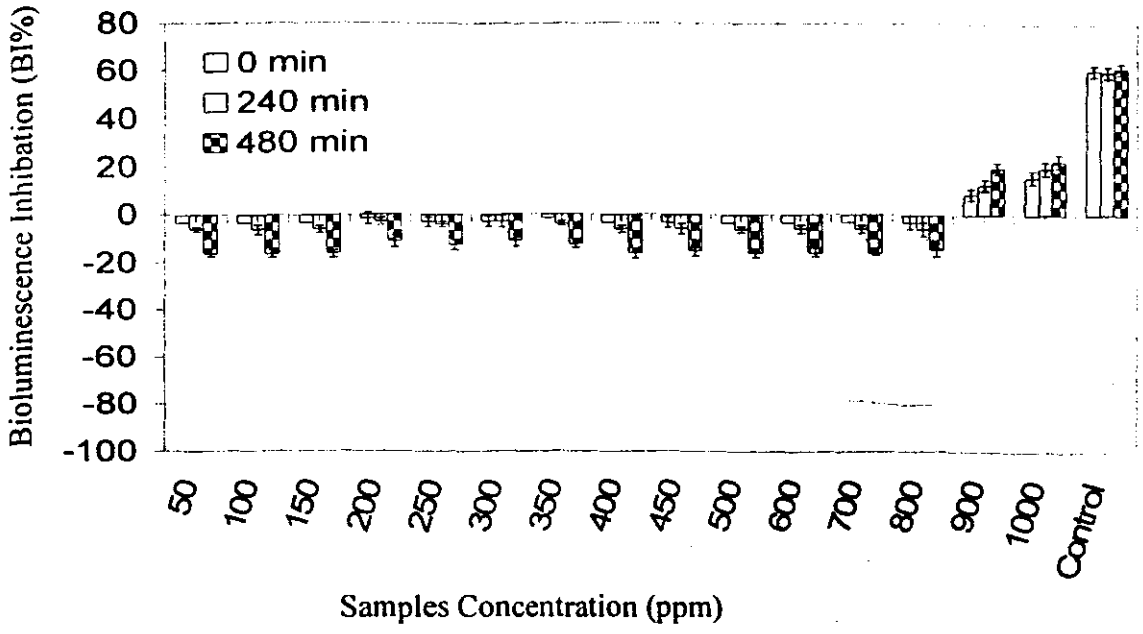

Fig. 2. Changes in bioluminescence inhibition percentages of bioreporter *Acinetobacter* DF4/PUTK2 during toxicity monitoring of treated dye solution. The values of the assay are means of four replications. Error bars represent standard error of the mean (n = 4). Control indicates the bioreporter mixed with non-diluted dye sample.

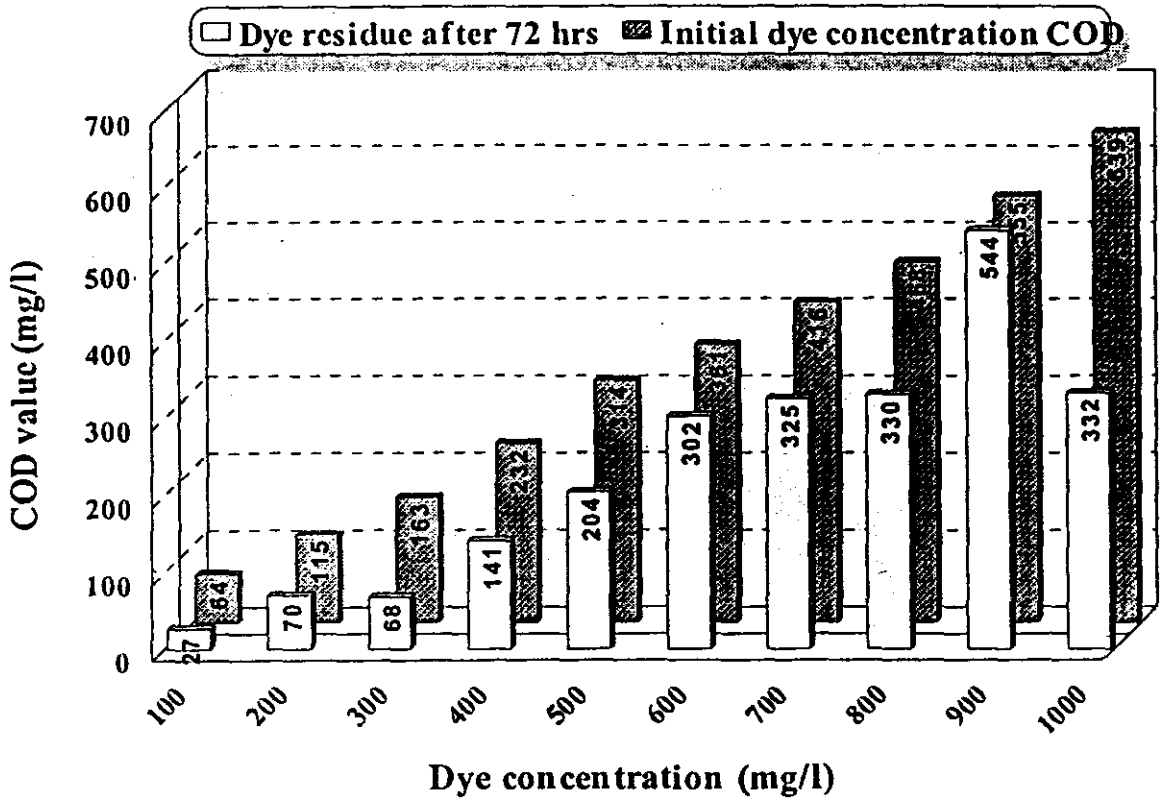

Fig. 3. Changes of COD in water based direct violet dye after biotreatment with *A. niger*.

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استخدام المجس الحيوى لتقييم الإزالة الحيوية بالفطريات لإزالة التاثير السام لأحد صبغات النسيج

وفاء محمد عبد الرحيم*، دسوقى عبد الحليم** و حسن معوض*

*قسم الميكروبيولوجيا الزراعية – المركز القومى للبحوث – القاهرة و**قسم التكنولوجيا الحيوية البيئية - معهد الهندسة الوراثية والتكنولوجيا الحيوية – مدينة مبارك للأبحاث العلمية والتطبيقات التكنولوجية – الاسكندرية – مصر .

عرفت العملية الحيوية كوسيلة سهلة من الوسائل ذات الكفاءة العالية لمعالجة مدى واسع من المخلفات العضوية الصناعية. وتعتبر الصبغات النسجية من المركبات العضوية صعبة التحلل التى تشكل مكوناً أساسيا من المخلفات الصناعية. وعادة ما تخرج متبقيات تلك الصبغات مع مخلفات الصناعات بدون معالجة حيث تصل فى النهاية إلى التربة او البيئة المائية. ومن المعروف إن الأصباغ مركبات كيميائية معقدة مقاومة للتحلل الحيوى.

وقد تم الحصول على أعلى إنتاج للكتلة الحية وافضل معدل نمو لفطر الاسبرجلس نيجر 20 *Aspergillus niger* حيث هدفت هذه الدراسة إلى إختبار قدرة سلالة من سلالات فطر الاسبرجلس نيجر *Aspergillus niger* على إزالة الصبغة البنفسجية من النوع المباشر بتركيزات تراوحت من ١٠٠ إلى ١٠٠٠ مجرام /لتر. وقد تمت متابعة قدرة السلالة على إزالة اللون فى محاليل الصبغة المختبرة وكذلك دراسة سمية المحلول الناتج من المعالجة.

وقد أظهرت النتائج ان السلالة الفطرية كانت قادرة على إزالة الصبغة بنسب تراوحت بين ٧٠٪ خلال ساعتين. إلا أن إزالة اللون كانت اقل عندما زادت تركيزات الصبغة عن ٨٠٠ مجرام / لتر، ولو ان إزالة اللون ما زالت مرتفعة نسبيا وتراوحت بين ٤٦,٩- ٧٣,٢ ٪ من الصبغة الموجودة بالمحلول. وقد زاد معدل إزالة الصبغة فى المحاليل المحتوية على تركيزات مرتفعة بمرور الزمن حتى سجلت تقريباً ٧٣ ٪ بعد ٧٢ ساعة من التحضين. وإتضح من دراستنا السابقة أن إزالة اللون تستند فى المراحل الأولى على عملية امتصاص الصبغة على الكتلة الحية للسلالة الفطرية ثم يتبع ذلك عملية تكسير للصبغة بعد الامتصاص.

وتم فى هذه الدراسة أيضاً متابعة سمية الصبغات باستخدام تقنية المجسات الحيوية الضوئية باستخدام bioluminescence biosensor. bioreporter *Acinetobacter* DF4/PUTK2 لتقييم التأثير السمى لبقايا الصبغة البنفسجية من النوع المباشر بعد معالجتها حيوياً بواسطة فطر الاسبرجلس نيجر للتأكد من إنحسار سميتها بعد المعالجة. واظهرت النتائج المتحصل عليها ان المعالجة الحيوية لمعظم التركيزات المختبرة باستخدام فطر الأسبرجلس أدت إلى نقص ملحوظ فى تثبيط نمو bioluminescence ماعدا أعلى ثلاث تركيزات (٨٠٠، ٩٠٠، ١٠٠٠ مجم/ لتر) . ويدل ذلك على ان المعالجة الحيوية لهذه الصبغة صحابها انخفاض كبير لسمية المحاليل بعد المعالجة. كما أوضحت النتائج ايضاً أن المعالجة الفطرية ساهمت فى خفض قيم ال Chemical Oxygen Demand (COD) فى المحلول المعالج نتيجة لإزالة الحمل العضوى الناتج عن تواجد الصبغة.