Biodegradation (2012) 23:243–251
DOI 10.1007/s10532-011-9503-z

ORIGINAL PAPER

Assessment of the efficacy of *Aspergillus* sp. EL-2 in textile waste water treatment

Ola M. Gomaa · Hussein Abd El Kareem · Reham Fatahy

Received: 21 February 2011 / Accepted: 29 July 2011 / Published online: 7 August 2011

Abstract Fungal biomass has the ability to decolorize a wide variety of dyes successfully through a number of mechanisms. A brown rot isolate, previously identified as *Aspergillus* sp. EL-2, was used in the aerobic treatment of textile waste water efficiently. In the current work, the treated waste water was tested chemically using more than one combined treatment. Microbial toxicity, phytotoxicity, genotoxicity and cytotoxicity were also studied to assess the toxicity level for each treatment. The obtained data suggest that the contribution of more than one mode of treatment is essential to ensure complete destruction of the by-products. The use of gamma irradiation (25 kGy) after the bioremediation step led to the decrease of the by-products of biodegradation as observed by visible spectrum and Fourier transfer infra red spectroscopy (FT-IR). The toxicity assessment presented variable results indicating the need for more than one toxicity test to confirm the presence or absence of hazardous compounds. Brown rot fungus could be used efficiently in the treatment of textile waste water without the risk of obtaining high carcinogenic or genotoxic compounds, especially if combined treatment is employed.

O. M. Gomaa (✉) · H. A. E. Kareem · R. Fatahy
Microbiology Department, National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt
e-mail: ola_gomaa@hotmail.com

Keywords Textile waste water · Assessment · Brown rot fungi · Gamma radiation · Combined treatment · Toxicity tests

Introduction

The increase in industrial activities is undoubtedly concomitant with an increase in environmental pollution, the major concern in these environmental problems are the decrease in potable water versus the release of waste water; therefore, the choice to employ treated waste water for non-potable uses has become the answer and leave the clean water for drinking purposes. The textile industry in Egypt is one of the oldest industries, it includes 3,000 factory and is among the major three industries releasing tons of waste water (EEAA report 2003). The chemical structure of synthetic dyes used in the textile industry are based primarily on substituted aromatic and heterocyclic groups such as aromatic amines, these aromatic amines are suspected carcinogenic and/or genotoxic compounds (Nyanhongo et al. 2002).Therefore, improper disposal of textile dyes is considered a major environmental problem, which leads to disturbances in the ecosystem (Ambrosio and Campos-Takaki 2004).

In the past, only one method of conventional waste water treatment was employed for color removal, methods such as chemical or physico-chemical treatment (coagulation, electrolysis, ozonation…etc.,) could be considered as inefficient, expensive, or of

limited applicability (Merzouk et al. 2009). On the other hand, biological methods are cost effective and applicable (Chen et al. 2003b; Sirinivasan and Murthy 2009; Jadhav et al. 2010; Ramalingam et al. 2010). Both white rot and brown rot fungi could be employed efficiently in biological treatment of textile dyes. But biological treatments also have their drawbacks which involve the transfer of the dye onto biomass via adsorption or the incomplete destruction of the dye molecule which leaves carcinogenic by-products as potential hazard (Kaushi and Malik 2009). The use of more than one treatment method might provide complete destruction of the dye without producing toxic waste (Brosillon et al. 2008). This new trend is gaining more attention due to the ability to combine the positive outcome of one or more method(s) and/or eliminating the drawbacks of another.

Radiation technology has been recognized as a promising process for waste water treatment for its simplicity and efficiency (Ma et al. 2007), given that if combined with another conventional method of treatment, an additional economical advantage would be gained (Földváry Cs and Wojnárovits 2007). While color removal and complete destruction of dye molecules is important, the toxicity assessment of dyes and dye intermediates has become an extremely important matter, especially that most dyes have no safety data sheet (Mathur et al. 2005). Therefore, detoxification of the treated effluent should be coupled to color removal (Di Gregorio et al. 2010). The carcinogenic tests could be performed through detecting microbial changes, phytotoxicity or chromosomal aberrations in mammalian cells (Chen et al. 2003a; Schnieder et al. 2004; Mathur et al. 2005).

The brown rot fungus *Aspergillus* sp. EL-2, characterized in a previous work (Gomaa et al. 2011) as a promising and efficient organism in bioremediation of textile waste water, is further investigated in the current work in terms of assessment of the level of toxicity of single and combined treated textile waste water for its potential use in non-potable applications.

Materials and methods

Textile waste water

The textile effluent was collected in sterile containers from an Egyptian company for textile dyeing and printing located in the 6th of October district, 40 km outside Cairo, Egypt. The samples were obtained at the end of a dyeing process of polyester. The dye used was disperse blue 60 which had a final turquoise color; the samples were stored at 4°C. The pH value was 6.1. The maximum absorbance was detected using a Schimadzu UV 2100 spectrophotometer, the absorbance maxima was scanned in the range from 400 to 700 nm. The waste water was used for the cultivation of the fungi without any treatment or dilution.

Microorganism and cultivation conditions

A previously isolated brown rot fungus, identified earlier as *Aspergillus* sp. EL-2 capable of decolorizing textile waste water (Gomaa et al. 2011) is used in the following study. The fungus was grown on GYP medium which consisted of glucose (20 g/l); yeast extract (5 g/l); peptone (5 g/l) and magnesium sulfate (1 g/l). Once inoculated, flasks were incubated on a rotatory shaker at 150 rpm and 30°C for 48 h. This pre-inoculum was used to inoculate the waste water for the decolorization experiments.

Experimental set up

Four groups were set, A: waste water with no treatment, this was used as the negative control, B: waste water inoculated with *Aspergillus* sp. EL-2, C: waste water inoculated with *Aspergillus* sp. EL-2 and another *Aspergillus* sp. (isolated from soil) for a combined effect, D: waste water treated with *Aspergillus* sp. EL-2 and treated with a high radiation dose of 25 kGy as a combined biological and physical treatment. The flasks were incubated at 30°C and 150 rpm for 48 h, after which all flasks were filtered using Mira cloth to remove the fungal pellets and the filtrate was used in the following experiments.

Gamma radiation

A 50 ml of biologically treated cultures were used for gamma radiation experiments. Gamma irradiation was performed at the Indian cobalt source located at the National Center for Radiation Research and Technology (NCRRT) for the liquid cultures. The cultures were subjected to 25 kGy at a dose rate of 1.93 Gy/s.

Analysis of the waste water

Decolorization and waste water spectrum

The dye decolorizing ability of the local strain under all experimental conditions was calculated by the following equation:

$$\frac{A_\circ - A}{A_\circ} \times 100$$

$A_\circ$ is the initial dye absorption on the day of inoculation, A is the final dye absorption after 48 h of incubation. Spectrophotometric assays were performed using a Schimadzu UV 2100 spectrophotometer. The decolorization experiments were performed in triplicates and the results recorded are the mean value of three independent experiments.

The remaining filtrates of all flasks were compared by spectroscopic analysis (400–700) nm. The spectrum was recorded as absorbance and was plotted against wavelength.

Fourier transfer infra red spectroscopy (FT-IR)

The IR spectra of all the filtrates were recorded using FT-IR spectrophotometer (Mattson 1000 FT-IR, England). Ethyl acetate was added to the culture filtrates in the ratio of 1:1 to extract the dyes, 1 ml of each culture extract was dropped on to clean glass slide covers, and were left to dry at room temperature. Cover slides were placed above the beam, the FT-IR spectra were recorded using refractive measurements and plotted as wave number versus transmittance using WinFirst software.

Toxicological studies

Microbial toxicity

Escherichia coli was used to detect microbial toxicity according to Ambrosio and Campos-Takaki (2004). *E. coli* was grown in nutrient broth media overnight, 1 ml (cell count 10^5) was poured in a plate and nutrient agar media was poured on top. The plates were incubated at 30°C overnight. About 4 mm discs of sterilized filter paper were immersed in waste water, bioremediated waste water and combined biological and gamma irradiated treated waste water, each group of filter paper were left to dry and then placed under sterile conditions on top of the *E. coli* grown Petri dishes. The toxic effect was monitored as the inhibitory zone forming around each disc. Each group of plates contained at least 4 filter paper discs.

Phytotoxicity test

Trigonella-foenum-graecum seeds were placed on sterile Petri dishes with cotton inside. The seeds were placed evenly onto the plates. Each group contained 10 seeds. The plates were irrigated with equal volumes (20 ml) of water, waste water, bioremediated waste water and combined biological and gamma irradiated treated waste water. The germination percentage and the shoot length were monitored every day for all groups.

Cytotoxicity assay

The cytotoxic effect of water samples against hepatocellular carcinoma (Hep-G2) cells (ATCC, VA, USA) was estimated by the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (Hansen et al. 1989). Cells (5×10^4 cells/well) were incubated with various volumes of the water samples at 37°C in a fetal bovine serum FBS-free medium, before submitted to MTT assay. The absorbance was measured with an ELISA reader (BioRad, München, Germany) at 570 nm. The data are expressed as the mean percentage of viable cells as compared to the respective control untreated cells. The half maximal growth inhibitory concentration IC50 values were calculated from the line equation of the dose-dependent curve of each sample compared with positive control (CP) paclitaxel**.**

Genotoxicity

Chromosome aberrations Chromosome preparation from mouse bone-marrow was carried out according to the method of Yosida and Amano (1965). Bone-marrow cells were collected from both femurs in 6–8 ml hypotonic solution (0.075 M) of KCl. The cell suspension was incubated for about 20 min at 37°C, and then centrifuged at 1,000 rpm for 10 min. The cells were re-suspended in freshly-prepared cold fixative (methanol: acetic acid; 3:1; V/V), and centrifuged again for 10 min at 1,000 rpm. The fixation step was repeated three times. The cells were

re-suspended in fixative and were spread by dropping the concentrated cell suspensions onto slides that had just been removed from a freezer. After complete drying, the slides were stained with 10% Giemsa in phosphate buffer (pH 6.8) for 35–40 min, washed for 10 min in phosphate buffer, and air dried. Hundred well-spread metaphases were analyzed per animal. Metaphases with gaps, chromosome or chromatid breakage, fragments, deletions as well as numerical aberrations (polyploidy) were recorded.

Mitotic index The prepared slides for chromosomal aberrations were used to determine the mitotic index (MI), which based on the scoring of 1,000 cells for each animal. The number of dividing cells including prophases and metaphases was recorded. The MI (number of dividing cells/1,000 cells) was calculated.

Results and discussion

Analysis of the treated textile waste water

Decolorization of textile waste water

The decolorization of the textile waste water under the different treatments is represented in Fig. 1, the results show that about 80% decolorization was obtained when *Aspergillus* sp. EL-2 was used alone, while using an additional *Aspergillus* isolate in the same culture medium caused a decrease in the decolorization which reached 18%, on the other hand, the use of a consecutive biological and physical approach represented by *Aspergillus* sp. EL-2 and gamma irradiation has resulted in over 90% decolorization. This result clearly demonstrates that combinatory treatments of biological and physical result in higher decolorization (Brosillon et al. 2008).

Visible spectrum

To analyze the changes occurring after each treatment, a visible analysis was performed for the liquid cultures after each treatment, this represents the removal of color. The analysis of the spectrum reveals the nature of decolorization being adsorption or biodegradation (Asad et al. 2007). The results shown in Fig. 2 demonstrates the obvious decrease in the absorbance of the spectrum, the untreated textile waste water being the highest and the combined biological and physical being the lowest. Gamma irradiation of aqueous media results in the formation of hydroxyl radicals which in turn attack recalcitrant compounds, this technique is considered very promising in the area of waste water treatment (Hu and Wang 2007). A decrease in the visible region spectrum indicates a decrease in the color which could indicate either biodegradation or bioadsorption, while the presence of a peak in the UV region indicates cleavage of the dye and increase in the aromatic amine formation is a confirmed indication of biodegradation. The obtained results indicate that

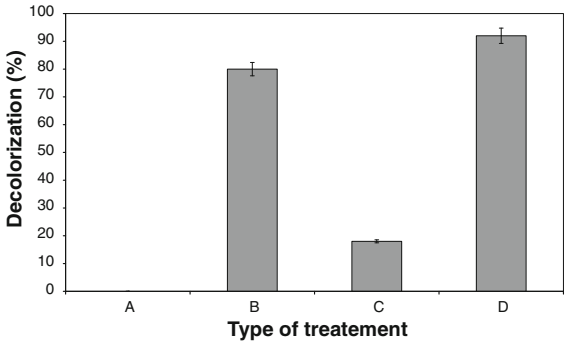

Fig. 1 The decolorization of textile waste water using different treatments: (*A*) *Aspergillus* sp. EL-2 treated waste water (*B*), combined *Aspergillus* sp. EL-2 and *Aspergillus* sp. treated waste water (*C*) and combined *Aspergillus* sp. EL-2 and gamma irradiated waste water (*D*)

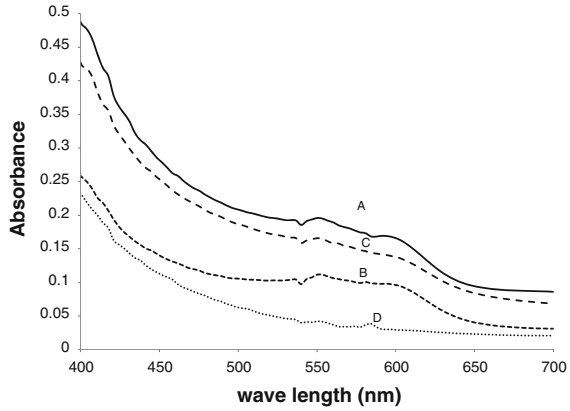

Fig. 2 Visible spectrum for untreated waste water (*A*), *Aspergillus* sp. EL-2 treated waste water (*B*), combined *Aspergillus* sp. EL-2 and *Aspergillus* sp. treated waste water (*C*) and combined *Aspergillus* sp. EL-2 and gamma irradiated waste water (*D*)

biodegradation and/or bioadsorption are possible modes of decolorization of textile waste water; therefore, another analysis is required.

FT-IR analysis

FT-IR spectroscopy was used to study the functional groups present after each treatment to indicate if biodegradation is involved in the removal process. Figure 3 shows that spectra A, B and C have faint weak intensities in the 3,500–400 nm region which represent the free –OH, while intense sharp peaks are present in the 2,215–2,240 nm representing stretching C=N, 1,590–1,750 nm represent conjugated C=O and 1,490 nm represent CH_2 bending. Spectrum D, on the other hand, lacks the presence of any sharp or intense peaks at the mentioned wavelengths. The results indicate that the efficient removal of color by *Aspergillus* sp. EL-2 is complemented by a complete destruction of aromatic compounds present after the biological treatment which could endorse some level of toxicity. Gamma irradiation is obviously useful in the destruction of the compounds present, especially that the radiation dose is 25 kGy which is the sterilization dose. According to Ma et al. (2007), the $OH^{\bullet}$ attacks the present compounds resulting in ring opening. The need for high gamma radiation doses for azo dye degradation is confirmed by Krapfenbauer et al. (2000) who stated that for complete dye destruction and in the presence of air, a high irradiation dose is required.

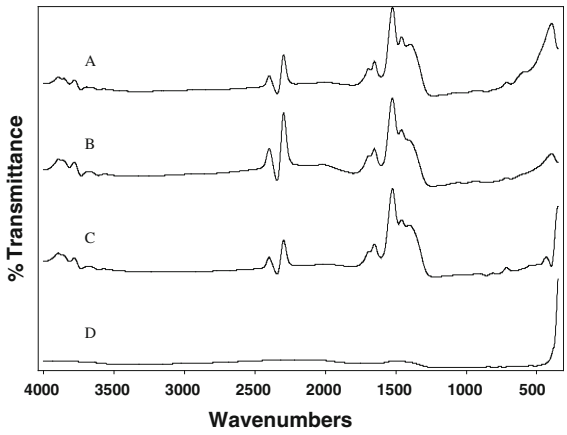

Fig. 3 FT-IR spectrum for waste water (*A*), *Aspergillus terreus* treated waste water (*B*), mixed culture of *Aspergillus terreus* and *Aspergillus* sp. (*C*) and combined treatment of *Aspergillus terreus* and gamma irradiation (*D*)

Toxicity studies of the treated textile waste water

Microbial toxicity

Microbial toxicity was performed using *E. coli* cultures, the negative results (Table 1) obtained under all treatments indicate the tolerance of *E. coli* to the textile waste water and its by-products, but does not prove lack of toxicity; therefore, other toxicity tests were performed. Microbial mediated toxicity is considered a mode of detection of the level of toxicity of some compounds using *E. coli* (Pasco et al. 2008) or *Aeromonas hydrophila* as a model system for assessing toxicity (Chen et al. 2009).

Phytotoxicity

Phytotoxicity test was performed using each treatment on a number of *Trigonella-foenum-graecum*. Table 2 illustrates the different germination percentage, shoot lengths of seeds after watering for 8 days with the different treated waste water. The results show that when combined treatment is performed the root length resembles that of beans watered with tap water. Seed germination and plant growth are a common technique to evaluate phytotoxicity (Kapanen and Itavaara 2001). This experiment reveals that the by-products generated after different combined treatments could be less toxic than using a single treatment, and definitely less toxic than using the untreated textile waste water. This is another verification of the need to use more than one mode of treatment to ensure complete degradation and not only color removal. These results are in agreement with Di Gregorio et al. (2010) who stated that phytotoxicity decreases after efficient discoloration. Figure 4 represents the growth

Table 1 Inhibition zone for *E. coli* cultures after different treatments of textile waste water

Treatment	A	B	C	D
Inhibition zone (mm)	−ve	−ve	−ve	−ve

(*A*), *Aspergillus terreus* treated waste water (*B*), mixed culture of *Aspergillus terreus* and *Aspergillus* sp. (*C*) and combined treatment of *Aspergillus terrues* and gamma irradiation (*D*)

−ve represents lack of formation of inhibition zone

Table 2 Phytotoxicity assay for germination percentage and shoot length of *Trigonella-foenum-graecum* seeds after different treatments for 8 days

Type of treatment	Germination mean percentage (%)	Shoot length (cm)	Observation
Control	100	4.5	Leaves appeared on all seeds, clean growth
A	20	0.5 ± 0.42***	No leaves. Fungus around the seeds, no leaves. Swollen shoots
B	80	2 ± 0.42***	Leaves appeared on some seeds, fungus around the seeds
C	70	1.5 ± 0.42***	Leaves appeared on some seeds, fungus around the seeds, shoots are brownish in color
D	90	3 ± 0.1*	Leaves appeared on most of the seeds, clean growth, normal looking shoots

The germination mean percentage is the average of three separate groups, each group consists of 10 seeds

Control represents tap water, *** $P < 0.001$: significance compared to Control. * $P < 0.01$: significance compared to control, *ns* not significant (*t* test)

of seeds when watered with *Aspergillus* sp. EL-2 (treatment B) and combined *Aspergillus* sp. EL-2 and gamma irradiation (treatment D). The brown spots showing in the first photo represents fungal growth which started to appear after 5 days of growth, while the second photo lacks fungal spores indicating that gamma irradiation as a combined step did not only destruct the by-products but also sterilized the waste water to eliminate any contamination after using fungi for the treatment of waste water, this is considered more safe if the treated waste water is to be used in irrigation.

Cytotoxicity

To study the cytotoxic effect of different water samples, a human cancer cell line (hepatocellular carcinoma Hep-G2 cells) was used. MTT assay indicated that the treatment of Hep-G2 with sample A resulted in a non-cytotoxic effect against cells with IC50 values >100 µl, but which increased at higher concentrations, while the other samples showed no effect on the growth of Hep-G2 cells (Fig. 5). The least cytotoxic effect was represented by treatment number B at higher concentrations, while the combined biological and physical treatment showed cytotoxic effect when the concentrations were above 150 µl.

Genotoxicity

Chromosome aberrations When mice were treated with sample A the mean percentage of chromosome aberrations, excluding gaps, were more than three folds higher than that of control ($P < 0.001$). This indicates that sample A is significant genotoxic water as compared with the control untreated mice cells and that its genotoxic effect is of comparative danger with

After treatment B After treatment D

Fig. 4 Photos of *Trigonella-foenum-graecum* seeds watered with *Aspergillus terreus* treated waste water (*B*) and combined (*Aspergillus terreus*/gamma irradiated) treated waste water (*D*)

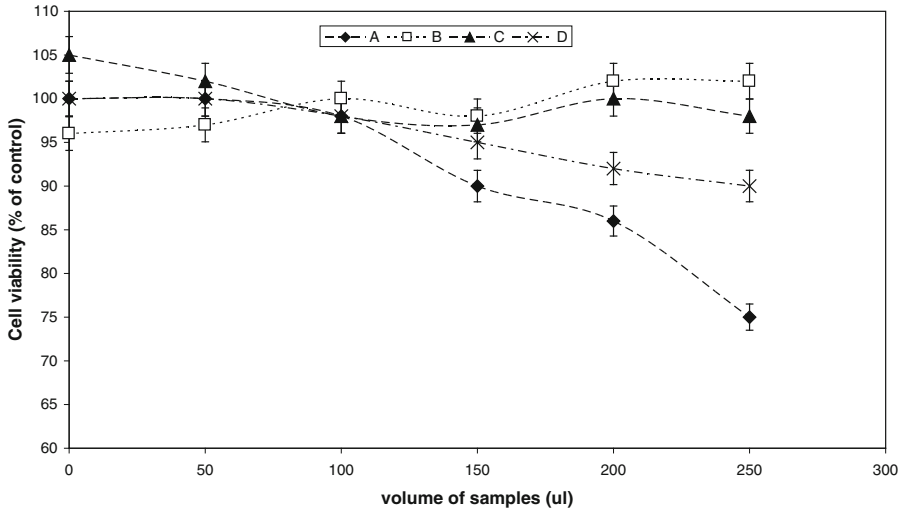

Fig. 5 Cytotoxicity test for the water samples as assayed by MTT

Table 3 Number and mean percentage of chromosome aberrations induced after 24 h injection with 200 µl samples

Dose 200 µl	Total abnormal metaphases				No. of different types of aberrations					
	Including gaps		Excluding gaps		Gaps	F and/or Br	Del	CF	MA	Tetrap.
	No.	Mean (%) ± SE	No.	Mean (%) ± SE						
Control	21	4.20 ± 0.82	15	3.00 ± 0.50	6	11	2	0	0	2
CP	154	30.8 ± 0.51***	134	26.8 ± 0.53***	20	29	5	0	89	11
A	49	9.8 ± 0.42***	41	8.20 ± 0.65***	8	26	8	1	0	6
B	32	6.4 ± 1.15**	21	4.20 ± 0.74**	11	13	4	0	0	4
C	39	7.8 ± 0.22*	29	5.80 ± 0.42*	10	22	2	1	0	4
D	30	4.4 ± 1.15**	18	3.20 ± 0.54**	10	12	4	0	0	4

The total number of scored cells is 500 (5 animals/group)

F fragments, *Br* breaks, *Del* deletions, *CF* centric fusions, *MA* multiple aberrations, *Tetrap* tetraploidy

*** $P < 0.001$: significance compared to Control. ** $P < 0.01$, *** $P < 0.001$: significance compared to Sample 1, *ns* not significant (*t* test)

that of the CP (Table 3). Chromosomal analysis of bone marrow cells derived from mice that were treated with samples B and C revealed that the mean percentages of aberrations were lower than that resulted from sample A, while sample D showed the closest results to the control samples. The significance of chromosome aberrations resulted from sample B and C reduced to $P < 0.01$ and $P < 0.05$ in comparing to sample A respectively. The main types of chromosome aberrations observed with these samples were chromosomal breaks and fragments (Table 3). The results obtained show that all the treatments were efficient for removal of toxicity, however, the combined biological and physical method proved to be the closest to the non-toxic control samples. Fish exposed to non-remediated dye effluent showed high DNA fragmentation, the genotoxicity levels decreased after two remediation processes (Grinevicius et al. 2009), this confirms that sometimes more than one mode of treatment is required to destruct the possible toxic compounds completely.

Mitotic index The mean percentage of MI was measured in bone marrow cells for all mice. This mean percentage was significantly inhibited in CP-treated and sample A mice compared to control. The rate of bone-marrow proliferation was remarkably increased in samples B, C and D, compared with the

Table 4 Number and mean percentage of MI and MNPCE induced after 24 h injection with 200 µl samples

Sample (injected dose 200 µl)	MI	
	No.	Mean (%) ± SE
Control	190	3.80 ± 0.11
CP	85	1.70 ± 0.16[a]***
A	90	1.90 ± 0.18[a]***
B	224	4.48 ± 0.29[ns]
C	217	4.34 ± 0.10[ns]
D	200	4.00 ± 0.23[ns]

[a] Highly significant

Total number of scored cells 5000 (5 animals/group)

*** $P < 0.01$: significance compared to Control. *ns* not significant (*t* test)

control but with insignificant manner, however, sample D was also the closest to the control samples (Table 4). These findings indicate that the samples did not affect the cell proliferation. These findings coincide with Jadhav et al. (2010) who stated that a decreased MI could be considered a reliable method to determine the presence of cytotoxic compounds, in the present study, all the treatments exhibited high MI indicating that any type of treatment was enough to ensure safe treated effluent. The level of increase or decrease in the MI could be used efficiently as a biomonitoring technique for environmental toxicity (Carita and Marin-Morales 2008). But the results shown from previous toxicity tests reveal contradiction to the previous statement; each toxicity test indicated a different toxicity level. The type and number of tests confirming mutagenicity conclude the accuracy of the results and determine the safety in a precise way (Kirkland et al. 2005), a battery of three tests was postulated to be enough (Kirkland and Speit 2008).

Conclusion

The use of brown rot fungi in decolorization is considered very promising because of its efficiency and low cost, but an additional treatment might be required for complete destruction of any possible remaining toxic compounds. Although the use of treated waste water in ornamental, woody plant irrigation or in industries which uses tons of water is a successful way to save clean water for drinking purposes, non-toxic water is required. The results obtained in this study show that (1) although decolorization by fungal isolates could be used as an efficient method for color removal, yet it doesn't indicate lack of toxicity; (2) microbial toxicity does not prove lack of toxicity; (3) phytotoxicity indicate the reuse of treated waste water in irrigation; (4) cytotoxicity results showed that bioremediation only was efficient in toxicity removal, genotoxicity showed that combined treatments were the least toxic, while MI showed all treatments to be safe. From all the concluded data, it is suggested that a number of toxicological parameters should be evaluated to ensure the waste water treatment efficacy, especially if the treated waste water is used in any human-related area.

Acknowledgment This work was supported by Grant 161 from the Science and Technology Development Fund (STDF), Cairo, Egypt. The authors would like to thank the Cancer Research Lab. at the road to Nobel, National Research Center, Cairo, Egypt for providing their lab facilities for the cytotoxicity and genotoxicity section of the manuscript.

References

Ambrosio ST, Campos-Takaki GM (2004) Decolorization of reactive azo dyes by *Cunninghamella elegans* UCP 542 under co-metabolic conditions. Bioresour Technol 91: 69–75

Asad S, Amoozegar MA, Pourbabaee AA, Sarbolouki MN, Dastgheib SMM (2007) Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresour Technol 98:2082–2088

Brosillon S, Djelal H, Merienne N, Amrane A (2008) Innovative integrated process for the treatment of azo dyes: coupling of photocatalysis and biological treatment. Desalination 222:331–339

Carita R, Marin-Morales MA (2008) Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chemosphere 18:311–316

Chen SC, Kao CM, Huang MH, Shih MK, Chen YL, Huang SP, Liu TZ (2003a) Assessment of genotoxicity of benzidine and its structural analogues to human lymphocytes using comet assay. Toxicol Sci 71:283–288

Chen KC, Wu JY, Liou DJ, Hwang SCJ (2003b) Decolorization of the textile azo dyes by newly isolated bacterial strains. J Biotechnol 101:57–68

Chen BY, Lin KW, Wan YM, Yen CY (2009) Revealing interactive toxicity of aromatic amines to azo dye decolorizer *Aeromonas hydrophila*. J Hazard Mater 166: 187–194

Di Gregorio S, Balestri F, Basile M, Matteini V, Gini F, Giansanti S, Tozzi MG, Basosi R, Lorenzi R (2010)

Sustainable discoloration of textile chromo-baths by spent mushroom substrate from the industrial cultivation of *Pleurotus ostreatus*. J Environ Prot 1:85–94

EEAA report (2003) Summary of sectional reports of environmental agency. Sustained Environmental Management (SEAM) Project "Clean Production" (CP), prepared by Central Unit for Water Quality, Ministry of Water Resources and Irrigation, Cairo, Egypt

Földváry Cs M, Wojnárovits L (2007) The effect of high-energy radiation on aqueous solution of Acid Red 1 textile dye. Radiat Phys Chem 76:1485–1488

Gomaa OM, Momtaz OA, Abd El Kareem H, Fathey R (2011) Isolation, identification and biochemical characterization of a brown rot fungus capable of textile dye decolorization. World J Microbiol Biotechnol 27:1641–1648

Grinevicius VMAS, Geremias R, Laus R, Bettega KF, Laranjeiras MCM, Fávere VT, Filho DW, Pedrosa RC (2009) Textile effluents induce biomarkers of acute toxicity, oxidative stress and genotoxicity. Arch Environ Contam Toxicol 57:307–314

Hansen MB, Nielsen SE, Berg K (1989) Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods 119: 203–210

Hu J, Wang J (2007) Degradation of chlorophenols in aqueous solution by γ-radiation. Radiat Phys Chem 76:1489–1492

Jadhav JP, Kalyani DC, Telke AA, Phugare SS, Govindwar SP (2010) Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals, and toxicity from textile dye effluent. Bioresour Technol 101(1):165–173

Kapanen A, Itavaara M (2001) Ecotoxicity tests for compost applications. Ecotoxicol Environ Saf 49:1–16

Kaushi P, Malik A (2009) Fungal dye decolourization: recent advances and future potential. Environ Int 35:127–141

Kirkland DJ, Speit G (2008) Evaluation of the ability of a battery of three in vitro genotoxicity tests to discriminate rodent carcinogens and non-carcinogens III. Appropriate follow-up testing in vivo. Mutat Res 654:114–132

Kirkland DJ, Henderson L, Marzin D, Müller L, Parry JM, Speit G, Tweats DJ, Williams GM (2005) Testing strategies in mutagenicity and genetic toxicology: an appraisal of the guidelines of the European Scientific Committee for cosmetics and non-food products for the evaluation of hair dyes. Mutat Res 588:88–105

Krapfenbauer K, Wolfger H, Getoff N, Hamblett I, Navaratanm S (2000) Pulse radiolysis and chemical analysis of azo dyes in aqueous solution I. *p*-Phenylazoaniline. Radiat phys Chem 58:21–27

Ma H, Wang M, Yang R, Wang W, Zhao J, Shen Z, Yao S (2007) Radiation degradation of Congo red in aqueous solution. Chemosphere 68:1098–1104

Mathur N, Bhatnagar P, Bakre P (2005) Assessing mutagenicity of textile dyes from Pali (Rajasthan) using Ames Bioassay. Appl Ecol Environ Res 4:111–118

Merzouk B, Gourich B, Sekki A, Madani K, Vial Ch, Barouki M (2009) Studies on decolorization of textile wastewater by continuous electrocoagulation process. Chem Eng J 149:207–214

Nyanhongo GS, Gomes J, Gubitz GM, Zvauya R, Read J, Steiner W (2002) Decolorization of textile dyes by laccases from a newly isolated strain *Trametes modesta*. Water Res 36:1449–1456

Pasco NF, Goonerate R, Daniel RM, Czollner A, Scott AJ (2008) Toxicity assessment of chlorophenols using a mediated microbial toxicity assay. Int J Environ Anal Chem 88:1063–1075

Ramalingam SN, Shanmugapriya S, Shakthipriyadarshini S, Sadasivam S, Shanmugapraksh M (2010) Decolorization of textile dyes by *Aspergillus tamarii*, mixed fungal culture and *Penicillium purpurogenum*. J Sci Ind Res 69: 151–153

Schnieder K, Hafner C, Jager I (2004) Mutagenicity of textile dye products. J Appl Toxicol 24:83–91

Sirinivasan SV, Murthy DV (2009) Statistical optimization for decolorization of textile dyes using *Trametes versicolor*. J Hazard Mater 65(1–3):909–914

Yosida TH, Amano K (1965) Autosomal polymorphism in laboratory bred and wild Norway rats, *Rattus norvegicus*. Misima Chromosoma 16:658–667