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Full Length Research Paper

Application of *Aspergillus niger* SA1 for the enhanced bioremoval of azo dyes in Simulated Textile Effluent

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Biological remediation is always envisaged as cost effective and eco-friendly for the treatment of recalcitrant dyes and effluents. *Aspergillus niger* SA1, a brown rot fungi, isolated from storage pond of textile wastewater, showed a great mineralizing ability for azo dyes, acid red (AR) 151 and orange (Or) II. Decolorization assays were carried out for 24 h, by taking 100 ml of dye containing Simulated Textile Effluent (STE) with 5 g of freshly grown fungal pellets. Decolorization of AR 151 was well over 95% under different conditions, however, it reduced to 52% when treated with pre-used fungal biomass under shaking condition. In case of Or II, results were 50 and 61% under static while 65 and 85% under shaking condition with fresh and pre-used fungal biomass respectively. Primarily, dyes removal in STE appeared due to biosorption/bioadsorption of the fungal biomass. However, discoloration of dyes onto the biomass with subsequent formation and then decline in their products in STE suggested clearly that dyes were basically metabolically degraded by the fungal strain.

Key words: Acid red 151, *Aspergillus niger,* azo dyes, decolorization, degradation, orange II.

INTRODUCTION

Various types of synthetic dyes and pigments are extensively used (approximately 100,000 tons/year) worldwide in textile sector (Moreira et al., 2000; Park et al., 2006; Soares et al., 2002). Huge amount (5-50%) of dyes (specifically reactive azo), on account of their poor absorbability into different fibers are continuously being exhausted in wastewater streams from textile industries (McMullan et al., 2001). Predominantly Synthetic dyes, including sulfonated and their biodegradation products contain structural elements, which are unknown or rare in nature. So, these chemicals are considered as major recalcitrant organic compounds in wastewater stream of textile industry. These chemicals not only have a negative aesthetic effect on water bodies but these also resist microbial degradation, thereby reducing self-purification ability of the water bodies. Furthermore, dyes and associated chemicals have also been found contributing aquatic toxicity in terms of mutagenicity (Grover et al., 1996; Reid et al., 1984; Rosenkranz and Klopman, 1990; Wagner, 1993).

It is the complex changing nature and huge volume of textile effluent, which makes it difficult to be treated with a single conventional treatment technology (McMullan et al., 2001; O'Neill et al., 1999; Shaul et al., 1991). A variety of physicochemical treatments have been devised previously for the dyes and textile wastewater. However, these suffered from some serious drawbacks in terms of their limited applications or their high cost. Besides, chemical treatments created an additional chemical load in water bodies that eventually resulted in sludge disposal problems. Several factors determine the technical and economic feasibility of each single dye removal technique. These include; dye type and its concentration, wastewater composition, operation costs (energy and material), environmental fate and handling costs of generated waste products (Cooper, 1993; Grau, 1991; Robinson et al., 2001; Vandevivere et al., 1998). The domain of biological remediation has been gaining a great deal of attention during the last couple of decades

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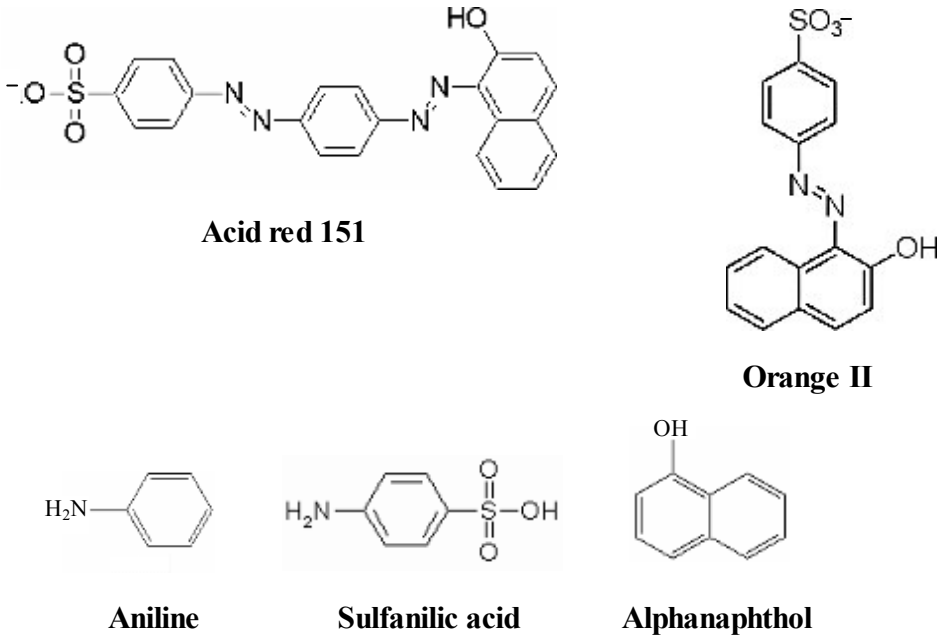

Figure 1. Structure of dyes and related products.

for its cost-effective and eco-friendly evolving nature. In this context, researchers have paid much attention in exploring microbial and plant remediation techniques for water and soil polluted with different contaminants. Biological dye removal techniques are either based on partial or complete biodegradation of dyes by pure and mixed cultures of bacteria, fungi and algae (Arora and Chander 2004; Bhatt et al., 2000; Chen et al., 2003; Ferreira et al., 2000; Machado et al., 2006; Sandhya et al., 2005; Semple et al., 1999; Wesenberg et al., 2003; Zheng et al., 1999). However, a great deal of expertise is a prerequisite in screening, selection and application of right organism(s) against specific dyes under different physico-chemical conditions to achieve optimum results.

The biodegradation of dyes was first reported in White-rot (Glenn and Gold, 1983) and since then many scientists have focused their efforts in exploring the bio-mineralization properties of different fungi. Virtually, dyes from all chemically distinct groups have been found prone to fungal oxidation due to their lignin modifying systems (Arora and Chander, 2004; Fu and Viraraghavan, 2001; Libra et al., 2003). By far, the fungal decolorization/ degradation of dyes has been reported either through biosorption (Fu and Viraraghavan, 2000) or enzymatic mineralization (lignin peroxidase, manganese peroxidase, manganese independent peroxidase and laccases) (Ferreira et al., 2000; Wesenberg et al., 2003; Wong and Yu, 1999; Zheng et al., 1999). So, considering the metabolizing properties, fungi were either used directly or their extracts (cultural filtrate) containing enzymes were exercised for the degradation of dyes. Reports have, however, supported application of fungal biomass (pre-grown) than cell-free system (containing enzymes) for the treatment of dyes, specifically, when fungi showed adsorption as well as enzymatic degradation abilities for dyes (Aksu and Tezer, 2000; Braun and Vecht-Lifshitz, 1991; Coulibaly et al., 2003; Rojek et al., 2004; Zhau and Zimmerman, 1993). In this context, White-rot fungi have been mostly used in different types of reactors such as aerated reactor (Prout, 1990) packed bed (Schliephake et al., 1993) and rotating biological contactors (Alleman et al., 1995), though each reactor type has shown some limitation, considering the waste array of dyes and the time required for their treatment.

The present study focused on the role of a brown-rot fungus, *Aspergillus niger* SA1, for the decolorization/ degradation of dyes and related products. Two important azo dyes including AR 151 and Or II were used in the research work because these dyes and their products (Figure 1) proved strong recalcitrant and hazardous in nature. Pre-grown biomasses (wet pellets) of fungus were repeatedly used for the removal of dyes in STE in batch mode static/shaking conditions.

MATERIALS AND METHODS

Chemicals

Dyes and chemical compounds used in the research work were obtained from BDH Laboratory Chemical Division, Poole, Dorset, England, Sigma chemicals Co., St, Lois and E. Merck, Darmstadt, Germany.

Composition of simulated textile effluent (STE)

Simulated textile effluent was made by adding per liter of distilled

water; acetic acid (99.9%) 0.150 ml, $(NH_2)_2CO$ 100.0 mg, KH_2PO_4 67.0 mg, $NaHCO_3$ 840.0 mg, $MgSO_4.7H_2O$ 38.0 mg, $CaCl_2$ 21.0 mg, $FeCl_3$ $6H_2O$ 7.0 mg and dye 20 mg. pH of the medium was adjusted to 8 by using 0.1M HCl and NaOH (Luangdilok and Panswad, 2000).

Isolation of fungal strain

Fungal strain used in the present study was previously isolated and identified by Ali et al. (2008 a, b).

Production of wet biomass of fungi

A freshly grown fungal culture (on Saboraud dextrose agar) on petri plate was taken as an inoculum source for its bulk production. The fungal mycelia along with spores were scratched from the solid medium and then taken in 1000 ml cotton plugged flat bottom Erlenmeyer flask containing 500 ml Saboraud dextrose broth. Enhanced fungal growth was obtained by operating the culture on a shaker incubator at 30°C for 8 days. The fungal biomass (wet pellets) produced was filtered out from the liquid medium by using Whattman filter paper No. 1. It was washed twice with deionized water before application in the experiments.

Decolorization assays

Decolorization assay was carried out by taking an aliquot of 100 ml of STE containing 20 mgl^{-1} of a dye in 250 ml cotton plugged Erlenmeyer flask. Each dye containing solution was treated with 5 gm of freshly grown fungal biomass (wet pellets) for 24 h under static or shaking (orbital shaker at 100 rpm) condition. After each experiment, the fungal biomass was washed with deionized water, stored for 24 h in the incubator (30°C), and then re-used in the subsequent experiment.

Analytical

Residual amount of dye and different products in each experiment were analyzed by taking samples of treated dye containing STE in epindorff tubes (size 2.5 ml) at regular intervals. Samples were initially filtered through Whattman filter paper No. 1, filtrate was centrifuged at 10,000 rpm for 10 min on a micro-centrifuge and the supernatant was then filtered through 0.2 µm syringe filter to remove any tiny particulate material in it. The residual amount of AR 151 (λ max = 225 nm) and Or II (λ max = 231 nm) was monitored by analyzing them though High Performance Liquid Chromatography (HPLC) (Agilent 1100 ChemStation HPLC, equipped with a diode array detector and a 100 RP-18 column (4 mm i.d. x 250 mm length) containing 5 µm packed particles (Merck KgaA, Darmstadt, Germany)). The solvent system (mobile phase) used for Or II was acetonitrile and 0.03 M ammonium carbonate buffer (30:70 %) and for AR 151, it was acetonitrile and H_2O (30: 70). The flow rate was kept 1.5 ml mn^{-1} during analysis.

Result obtained in each experiment was expressed as mean with standard error. Results obtained in different operational conditions (static and shaking with fresh and pre-used biomass) were statisti-cally compared with each other by using T-Test (Paired; two samples for means/2 samples; assuming equal variance). Correla-tion (Bi-variant) indices of decolorizations of each dye and its associated products formed in different experiments were also determined. Probabilities (p-values) of <0.05 and <0.01 were considered significant and highly significant, respectively.

Precaution

The entire experimental work was carried out under standard sterilize conditions. Each experiment was conducted in triplicate in order to ovoid errors.

RESULT

The present study clearly validated the role of an indigenous brown-rot fungal isolate *A. niger* SA1 for achieving enhanced decolorization/degradation of azo dyes AR 151 and Or II. Application of biomass in STE containing AR 151 or Or II yielded 50-100% results in 24 h in different condition studied. Besides, removal of AR 151 was considerably higher than Or II. Primarily, fungal biosorption/ bio-adsorption appeared to be associated with dyes removal in STE (specifically in case of AR 151). However, HPCL analysis of the treated STE samples showed that dyes were degraded into their constituent products. Furthermore, culture stability (static/shaking), mode of application of biomass (fresh or pre-used) and dyes types were found to be playing a major role on the metabolizing properties of fungus related to dyes removal (Figures 2-4).

Decolorization of AR 151 (20 mgl^{-1}) was 97-98% in 24 h in most of the conditions employed; however, it declined considerably (52.62%) (significantly at $p < 0.01$) with pre-used biomass under shaking condition. Under stationary mode, the final residual amount of aniline was maximum (10.97 mgl^{-1}) followed by sulfanilic acid (5.55 mgl^{-1}) with fresh biomass and only aniline (8.67 mgl^{-1}) was quantified with pre-used biomass. The production of aniline kept on increasing during the experiments, though the amount of remaining two products started decreasing even after 2 h. However, under shaking condition, the only product quantified at 24^{rth} h was sulfanilic acid both with fresh (18.55 mgl^{-1}) and pre-used (3.25 mgl^{-1}) biomass (Figure 2).

In case of Or II, the fungal decolorization abilities appeared to be declining when they were used in repeated batch modes under static/shaking condition (Figure 3). Nevertheless, decolorization of dye remained more than 50% under all treatment conditions in 24 h. It was significantly higher ($p < 0.01$) in shaking (84.91 and 64.86%) compared with static (61.08 and 50.62%) condition both with fresh and pre-used biomass respectively (Figure 3). The total residual amount of three products in Or II treatment kept considerably lower that is. <10 mgl^{-1} and started decreasing in the initial 1-2 h. The major product analyzed at 24^{rth} h was aniline (2.71 and 5.78 mgl^{-1}) in static, while sulfanilic acid (2.7 and 1.4 mgl^{-1}) in shaking conditions with fresh and pre-used biomass respectively.

Correlation indices showed a strong positive correlation ($r = 0.793$) (though non-significant at $p = 0.05$) between decolorization (percent) of AR 151 and total residual amounts (at 24^{rth} h) of 3 products under different treatment conditions. Contrarily, correlation was moderate negative ($r = -0.332$) in case of Or II, which

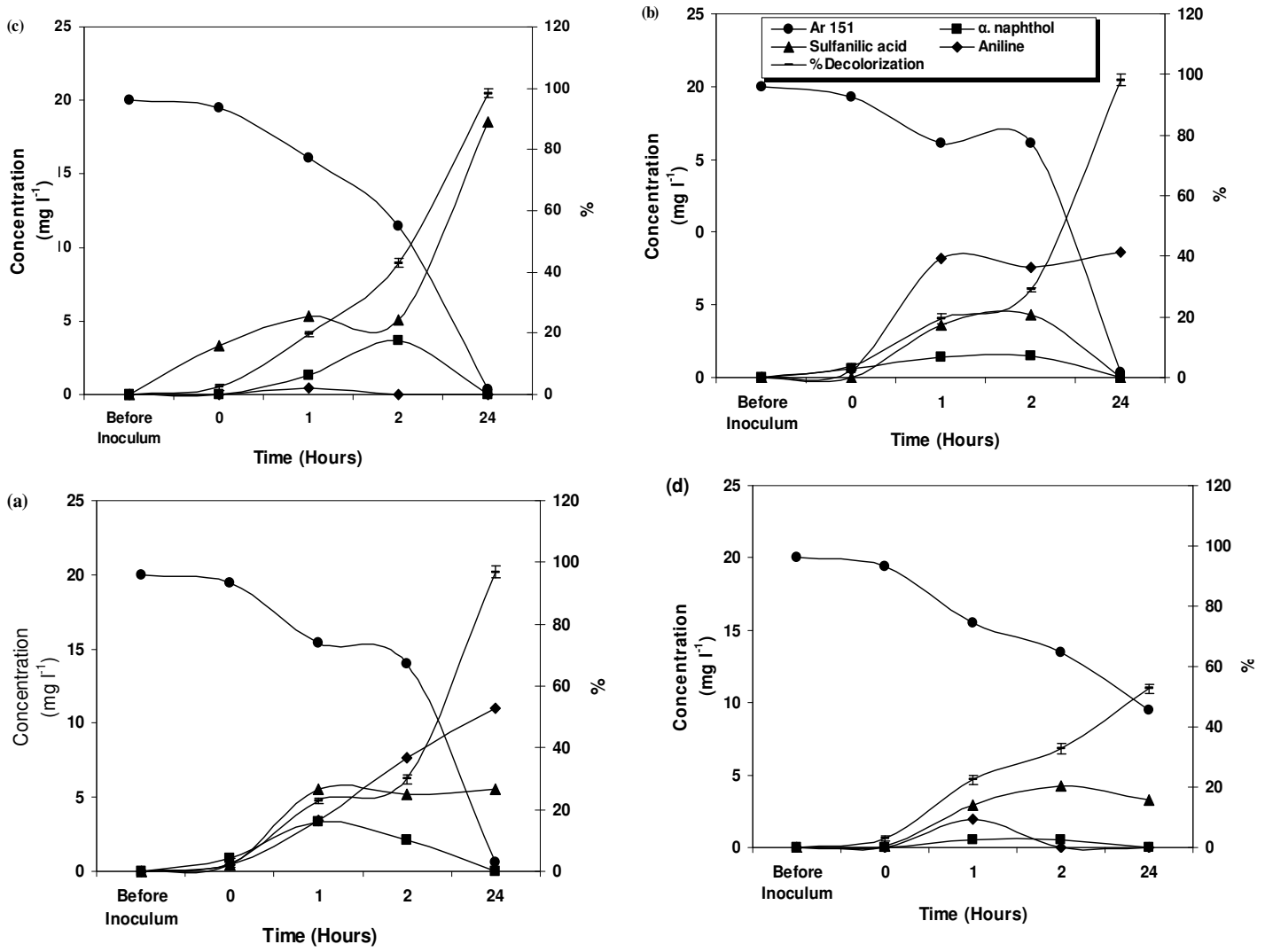

Figure 2. Time course of decolorization of AR 151 and different products formation by fresh and pre-used biomass of *A. niger* SA1; in static (a and b) and in shaking (c and d) conditions, respectively.

indicated better mineralization of products (total) than was analyzed in case of AR 151 while considering the amount of respective decolorizations (percent) in two dyes.

DISCUSSION

Application of biomass (pre-grown) of *A. niger* SA1, proved and emerged as a superior approach in achieving enhanced decolorization/degradation (up to 98% in AR 151 and 85% in Or II in 24 h) of azo dyes in STE (Figures 2 and 3). This approach is essentially required when fungus shows both metabolically mediated biosorption/ bioadsorption as well as biodegradation of dyes. Previously, the same fungal strain when seeded as an inoculum source to grow with AR 151 or Or II in STE (with glucose as an additional C source) exhibited a significant decline in its biomass production. Furthermore, it took about 6-8 days to produce 80-90% results (Ali et al., 2008 a, b). So, it is inferred from the present and previous results that the growth and metabolizing abilities of fungal strain can be restricted to certain extent when it is allowed to grow in presence of a specific azo dye.

Apparently, dyes removal in the present study was seen merely due to biosorption/bioadsorption of fungal hyphae (Figure 4). Likewise, few other studies have also clearly mentioned biosorption/bioadsorption of certain brown-rot fungi (*A. niger* and *A. foetidus*) (Ali et al., 2008a; Fu and Viraraghavan 2000; Knapp and Newby 1995; Sumathi and Manju 2000) as the primary dyes removal phenolmenon coupled with electrostatic pull between the positively charged cell wall and negatively charge dyes (Aksu et al., 1999; Aksu and Tezer, 2000). In this context, divalent dye (Acid Red 114) was found more attracted towards the fungal cell wall (of *M. verrucaria*) than the

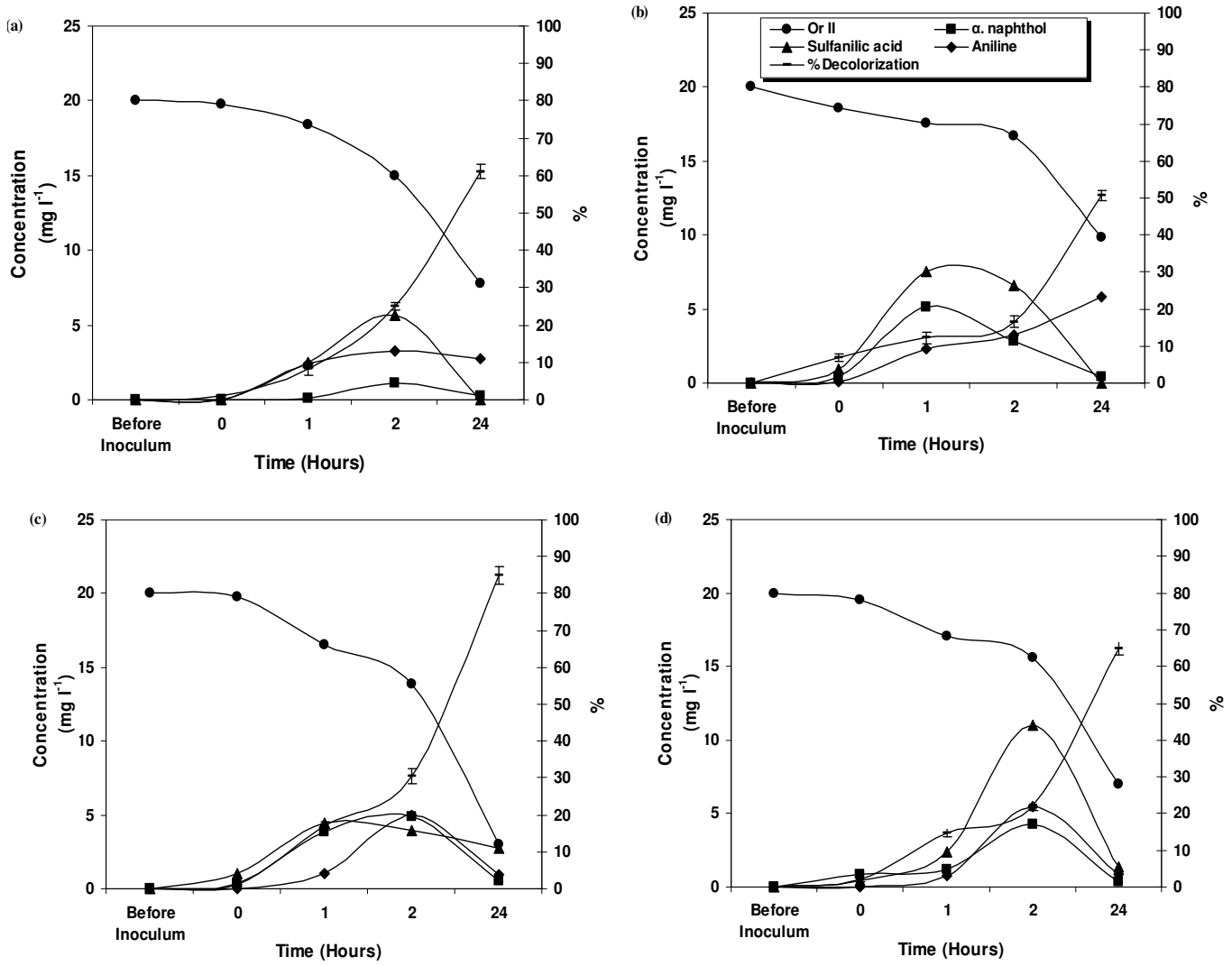

Figure 3. Time course of decolorization of Or II and different products formation by fresh and pre-used biomass of *A. niger* SA1; in static (a and b) and in shaking (c and d) conditions respectively.

monovalent dye (Acid Orange II) (Laszlo, 1994). Similar electrostatic attraction seemed to be responsible in the greater removal of AR 151 (50-100%) than Or II (50-85%) in STE.

A comprehensive reduction of dyes after taken into/onto the fungal biomass (Figure 4) with formation and further mineralization of their reduction products (analyzed through HPLC) indicated degradation of dyes (Figures 2 and 3); a metabolically triggered phenomenon linked with certain fungal oxidases (Ferreira et al., 2000; López et al., 2004; Rojek et al., 2004; Wesenberg et al., 2003). Similarly, Balan and Monterio (2001) associated bioremo-val of Indigo with adsorption as well as extracellular fungal activity. In this context, Rojek et al. (2004) specifically linked 60-70% of dye removal with biosorption (physicochemical/metabolically-dependent) and 30-40% to biodegradation in case of *P. chrysosporium*. In addition, biodegradation of metal complex dyes by *Trametes versicolor* has been categorized into a number of stages including; the primary adsorption of the dyes into the hyphae, followed by breaking of the metal complex bonds and then enzymatic degradation (90%) of the dye took place (Blanquez et al., 2004).

Culture stability has been found playing an important role in the production, stability and dyes catabolizing properties of the certain fungal enzymes (Glenn and Gold, 1983; Kirby et al., 2000; Kuwahara et al., 1984). It was stable (static) culture of *A. niger* SA1 that exhibited higher removal of AR 151 and its associated products. On the contrary, the same fungus showed higher removal of Or II under agitated (shaking) condition. Static culture might have also created an anoxic condition in the broth surrounding (in/out) the fungal pellets, thereby facilitating bio-chemically mediated anaerobic reduction of dyes.

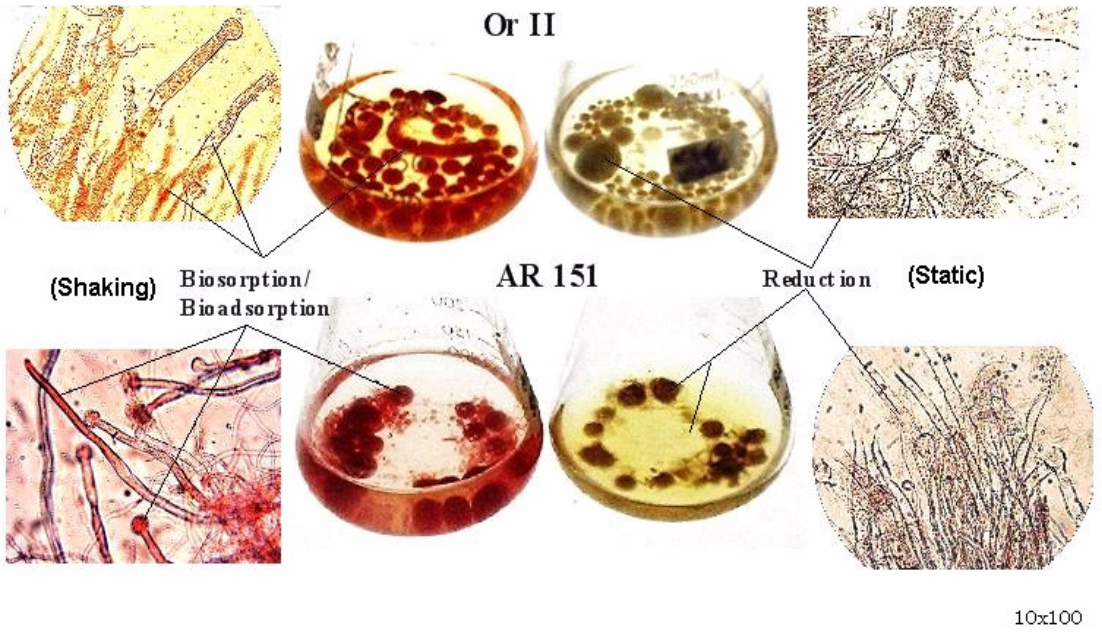

Figure 4. Decolorization (reduction) of AR 151 and Or II after biosorption/bioadsorption by *A. niger* SA1 in static/shaking conditions.

Nevertheless, enhanced degradation of dyes by fungal peroxidases has generally been linked to shaking cultures (aerated), where nutrients and oxygen are easily accessible to the cells for oxidative metabolism (Arora and Chander, 2004; Fu and Viraraghavan, 2001; Jarosz-Wilkolazka et al., 2002; Libra et al., 2003; Machado et al., 2006; Wesenberg et al., 2003). This aforementioned logic clearly supported results obtained in case of Or II, where decolorization was considerably higher (15-25%) with comparatively less amount of total residual products under shaking compared to static condition. Despite all, dyes removal abilities of *A. niger* SA1 appeared to be declining (10-50%) in almost all cases (with an exception of AR 151 under static mode) when used in two consecutive experiments (Figures 2 and 3). Sam and Yesilada (2001), however, reported a little decrease in decolorization ability of *Coriolus versicolor* and *Funalia trogii* for Or II in repeated batch mode application. So, it would have been a better choice if biomass were used intermittently rather than being applied continuously in the experiments. In periodic applications, fungal biomasses might regain their metabolizing abilities for further application in bioremediation experiments.

Previous reports have depicted both partial and complete biodegradation of dyes including AR 151 and Or II (Blanquez et al., 2004; Coughlin et al., 1997 and 1999; López et al., 2004; Quezada et al., 2000; Sam and Yesilada, 2001). In this respect, the possible degradation pathways of these dyes have mostly been considered uncertain due to their end products of unstable chemical nature (Field et al., 1995; Kudlich et al., 1999). Still, Lopez et al. (2004), in one of their study analyzed nine transformation products of Or II, suggesting both its symmetrical and asymmetrical cleavage (with reference to azo bond). Current research work predicts similar degradation patterns in case of both dyes which resulted in the formation of products like a naphthol, sulfanilic acid and aniline.

Conclusion

Present study has revealed that *A. niger* SA1 a brown-rot fungus has similar capabilities like white-rot in the efficient and comprehensive degradation of pollutants like dyes. Besides, the fungal isolate has also shown a trend towards its long term applicability in bio-reactors; however, it could be better if used intermittently so that it regains its metabolizing properties.

REFERENCES

Aksu Z, Tezer S (2000). Equilibrium and kinetic modeling of biosorption of Remazol Black B by *Rhizopus arrhizus* in a batch system: effect of temperature. Process Biochem. 36: 431-439.

Aksu Z, Calik A, Dursun AY, Demircans Z (1999). Biosorption of iron (III)-cyanide complex anions to *Rhizopus arrhizus*: application of adsorption isotherms. Process Biochem. 34: 483-491.

Ali N, Hameed A, Ahmed S, Khan AG (2008a). Decolorization of structurally different textile dyes by *Aspergillus niger* SA1. World J. Microbiol. Biotechnol. 24: 1067-1072.

Ali N, Ikramullah, Lutfullah G, Hameed A, Ahmed S (2008b). Decolorization of Acid red 151 by *Aspergillus niger* SA1 under different physicochemical conditions. World J. Microbiol. Biotechnol. 24: 1099-1105.

Alleman BC, Logan BE, Gilbertson LR (1995). Degradation of pentachloro-phenol by fixed films of White-rot fungi in rotating tube bioreactor. Water Res. 29(1): 61-67.

Arora DS, Chander M (2004). Decolorization of diverse industrial dyes by some *Phlebia* spp. and their comparison with *Phanerochaete chrysosporium*. J. Basic. Microbiol. 44(5): 331-338.
Balan DSC, Monterio RTR (2001). Decolorization of textile dye indigo by lignolytic fungi. J. Biotechnol. 89: 141-145.
Bhatt M, Patel M, Rawal B, Novotny C, Molitoris HP, Sasek V (2000). Biological decolorization of synthetic dye RBBR in contaminated soil. World J. Microbiol. Biotechnol. 16: 195-198.
Blanquez P, Casas N, Font X, Gabarrell X, Sarra M, Caminal G, Vicent T (2004). Mechanism of textile metal dye biotransformation by *Trametes versicolor*. Water Res. 38(8): 2166-2172.
Braun S, Vecht-Lifshitz SE (1991). Mycelial morphology and metabolite production. Trends Biotechnol. 9: 63-68.
Chen K, Wua J, Liou D, Hwang SJ (2003). Decolorization of the Textile dyes by newly isolated bacterial strains. J. Biotechnol. 101: 57-68.
Cooper P (1993). Removing color from dyehouse waste waters: A critical review of technology available. J. Soc. Dyers Colorist. 109: 97-100.
Coughlin MF, Kinkk BK, Tepper A, Bishop PL (1997). Characteristics of aerobic azo dyes degrading bacteria and their activity in biofilms. Water Sci. Technol. 36(1): 215-223.
Coulibaly L, Germain G, Spiros AN (2003). Utilization of fungi for biotreatment of raw wastewaters. Review. Afr. J. Biotechnol. 2(12): 620-630.
Ferreira VS, Magalhaes DB, Kling SH, Silva JG, Bon EPE (2000). N-demethylation of methylene blue by lignin peroxidase from *Phanerochaete chrysosporium*: Stoichiometric relation for H_2O_2 consumption. Appl. Biochem. Biotech. 4(86): 255-265.
Field JA, Stams AJM, Kato M, Schraa G (1995). Enhanced biodegradation of aromatic pollutants in co-cultures of anaerobic and aerobic bacterial consortia. A. V. Leeuwen. Int. J. Gen. Mol. Microbiol. 67: 47-77.
Fu YZ, Viraraghavan T (2000). Removal of a dye from aqueous solution by the fungus Aspergillus niger. Water Qual. Res. J. Canada. 35: 95-111.
Fu Y, Viraraghavan T (2001). Fungal decolorization of dye wastewaters: Review. Bioresour. Technol. 79: 251-262.
Glenn JK, Gold M (1983). Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 45: 1741-1747.
Grau P (1991). Textile industry wastewaters treatment. Water Sci. Technol. 24: 97-103.
Grover IS, Kaur A, Mahajan RK (1996). Mutagenicity of some dye effluents. National Acad. Sci. Lett. Ind. 19(7-8): 149-158.
Kirby N, Marchant R, McMullan G (2000). Decolorization of synthetic textile dyes by *Phlebia tremellosa*. FEMS. Microbiol. Lett. 188(1): 93-96.
Knapp JS, Newby PS (1995). The microbiological decolorization of an industrial effluent containing a diazo-linked chromophore. Water Res. 29: 1807-1809.
Kudlich M, Hetheridge MJ, Knackmuss HJ, Stolz A (1999). Autoxidation reactions of different aromatic o-aminohydroxynaphthalenes that are formed during the anaerobic reduction of sulfonated azo dyes. Environ. Sci. Technol. 33(6): 896-901.
Laszlo JA (1994). Removing acid dyes from textile wastewater using biomass for decolorization. American Dyestuff Reporter.
Libra JA, Borchert M, Banit S (2003). Competition strategies for the decolorization of a textile reactive dye with the White-rot fungi *Trametes versicolor* under non-sterile conditions. Biotech. Bioeng. 82(6): 736-744.
López C, Valade A, Combourieu B, Mielgo I, Bouchon B, Lema JM (2004). Mechanism of enzymatic degradation of the azo dye Orange II determined by ex-situ ^{1}H nuclear magnetic resonance and electrospray ionization-ion trap mass spectrometry. Analy. Biochem. 335: 135-149.
Luangdilok W, Panswad T (2000). Effect of chemical structures of reactive dyes on color removal by an anaerobic-aerobic process. Water Sci. Technol. 42(3-4): 377-382.
Machado KMG, Compart LCA, Morais RO, Rosa LH, Santos MH (2006). Biodegradation of reactive textile dyes by Basidiomycetous fungi from Brazilian ecosystems. Brazil. J. Microbiol. 37: 481-487.
McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat IM, Marchant R, Smyth WF (2001). Microbial decolorization and degradation of textile dyes: Mini Review. Appl. Microbiol. Biotechnol. 56: 81-87.
Moreira MT, Mielgo I, Feijoo G, Lema JM (2000). Evaluation of different fungal strains in the decolourisation of synthetic dyes. Biotechnol. Lett. *22*: 1499-1503.
O'Neill C, Hawkes FR, Hawkes DL, Esteves S, Wilcox S (1999). Color in textile effluents sources, measurement, discharge consents and simulation: Review. J. Chem. Technol. Biotechnol. 74: 1009-1018.
Park Ch, Lee M, Lee B, Kim SW, Chase HA, Lee J, Kim S (2006). Biodegradation and biosorption for decolorization of synthetic dyes by *funaliatrogii*. J. Biochem. Engin. In press. pp. 1-7.
Prout A (1990). Bench scale development and evaluation of a fungal bioreactor for color removal from bleach effluents. Appl. Microbiol. Biotechnol. 32: 490-493.
Quezada M, Linares I, Buitrón G (2000). Use of a sequencing batch biofilter for degradation of azo dyes (acids and bases). Water Sci. Technol. 42(5-6): 329-336.
Reid TM, Morton KC, Wang CY, King CM (1984). Mutagenicity of azo dyes following metabolism by different reductive/oxidative systems. Environ. Mutagen. 6: 705-717.
Robinson T, McMullan G, Marchant R, Nigam P (2001). Remediation of dyes in Textile effluent: A critical review on current treatment technologies with a proposed alternative. Biores. Technol. 77: 247-255.
Rojek K, Roddick FA, Parkinson A (2004). Decolorization of natural organic matter by *Phanerochaete chrysosporium*: the effect of environmental conditions. Water Sci. Technol., Water Suppl. 4(4): 175-182.
Rosenkranz HS, Klopman G (1990). Structural basis of the mutagenicity of 1-amino-2-naphthol-based azo dyes. Mutagenesis. 5(2): 137-146.
Sam M, Yesilada O (2001). Decolorization of Orange II dye by White-rot fungi. Folia Microbiol. (Praha). 46(2): 43-5.
Sandhya S, Padmavathy S, Swaminathan K, Subrahmanyam YV, Kaul SN (2005). Microaerophilic-aerobic sequential batch reactor for treatment of azo dyes containing simulated wastewater. Process Biochem. 40: 885-890.
Schliephake K, Lonergan GT, Jones CL, Mainwarning DE (1993). Decoloriza-tion of a pigment plant effluent by *Pycnoporus cinnabarinus* in a packed bed bioreactor. Biotechnol. Lett. 15(11): 1185-1188.
Semple KT, Cain RB, Schmidt S (1999). Biodegradation of aromatic compounds by microalgae. FEMS. Microbiol. Lett. 170: 291-300.
Shaul GM, Holdsworth TJ, Dempsey CR, Dostal KA (1991). Fate of water soluble azo dyes in the activated sludge process. Chemosphere, 22: 107-119.
Soares GM, Amorim MT, Hrdina R, Ferreire MC (2002). Studies on the biotransformation of novel diazo dyes by laccase, Process Biochem. 37: 581-587.
Sumathi S, Manju BS (2000). Uptake of reactive Textile dyes by *Aspergillus foetidus*. Enz. Microbiol.Technol. 27(6): 347-355.
Vandevivere PC, Bianchi R, Verstraete W (1998). Treatment and reuse of wastewater from the Textile wet-processing industry: Review of emerging technologies. J. Chem. Technol. Biotechnol. 72(4): 289-302.
Wagner S (1993). Improvements in products and processing to diminish environmental impact. COTTECH Conference, Raleigh, N.C, pp. 11-12.
Wesenberg D, Kyriakides I, Agathos SN (2003). White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol. Adv. 22: 161-187
Wong YX, Yu J (1999). Laccase catalyzed decolorization of synthetic dyes. Water Res. 33: 3512-3520.
Zhau W, Zimmerman W (1993). Decolorization of industrial effluents containing reactive dyes by actinomycetes. FEMs. Microbiol. Lett. 107: 157-162.
Zheng Z, Levin RE, Pinkham JL, Shetty K (1999). Decolorization of polymeric dyes by a novel Penicillium isolate. Proc. Biochem. 34: 31-37.