

2006

## Membrane coupled fungi reactor - an innovative approach to bioremediation of hazardous dye wastewater

Faisal I. Hai

*University of Wollongong*, [faisal@uow.edu.au](mailto:faisal@uow.edu.au)

Kazuo Yamamoto

*University of Tokyo*

Kensuke Fukushi

*University of Tokyo*

Follow this and additional works at: <https://ro.uow.edu.au/scipapers>



Part of the [Life Sciences Commons](#), [Physical Sciences and Mathematics Commons](#), and the [Social and Behavioral Sciences Commons](#)

---

### Recommended Citation

Hai, Faisal I.; Yamamoto, Kazuo; and Fukushi, Kensuke: Membrane coupled fungi reactor - an innovative approach to bioremediation of hazardous dye wastewater 2006.  
<https://ro.uow.edu.au/scipapers/4848>

---

## Membrane coupled fungi reactor - an innovative approach to bioremediation of hazardous dye wastewater

### Abstract

Virtually all the known physico-chemical and biological techniques have been explored for treatment of extremely recalcitrant dye wastewater; none, however, has emerged as a panacea. A single universally applicable end-of-pipe solution appears to be unrealistic, and combination of appropriate techniques is deemed imperative to devise technically and economically feasible options. An in-depth evaluation of wide range of potential hybrid technologies delineated in literature along with plausible analyses of available cost information has been furnished. In addition to underscoring the indispensability of hybrid technologies, this paper also endorses the inclusion of energy and water reuse plan within the treatment scheme, and accordingly proposes a conceptual hybrid dye wastewater treatment system.

### Keywords

GeoQUEST

### Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

### Publication Details

Hai, F. Ibney., Yamamoto, K. & Fukushi, K. (2006). Membrane-coupled fungi reactor - An innovation approach to bioremediation of hazardous dye wastewater. *Environmental Sciences*, 13 (6), 317-325.

# Membrane coupled fungi reactor - an innovative approach to bioremediation of hazardous dye wastewater

Faisal Ibney Hai<sup>a</sup>, Kazuo Yamamoto<sup>b</sup> and Kensuke Fukushi<sup>b</sup>

<sup>a</sup>Department of Urban Engineering, <sup>b</sup>Environmental Science Center, The University of Tokyo,

7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan.

Tel. +81-3-5841-6255; Fax +81-3-5841-8533; E-mail: [faisal@env.t.u-tokyo.ac.jp](mailto:faisal@env.t.u-tokyo.ac.jp)

## Abstract

Owing to the inherent shortcomings of the conventional biological dye-effluent treatment processes, researchers have proposed diverse intriguing approaches, which, however, await practical implementation. This study demonstrates the feasibility of an innovative membrane coupled fungi reactor. Preliminary batch tests divulged the noteworthy role of biosorption along with biodegradation in decoloration, and also confirmed excellent decoloration even under concurrence of hardly biodegradable polyvinyl alcohol with recalcitrant dye in wastewater. Conversely, the continuous reactor accomplished stable 97% TOC and 99% color removal with an HRT of 15 hrs. Remarkable diminution in UV-absorbance of membrane-permeate, and detection of short chain aliphatic acid in it provided evidence of subsequent biodegradation of the aromatic group following the breakdown of the color-imparting chromophoric group of the dye.

**Key words:** Bioremediation, Hazardous dye wastewater, Membrane coupled fungi reactor

## Introduction

Large amounts of dyes are annually produced and applied in many different industries including the textile, cosmetic, paper, leather, pharmaceutical and food industries. There are more than 100,000 commercially available dyes with an estimated annual production of over  $7 \times 10^5$  tons, fifteen percent of which is lost during the dyeing process. Residual dyes along with other auxiliary chemical reagents used for processing simultaneously impose massive load on wastewater treatment systems, eventually leading to poor color and COD removal performances. Conversely, the presence of even trace concentration of dyes in effluent is highly visible and the release of such colored wastewater in the ecosystem is a remarkable source of esthetic pollution, eutrophication and perturbations (due to toxicity and persistence) in aquatic life [1].

Several physico-chemical decolorization techniques have been reported (e.g. adsorption, chemical transformation, incineration, photocatalysis, ozonation or membrane separation), none, however, has appeared as a panacea due to high cost, low efficiency and inapplicability to a wide variety of dyes. Biodegradation is an environmental friendly and cost competitive alternative; but the conventional aerobic treatments have been proved ineffective while highly toxic aromatic amines can be formed by reductive fission under anaerobic conditions. Accordingly researchers have put forward a broad spectrum of intriguing bioremediation schemes as encapsulated in **Table.1**. At the present stage of development, the available reports, however, serve as rather 'preliminary proof of concept', and barriers against their practical implementation prevail. For instance, although literature is replete with reports demonstrating the excellent capacity of white-rot fungi to degrade recalcitrant dye effluent, so far, their application in large-scale waste treat-

Hai, F. Ibney., Yamamoto, K. & Fukushi, K. (2006). Membrane-coupled fungi reactor - An innovation approach to bioremediation of hazardous dye wastewater. *Environmental Sciences*, 13 (6), 317-325. 1

**Current address:** School of CME, University of Wollongong, Australia ([faisal@uow.edu.au](mailto:faisal@uow.edu.au)); <http://www.uow.edu.au/~faisal/>

ment has been impeded by the lack of bioreactor systems that can sustain steady production of high levels of enzymes for a prolonged period together with a controlled growth of fungi.

Based on a comprehensive state of the art review of the potential bioremediation approaches to abatement of hazardous dye wastewater, this study proposes a submerged membrane bioreactor implementing white-rot fungi and accordingly demonstrates its feasibility through bench scale batch and continuous flow reactor investigations.

**Table 1.** Bioremediation approaches to dye wastewater at a glance

Category	Examples	Selected Reference
Conventional biological processes	<ul style="list-style-type: none"> <li>Activated sludge, fluidized biofilm, different fixed film systems or a combination thereof.</li> </ul>	2-5
Standalone or sequential application of innovative biological processes	<ul style="list-style-type: none"> <li>Anaerobic decoloration followed by aerobic mineralization</li> </ul>	6
	<ul style="list-style-type: none"> <li>Integration of textile production with wastewater treatment e.g., two phase anaerobic treatment wherein acidic phase bioreactor is also shared for textile production.</li> </ul>	7
	<ul style="list-style-type: none"> <li>Activated sludge pretreatment (to reduce organic nitrogen) before fungi decoloration</li> </ul>	8
	<ul style="list-style-type: none"> <li>Fungi pretreatment before anaerobic treatment</li> </ul>	9
	<ul style="list-style-type: none"> <li>Combined fungi (biofilm) and activated sludge culture</li> </ul>	10
Hybrid systems having biological process as the core	<ul style="list-style-type: none"> <li>Activated algae reactor (with mixed population of algae and bacteria)</li> </ul>	11
	<ul style="list-style-type: none"> <li>Chemical coagulation step preceded by or antecedent to or even concomitant with biological treatment</li> </ul>	3,12
	<ul style="list-style-type: none"> <li>Direct addition of activated carbon in biological reactor yielding enhanced microbial degradation and bioregeneration of adsorbent</li> </ul>	13
	<ul style="list-style-type: none"> <li>Biological activated carbon bed</li> </ul>	14,15
	<ul style="list-style-type: none"> <li>Partial breakdown by advanced oxidation process prior to biological mineralization</li> </ul>	16
	<ul style="list-style-type: none"> <li>Membrane filtration of biologically treated wastewater</li> </ul>	17
	<ul style="list-style-type: none"> <li>Membrane bioreactor, MBR</li> </ul>	<b>This study</b>

## Materials and methods

Feasibility of the proposed MBR system, implementing *Coriolus versicolor*, was studied under controlled temperature and pH of  $29\pm 1^\circ\text{C}$  and  $4.5\pm 0.2$ , respectively, in a specially designed reactor (**Fig.1**) using a synthetic wastewater (TOC= 2g/L) containing dye (100 mg/L; Poly S119), starch (two common components in real textile wastewater) and other nutrients. De-

Hai, F. Ibney., Yamamoto, K. & Fukushi, K. (2006). Membrane-coupled fungi reactor - An innovation approach to bioremediation of hazardous dye wastewater. Environmental Sciences, 13 (6), 317-325. 2

**Current address:** School of CME, University of Wollongong, Australia ([faisal@uow.edu.au](mailto:faisal@uow.edu.au)); <http://www.uow.edu.au/~faisal/>

tails about the media have been documented elsewhere [18]. Mixed Liquor suspended solid (MLSS) concentration was measured according to the standard method. Transmembrane pressure (TMP), as an indicator of membrane fouling, was continuously monitored using a vacuum pressure gauge (GC 61, JUST).

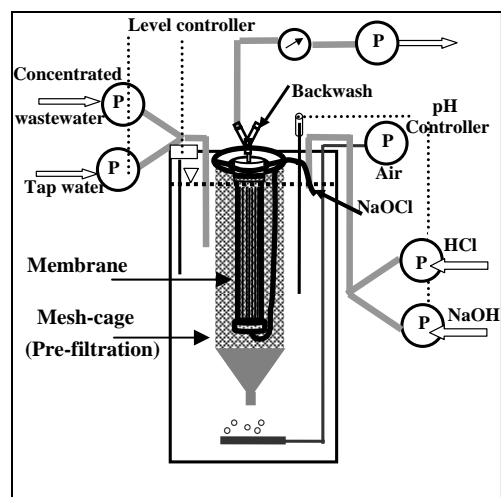
The bioreactor study was preceded by preliminary batch investigations to elucidate mechanism and extent of biological decoloration as well as the stability of the process during application with complex matrices of pollutants (dye along with polyvinyl alcohol, PVA). 500 ml conical flasks containing 250 ml of culture media (with specific amount of dye) were each aseptically inoculated with four pieces (approximately 1cm<sup>2</sup>) cut from actively growing culture on an agar plate and was incubated at the optimum growth temperature of 28 °C in aerobic condition (air diffusion through foam stopper) on a shaker at a speed of 90 rpm for specified period. After inoculation and at the indicated intervals of incubation, 2 ml of the media was removed and analyzed.

Pollutant removal performance was assessed by measuring Total organic carbon (TOC) concentration and absorbance at the peak wavelength (472 nm) of the dye, while high performance liquid chromatography (HPLC-UV) detecting short chain volatile fatty acids (VFAs) provided insight into degree of biodegradation. For the HPLC analysis, 0.025% (v/v) H<sub>2</sub>SO<sub>4</sub> solution was used as the eluent in conjunction with a Shimadzu, SCR-101H column. The corresponding injection volume, flow rate and column-temperature were 100 µl, 1 ml/min and 50°C, respectively. Fungal enzymatic ('Laccase') activity was measured according to Paszczynski et al.[19] by monitoring the OD<sub>468</sub>-change due to the oxidation of 2,6-dimethoxyphenol (DMP) at 25°C over 2 min using a spectrophotometer. PVA (n =500) degradation was quantified according to a spectrometric method based on the green color (690 nm) produced by the reaction of PVA with iodine in the presence of boric acid as described by Finley [20].

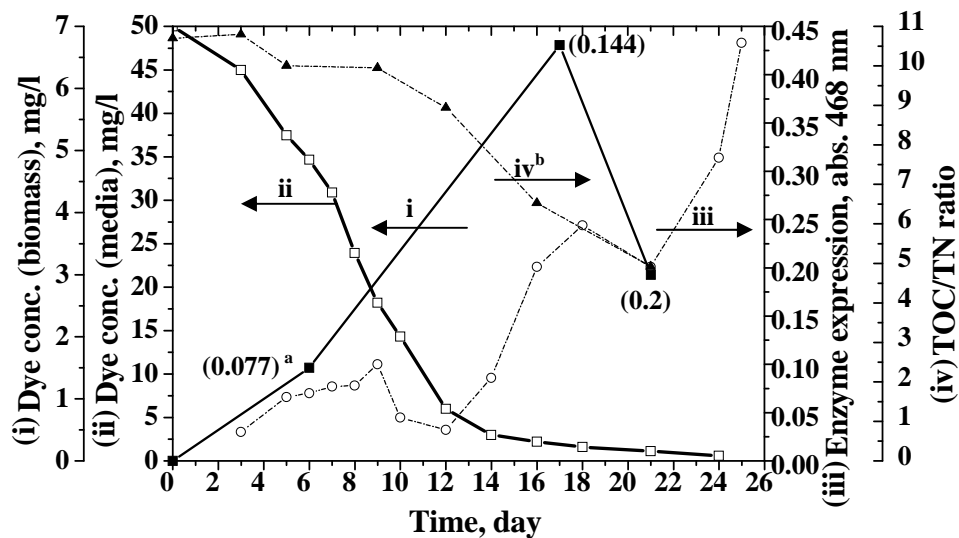
## Results

In line with the available reports, preliminary batch test explorations with the collected strain (*Coriolus versicolor*) revealed reasonable color and TOC removal performances (almost complete and 66%, respectively, within three weeks) concurrent with detectable level of expression of extracellular enzyme (**Fig.2**). In the course of depletion of the nutrients the enzyme profile exhibited a progressively increasing trend.

Biosorption, as a function of biomass and level of enzyme secretion, played significant role in media decoloration. For instance, on day 17 while the media appeared virtually colorless, significant amount of dye (Poly S119) still remained adsorbed on biomass, in absence of which the media would have exhibited a color equivalent to 6.7 mg/L of dye. The extent of biosorption, however, gradually subsided at the later stage with progressive improvement in level of enzyme secretion. Similar encouraging results were achieved with another dye Poly R478 (data not shown).



**Fig.1.** Schematic of the bench scale bioreactor system.



**Fig.2** Decoloration and enzyme profile in course of nutrient depletion (Batch test)

<sup>a</sup> Values within parentheses indicate corresponding dry biomass wt. (gm)

<sup>b</sup> Initial TOC/TN=2000/187; Final (3 weeks) TOC/TN=680/138 (mg/L).

Probable inhibition of decoloration of dye in presence of polyvinyl alcohol, another hardly biodegradable common pollutant in textile wastewater, was also assessed by adding it in amount of 500 mg/l in accordance with its actual concentration in such wastewater. The decoloration in presence of PVA appeared to be slower- indicating possible inhibition; the extent of such inhibition, however, was not severe. At the end of three weeks while the media with only dye (Poly R 478) exhibited a decoloration of 90.2%, the extent of decoloration in the media containing dye and PVA was 84.2%, with a concurrent 86% removal of PVA (**Table. 2**).

**Table 2.** Dye<sup>a</sup> and TOC removal<sup>b</sup> in presence of polyvinyl alcohol, (PVA)<sup>c</sup>

	Removal, %		
	Dye	TOC	PVA
Media with only dye	90.2	80.5	--
Dye along with PVA	84.2	62.4	86

<sup>a</sup> Poly R 478 (0.1g/L); <sup>b</sup>Batch test lasting for 3 weeks; <sup>c</sup>0.5 g/L

**Table 3.** Dye and TOC removal in continuous fungal MBR

	Concentration, mg/L		Removal, %
	Influent	Permeate <sup>a</sup>	
Dye	100	1-3	97-99
TOC	2000	40-60	97-98

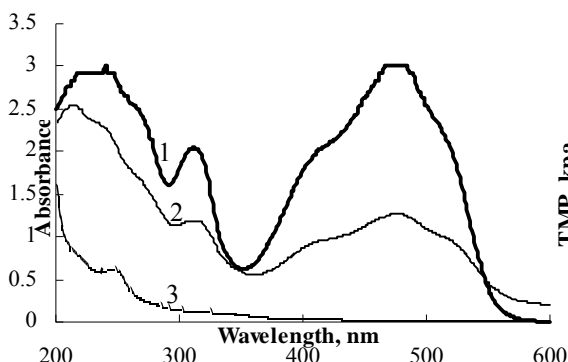
<sup>a</sup> Membrane-permeate was devoid of long chain aromatic and aliphatic acids and only contained acetic acid in avg. concentration of 140mg/L.

Conversely, the continuous reactor accomplished around 97% TOC and 99% color removal from the synthetic wastewater (TOC= 2g/L; Dye= 100 mg/L) for a prolonged period of observation under applied HRT and an average flux of 15 hrs and 0.011 m/d, respectively (**Table. 3**). Remarkable diminution of absorbance of the membrane-permeate in the UV range (**Fig. 3**)

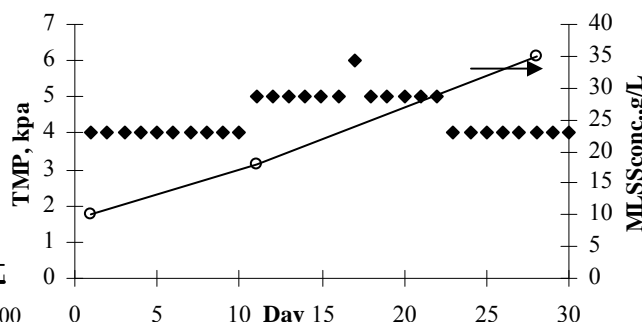
Hai, F. Ibney., Yamamoto, K. & Fukushi, K. (2006). Membrane-coupled fungi reactor - An innovation approach to bioremediation of hazardous dye wastewater. *Environmental Sciences*, 13 (6), 317-325.

**Current address:** School of CME, University of Wollongong, Australia ([faisal@uow.edu.au](mailto:faisal@uow.edu.au)); <http://www.uow.edu.au/~faisal/>

confirmed subsequent biodegradation of the aromatic group following the breakdown of the color-imparting chromophoric group of the dye. Detection of only short chain aliphatic acid like



**Fig.3.** Typical observed change in UV-VIS spectra, indicating dye-mineralization. (1) Influent, (2) Supernatant, (3) Permeate.



**Fig.4.** Mitigation of membrane fouling in proposed MBR: Time course of variation of transmembrane pressure along with rise in MLSS concentration.

acetic acid in the permeate by HPLC-UV analysis provided further evidence of considerable dye-mineralization.

In addition to the accomplishment of excellent stable pollutant removal, membrane-fouling (an inevitable consequence of interactions between membrane and the mixed liquor) was successfully precluded by placing a bundle of hollow fibers within a pre-filtration assembly, so as to avoid direct deposition of sludge onto it, together with its periodic high-pressure back-washing and low-dose chemical back-flushing (100 ml of chlorinated, 3000 ppm, permeate per  $\text{m}^2$  of membrane surface, every 3<sup>rd</sup> day) (**Fig.4**). Following slight fluctuations during the preliminary trials with the proposed fouling-mitigation strategy, the transmembrane pressure (TMP) remained stable around 4 kpa for an extended period of operation under progressively increasing MLSS concentration.

## Discussion

Biological treatment is a cost-competitive and eco-friendly alternative. Researchers are hence persistent in their pursuit of minimizing the inherent limitations of biological dye wastewater treatments. Several innovative attempts to achieve improved reactor design and/or to utilize special dye-degrading microbes or to integrate textile production with wastewater treatment, as summarized in **Table.1**, have been documented in literature. These approaches, however, await practical implementation. In this context, a membrane-coupled fungi reactor combining the excellent degradation capability of the white-rot fungi with the inherent advantages of a membrane bioreactor (MBR) was envisaged as a feasible system.

In this study, preliminary batch test exploration using a nutrient-sufficient media revealed almost complete color and 66%TOC removal within three weeks (**Fig.2**). Although the extracellular enzyme profile in the culture media exhibited a progressively increasing trend in the course of depletion of the nutrients, enzyme was detectable right from the first couple of days following the inoculation, and considerable extent of media decoloration was achieved long before the enzyme profile reached its peak. It is worth-mentioning here that, in line with the available reports

on necessity of nutrient-limitation (usually N) and presence of easily degradable carbon source for extracellular enzyme secretion by white-rot fungi, most of the studies [21] tend to utilize a media bearing irrationally high TOC/TN ratio (e.g.,  $\approx 100$ ), so that nitrogen is completely exhausted within first few days and an earlier onset of the peak enzymatic secretion, which enables a faster decoloration, is obtained. Such attempt, however, is of little practical significance keeping in mind that operation of continuous bioreactor necessitates maintenance of active biomass, which, in turn, requires appropriate TOC/TN ratio in feed wastewater.

Textile wastewater is a complex and highly variable mixture of many polluting agents ranging from inorganic and low molecular weight organic compounds to polymers [1]. PVA- a common constituent of textile wastewater, among others, has been reported to inhibit biological decoloration [22]. In this study too partial inhibition of dye decoloration efficiency in presence of hardly biodegradable compound PVA was observed (**Table.2**). It may however be stated that although inhibition to dye-decoloration imposed by the presence of PVA was evident, its extent was not severe, and, keeping in mind that batch test was preformed only with limited amount of biomass, the performance of the proposed bioreactor (MBR) may be expected to be better. Besides, as MBR offers the advantages of maintenance of high biomass and long SRT (independent of HRT), perhaps, a slightly reduced removal rate may be permitted.

In this study, MBR system was envisaged to be capable of coping with the impedances in implementation of white-rot fungi in large scale industrial waste treatment, such as, rather slow fungal degradation, loss of the extracellular enzymes and mediators with discharged water, and excessive growth of fungi. Indeed the laboratory scale fungal MBR sustained stable 99% color and 97% TOC removal from the utilized synthetic textile wastewater for a prolonged period (**Table.3, Fig.3**). The fact that membrane fouling was successfully precluded (**Fig.4**), adds to the preeminence of the proposed system. Nevertheless, a few further areas for optimization may be pointed out. For instance, probable enhancement of fungal decoloration by tuning the process parameters should be explored. Also assessment of performance with a more representative wastewater comprising of mixture of dyes of different structures along with other auxiliary chemicals would divulge the superiority of the proposed system.

## Conclusion

In view of the inherent shortcomings of the conventional biological dye-effluent treatment processes, this study proposed a membrane based biological dye wastewater treatment and accordingly demonstrated its technical feasibility. The specific conclusions that may be drawn from this study include,

- (i) Preliminary batch tests revealed the noteworthy role of biosorption along with biodegradation in decoloration; and also confirmed the process stability during application with wastewater containing complex matrices of pollutants (dye along with PVA).
- (ii) The stable decoloration in MBR was accompanied by remarkable extent of dye-mineralization.
- (iii) Membrane fouling, the major impedance against widespread use of MBR technology, was successfully avoided by employing cost-effective periodic chemical cleaning.

The proposed innovative approach shows great potential in terms of devising an excellent means to deal with hazardous dye wastewater.

## References

Hai, F. Ibney., Yamamoto, K. & Fukushi, K. (2006). Membrane-coupled fungi reactor - An innovation approach to bioremediation of hazardous dye wastewater. *Environmental Sciences*, 13 (6), 317-325.

**Current address:** School of CME, University of Wollongong, Australia ([faisal@uow.edu.au](mailto:faisal@uow.edu.au)); <http://www.uow.edu.au/~faisal/>



1. Hai, F.I., Yamamoto, K. and Fukushi, K. (2005): Hybrid treatment systems for dye wastewater. *Crit. Reviews. Env. Sci. Technol.* (In press)
2. Grau, P. (1991): Textile industry wastewaters treatment. *Wat. Sci. Tech.* 24: 97-103.
3. Yung-Kyu, P. and Chul-Hee, L. (1996): Dyeing wastewater treatment by activated sludge process with a polyurethane fluidized bed biofilm. *Wat. sci. Tech.* 34 (5-6):193-200.
4. Ahn, D-H., Chang, W-S. and Yoon, T-I. (1999):Dyestuff wastewater treatment using chemical oxidation, physical adsorption and fixed bed biofilm process. *Process Biochem.* 34: 429-439.
5. Zaoyan, Y., Ke, S., Guangliang, S., Fan, Y., Jinshan, D. and Huanian, M. (1992): Anaerobic-aerobic treatment of a dye wastewater by combination of RBC with activated sludge. *Wat. Sci. Tech.* 26(9-11), 2093-2096.
6. Zee, F.P.V. and Villaverde, S. (2005): Combined anaerobic–aerobic treatment of azo dyes—A short review of bioreactor studies. *Wat. Res.* 39: 1425-1440.
7. Feitkenhauer, H. and Meyer, U. (2001): Integration of biotechnological wastewater treatment units in textile finishing factories: from end of the pipe solutions to combined production and wastewater treatment units. *J. Biotechnol.* 89:185-192.
8. Miyata, N., Mori, T., Iwahori, K. and Fujita, M.(2000): Microbial decolorization of melanoidin-containing wastewaters: Combined use of activated sludge and the fungus *Coriolus hirsutus*. *J. Biosci. Bioeng.* 89: 145-150.
9. Feijoo G., Vidal, G., Moreira, M.T., Mendez, R. and Lema, J.M.(1995): Degradation of high molecular weight compounds of kraft pulp mill effluents by a combined treatment with fungi and bacteria. *Biotechnol. Lett.* 17(11): 1261-1266.
10. Kapdan, I.K. and Kargi, F. (2002): Simultaneous biodegradation and adsorption of textile dyestuff in an activated sludge unit. *Process Biochem.* 37:973-981.
11. Aziz, M.A. and Ng, W.J. (1993): Industrial wastewater treatment using an activated algae-reactor. *Wat. Sci. Tech.* 28(7), 71-76.
12. Dulkadiroglu, H., Dogruel, S., Okutman, D., Kabdasli, I., Sozen, S. and Orhon, D. (2002) Effect of chemical treatment on soluble residual COD in textile wastewaters. *Wat. sci. Tech.* 45(12): 251-259.
13. Pala, A. and Tokat, E. (2002): Color removal from cotton textile industry wastewater in an activated sludge system with various additives. *Wat. res.* 36: 2920-2925.
14. Walker, G.M. and Weatherley, L.R. (1997): A simplified predictive model for biologically activated carbon fixed beds. *Process Biochem.* 32: 327-335.
15. Qing, T., Chen, J., X, D. and Hua, Z.(2004): Study on the Enriched Oxygen BAC Method in the Advanced Treatment of Textile Dying-printing and Alkali Peeling Wastewater. *Proceedings of IWA World water congress, Marrakech, #28287.*
16. Mantzavinos, D. and Psillakis, E. (2004):Enhancement of biodegradability of industrial wastewaters by chemical oxidation pre-treatment. *J. Chem. Technol. Biotechnol.* 79: 431-454.
17. Rozzi, A., Antonelli, M. and Arcari, M. (1999): Membrane Treatment of Secondary Textile Effluents for Direct Reuse. *Wat. sci. Tech.* 40 (4-5): 409-416.
18. Hai, F.I, Yamamoto, K. and Fukushi, K. (2005): Different fouling modes of submerged hollow-fiber and flat-sheet membranes induced by high strength wastewater with concurrent bio-fouling. *Desalination*, 180: 89-97.

19. Paszczynski, A., Pasti, M.B., Goszczynski, S., Crawford, D.L. and Crawford, R.L. (1991): New approach to improve degradation of recalcitrant azo dyes by *Streptomyces* spp. and *Phanerochaete chrysosporium*. *Enzyme Microb. Technol.* 13: 378-384.
20. Finley, J.S. (1961): Spectrophotometric determination of polyvinyl alcohol in paper coatings. *Anal. Chem.* 33: 1925-1927.
21. Kapdan, I.K., Kargi, F., McMullan, G. and Marchant, R. (2000) Effect of environmental conditions on biological decolorization of textile dyestuff by *C. versicolor*. *Enzyme Microb. Technol.* 26: 381–387.
22. Harazono, K. and Nakamura, K. (2005): Decolorization of mixtures of different reactive textile dyes by the white-rot basidiomycete *Phanerochaete sordida* and inhibitory effect of polyvinyl alcohol. *Chemosphere* 59: 63–68.