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Bioremediation of Textile Effluent with Membrane Bioreactor Using the White-rot Fungus *Coriolus versicolor*

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Water is a prime candidate for the development of intensive water recycling strategies and the recovery of valuable chemical products in large industry. The present study performs the bench-scale submerged microfiltration bioreactor using the white-rot fungus *Coriolus versicolor* for treatment of textile dye wastewater and was confirmed the decoloration capacity of the fungus strain in agar-plate and aqueous batch studies. The temperature and pH of the reactor was controlled at 29±1° C and 4.5±2 respectively. The bioreactor was operated with an average flux of 0.05 m/d (HRT=15hrs) for a month. Extensive growth of fungi and their attachment to the membrane led to its fouling and associated increase of transmembrane pressure requiring periodic withdrawal of sludge and membrane cleaning. However, stable decoloration activity (approx. 98%) and TOC removal (>95%) was achieved using the entire system (fungi+membrane), while the contribution of the fungi culture alone to color and TOC removal, as indicated by the quality of the reactor supernatant, was 35-50% and 70%, respectively. The present study was assessed the decolorization efficiency of the collected white rot fungi strains through agar plate and liquid batch studies and, subsequently assessed the feasibility of a submerged microfiltration membrane bioreactor implementing the fungi culture for treatment of textile dye wastewater.

Key words: Textile Effluent, Bioreactor, White-rot fungi & Remediation.

The textile industry is one of the largest water consumers and is rated as the most polluting among all industrial sectors considering both volume and composition of effluent globally[1, 2]. It is a complex and highly variable mixture of many polluting substances ranging from inorganic compounds and elements to polymers and organic products[3, 4]. It induces persistent color coupled with organic load leading to disruption of the total ecological/symbiotic balance of the receiving water stream. Dyes with striking visibility in recipients may significantly affect photosynthetic activity in aquatic environment due to reduced light penetration and may also be toxic to some aquatic lives due to metals, chlorides, etc., associated with dyes or the dyeing process. It is difficult to remove dyes from effluents since dyes are stable to light, heat and oxidizing agents and are non-biodegradable[5, 6].

A number of secondary/ biological treatment have been reported like conventional treatment as filtration, adsorption on activated carbon (and other adsorbents), ion exchange, complex formation and chemical precipitation,

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flocculation and coagulation, chemical oxidation and reduction[7, 8], activated sludge, anaerobic/ aerobic, fixed biomass [9, 10], and advanced treatment – membrane filtration and photo decolourisation[11, 12]. However few have been accepted by the textile industries because of their lack of implementation 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 are ineffective for textile wastewater[13, 14, and 15]. Few anaerobic microorganisms can biodegrade dyestuffs by azoreductase activity, but highly biotoxic aromatic amines can be formed by reductive fission under anaerobic conditions [16, 17 and 18]. Though, wood rotting 'white-rot' fungi are able to degrade aerobically a wide variety of recalcitrant organic pollutants, including various types of dyes through extracellular secretion of non-specific oxidative enzymes as a secondary metabolic activity in C or N-limited medium[1, 19 and 20].

The application of white-rot fungi in large-scale for wastewater treatment has been impeded by the lack of bioreactor systems that can sustain steady production of high levels of ligninolytic enzymes for a long period together with a controlled growth of fungi [21, 22 and 23]. The extensively used systems were moved towards tank reactor and air-lift and bubble column, fixed bed bioreactor, rotating disk reactor and silicone-membrane reactor. Specifically few reports are there on dye decolorization in continuous bioreactors like Asgher et al., (2006)[13] reported 80% decolorization of a disperse dye (Red-553) in a continuous (10-20 days) fixed-film bioreactor. Zhang et al. (2006)[2] also investigated continuous decolorization of an azo dye, Orange II, in a packed-bed reactor, achieving high decolorization efficiency (97%). However, a number of operational problems such as formation of mycelia aggregates, electrode fouling and clogging emerged after a short time and made necessary the periodical removal of fungal biomass from the reactors. Mielgo et al. (2001)[24] proposed a pulsed flow bioreactor packed with immobilized fungi, which treated dye loads of 0.2 g dye/m^3.day at over 90% efficiency for several months. *In vitro* dye decolorization by manganese peroxidase in an enzymatic membrane reactor in continuous operation has been studied by Lopez et al. (2002)[25]. The system allowed a very fast decoloration with over 90% efficiency under high dye loading rate of 2.4 g dye/m^3.d. Fujita et al. (2000)[26] achieved 70% decolorization of heat-treatment liquor of waste sludge by a bioreactor using polyurethane foam-immobilized white-rot fungus equipped with a side stream ultrafiltration membrane. The overall feasibility of such a system, however, would depend upon the alleviation of the membrane-fouling problem. Selection and proper arrangement of an appropriate type of membrane, less vulnerable to fungi attachment and also easier to clean (for example, flat sheet type membrane), in a properly designed reactor ensuring efficient mass transfer with adequate aeration system is required.

This study was assessed the decolorization efficiency of the collected white rot fungi strains through agar plate and liquid batch studies and, subsequently assessed the feasibility of a submerged microfiltration membrane bioreactor implementing the fungi culture for treatment of textile dye wastewater.

MATERIALS AND METHODS

The white rot fungi, *Coriolus versicolor* strains used in this study had not been evaluated previously for decolorization and hence it was done using two polymeric dyes Poly R-478 and Poly S-119 (representatives of major commercial dyes). Feasibility of the proposed MBR system was studied in a specially designed reactor using a synthetic wastewater.

The study were used the white-rot fungi strains, *Coriolus versicolor*, NBRC 9791 and NBRC 30388 which obtained from the NITE Biological Resource Center (NBRC), Japan. The stock culture was grown on Potato Sucrose Agar (PSA) medium at 26.5°C (growth temperature range= 24-28 °C) as prescribed by NBRC. The culture was maintained at 4 °C and refreshed every 30–40 days. NBRC 9791 was used in the bioreactor experiment because of its superior performance in the batch test.

Poly R-478 (polyvinylamine sulfonated backbone with anthrapyridone chromophore, violet color) and Poly S-119 (polyvininylamine backbone with azo chromophore, orange color) were chosen for the present study. Then the peak absorbancein the visible range correspond to the wavelengths 520 nm and 472 nm for Poly R-478 and Poly S-119, respectively. Since textile effluent

contains a range of dyes, successful decoloration of a single dye does not adequately indicate the suitability of an organism for a decoloration process. However, these two polymeric dyes represent the majority of the synthetic dyes[27].

Degradation and decoloration studies

Solid medium in Petri plates were prepared using PSA medium to which an aliquot of an individual dye was added to a final concentration of 100 mg/l. Each plate containing one of the dyes and a control plate with no dye added were inoculated with NBRC 9791 and NBRC 30388 and kept for incubation at 26.5°C (IL 600 incubator, Yamato). In this study un- inoculated plates served as controls for abiotic decoloration. The experiment was performed in triplicate for each culture.

Preparation of liquid culture media

The synthetic media used which was almost the same as the low nitrogen media optimized by Kapdan & Kargi, (2002)[28] for *C. versicolor*. The only modification was replacement of glucose by starch, which is used in real textile wet processing. Then the media was made of 4.5 gl^{-1}starch, 0.4 gl^{-1} urea, 2 gl^{-1} KH_2PO_4, 0.099 g l^{-1} $CaCl_2$, 1.025 gl^{-1} $MgSO_4.7H_2O$, 0.001 gl^{-1} thiamine, 1 ml l^{-1} trace elements and desired concentration of the dyestuff. The TOC of the medium was around 2000 mg/l (dye TOC» 50mg/l). Stock trace elements solution was prepared by dissolving 0.125 g $CuSO_4.5H_2O$, 0.05 g H_2MoO_4, 0.061 g $MnSO_4.5H_2O$, 0.043 g $ZnSO_4.7H_2O$, 0.082 g $Fe_2(SO_4)_3.14H_2O$ in 1 l of milli-Q water. pH of the media was adjusted to 4.5 by HCl and NaOH

Batch studies and degradation pathway in aqueous Solution

300 ml flasks containing 200 ml of culture media (with 50 mg/l of dye) were aseptically inoculated with four pieces (approximately $1cm^2$) cut from the actively growing culture on an agar plate and incubated at the optimum growth temperature of 28 °C in aerobic condition (air diffusion through silicon stopper) on a shaker (BR-300LF, Taitec Bio-shaker) at a speed of 90 rpm for specified period. After inoculation and at the indicated intervals of incubation, 2 ml of the extracellular culture was removed and diluted properly (5 times for absorbance and 50 times for TOC measurement) with milli-Q water before measurement of absorbance and Total Organic Carbon.

Equipment and operating conditions of the bioreactor

A laboratory scale bioreactor, made of PVC pipes with a working volume of 12.5 liter was used. A schematic of the experimental set-up is depicted in Fig. 1.R_1. To facilitate complete mixing, the reactor was divided by a baffle (Fig.1.R_2) into two interconnected compartments-the larger one containing two hollow fiber polyethylene membranes (UMF 0234L1, Mitsubishi Reiyon), each having a surface area and pore size of 0.2 m^2 and 0.4mm, respectively. Air was provided from the bottom of the reactor by using three diffusers connected to air pumps. As shown in Fig. 1.R_2 the central diffuser (air flow 5l/min) and the other two diffusers (air flow from each 2.5l/min.) were operated alternately with a 5 min. cycle so that at any time the aeration rate in the reactor was 5l/min. This type of arrangement of the diffusers was expected to be effective for complete mixing along with membrane cleaning. The system was operated continuously under controlled temperature of 29±1°C. pH of the system was controlled at 4.5±2 by adding 0.3 N HCl and 0.3 N NaOH by pumps controlled by a pH controller. The media used in the unremitting experiment was the same as that used in the liquid batch test. pH- adjusted concentrated synthetic wastewater was diluted with pH-adjusted tap water and then supplied into the reactor by pumps controlled by a level controller (61F, Omron). The concentrated media was constantly stirred and also the temperature of the mixing tank was kept at 50°C to avoid settling of starch, which is poorly soluble in water under room temperature. The reactor was operated with an average flux of 0.05 m/d (HRT= 15 hrs.) and this produced 20 liters of effluent everyday. Effluent was filtered out through the membranes by suction pumps with a 5 min. on/off cycle (an instantaneous flux of 0.1 m/d across each membrane) for the first 9 days after when the cycle was changed to 9 min. on and 1 min. off to reduce the instantaneous flux (0.055 m/d) while maintaining the same average flux. The transmembrane pressure was monitored using vacuum pressure gauges (GC 61, JUST).

The system was first inoculated with fungi grown for two weeks in 1 liter Erlenmeyer flasks each containing 500 ml of the culture media and the reactor was operated in batch mode for a week after which the continuous operation was

started with a MLSS concentration less than 2000 mg/l. Specific amount of sludge was wasted from the reactor and membranes were cleaned (off site manual cleaning with water) when membrane fouling was so severe that the transmembrane pressure exceeded 60 kpa or so.

Analytical methods

Total organic carbon analyzer (TOC-V, Shimadzu C391-E058K) was used to analyze Total Organic Carbon as per standard methods (APHA 21th edition, 2005)[29]. The samples for TOC analysis in batch tests were homogenized (Branson sonifier 450) for 5 minutes (30% duty cycle, output control of 3) prior to the measurement. The samples were not filtered because starch are poorly soluble in water, would be retained by a filtering unit of 0.45mm. Samples from the membrane bioreactor were free from suspended solid and hence did not require any treatment before TOC measurement. The color (Dye) of the samples was measured by using a spectrophotometer (U-2010, Hitachi). The concentration of dyestuff was calculated from a calibration curve of 'absorbance versus concentration' and concentration values were used for calculations of decolorization efficiency. The sample from batch test for absorbance measurement was filtered through a Dismic-25 hydrophilic filtering unit (0.45mm, mixed cellulose ester). The absorbance measurement on the reactor supernatant and final effluent was made after centrifuging the sample (H-3R centrifuger, Kokusan) for 10 mins. at 3000 rpm. MLSS concentration was measured according to the APHA Standard methods[29].

RESULTS AND DISCUSSION

Preliminary assessment of dye decoloration was completed using solid medium. After five days, the study shown that the extent of mycelial growth on the agar plates was analogous for all cultures whether or not any dye was present and fungi grew extensively with white mycelia all over the agar plates. Both the top and bottom of the agar plate appeared almost colorless when the over-grown fungi mycelia on it were carefully removed after 20 days. It was the clear signal of decoloration capacity of the two strains studied here. No abiotic decoloration was perceived in uninoculated plates.

In this study, both the fungi strains showed good growth in liquid medium, the growth of NBRC 9791 being a bit faster. The fungi grew like white cotton balls in colorless culture media, and in media with dye the fungi mycelia turned colored due to absorption of dye. **Fig.2** shows the decoloration of Poly S-119 by both the fungi strains. A stable decrease of absorbance value was observed and almost complete disappearing of absorbance happened within two weeks. The study shown the decoloration rate of NBRC 9791 was faster than that of NBRC 30388 and the absorbance

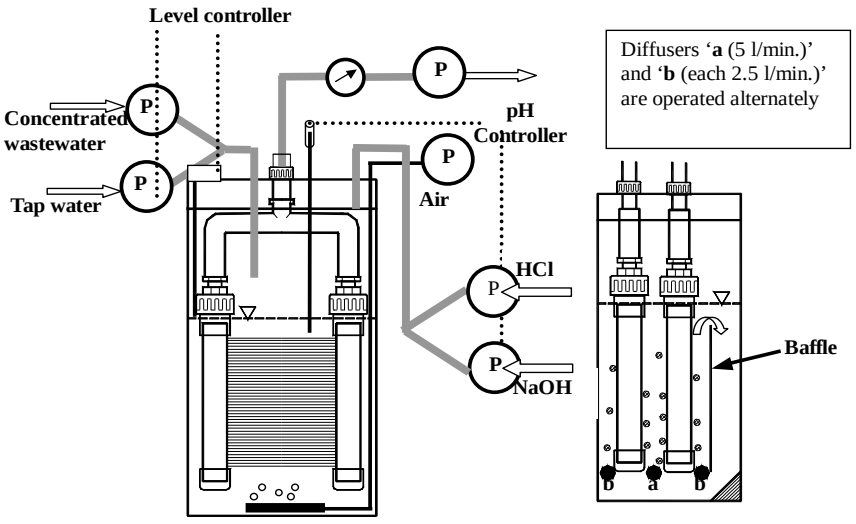


Fig.1. R_1 Schematic of the laboratory set up ; R_2 Side view of the reactor

value did not increase further even in 1 month which indicated stable decolorization without any release of dye from the fungi mycelia. Similarly efficient decoloration was exhibited in case of the dye Poly R-478 (results not shown).

The fungi have also shown stable TOC removal. The initial TOC of approximately 2000 mg C/l was reduced to almost its one-fourth within 2 weeks (**Fig.3)**. The TOC removal by fungi in media with or without dye was analogous, which indicated the suitability of the fungi for colored wastewater treatment. Over a month the final pH of the media inoculated with NBRC 30388 and NBRC 9791 were 5.61 and 5.3, respectively.

Performance of the membrane separated fungi reactor

Moghaddam et al. (2002)[30] have revealed that at low to moderate range (»5000 mg/l) of MLSS concentration, more presence of filamentous bacteria can reduce the severity of filter clogging in the coarse pore (50-200mm) filtration activated sludge process. They discoursed that this might be due to an additional external layer on the filter surface by the filamentous bacteria. This advantage, however, did not stand for "excessive abundance" of filamentous bacteria. In our study, although the initial MLSS concentration of the reactor was less than 2 g/l, it increased gradually and, despite of total sludge wastage of 7 liters in two steps (on 10th and 20th day), the MLSS concentration rose up to 29g/l (**Fig. 4**) within 25 days. Transmembrane pressure increased sharply (**Fig. 4**) due to severe membrane fouling by the fungi. The filamentous fungi were entwined with the membrane fibers in such a way that the fine air bubbles from the diffusers could not scrub the fungi off the membrane; rather the bubbles sometimes pushed the fungi more into the membrane. In this deference, a flat sheet type membrane module, with its characteristic flat shape and fiber less structure, might be suitable to prevent excess membrane attachment of fungi. There is also an opportunity to improve the design of the reactor and the aeration system to ensure

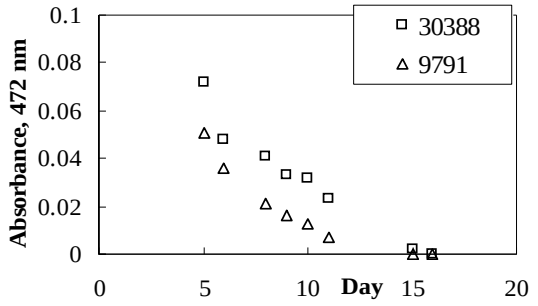


Fig. 2. Decolorization of Poly S119 by the two fungi strains (Batch test)

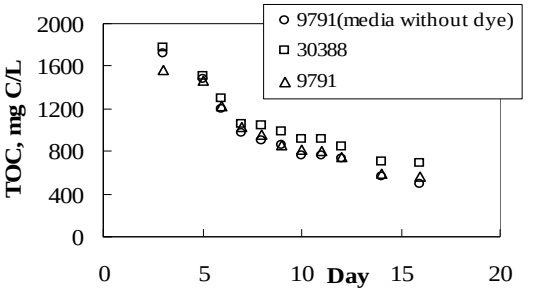
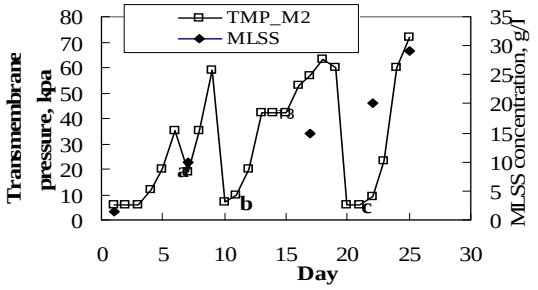
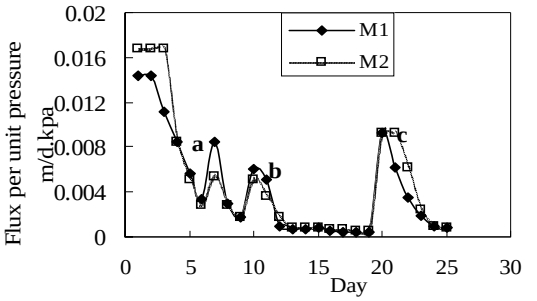


Fig. 3. TOC removal by the two fungi strains (Batch test)



a. Membrane cleaning, **b**. Sludge withdrawal and suction cycle change, **c**. Membrane cleaning and sludge withdrawal.

Fig. 4. Variation of MLSS concentration, and transmembrane pressure through membrane



a. Membrane cleaning, **b**. Sludge withdrawal and suction cycle change, **c**. Membrane cleaning and sludge withdrawal.

Fig. 5. Variation of flux per unit pressure through the membranes

enhanced mass transfer and scouring of sludge from the membrane surface[31, 32, 33 and 34].

The membranes were cleaned twice, first on day 7 (inside the reactor simply by brushing) and then on day 20 (outside of the reactor simply by water) during the 25-day operation period. Effluent was filtered out through the membranes by suction pumps with a 5 min. on/off cycle (an instantaneous flux of 0.1 m/d across each membrane) for the first 9 days after when the cycle was changed to 9 min. on and 1 min. off to reduce the instantaneous flux (0.055 m/d) while maintaining the same average flux (0.05 m/d). Higher 'flux per unit pressure' (**Fig. 5**) observed at the initial stage decreased later on. The 'flux per unit pressure' was recovered to some extent by membrane cleaning (day 7), suction cycle change and sludge withdrawal (day 10), and simultaneous cleaning and sludge withdrawal (day 20). After around three weeks, the fungi culture was observed to be composed predominately of fine particulate pellets rather than filamentous ones. However, this change did not influence the transmembrane pressure or color and TOC removal.

Removal of total organic carbon

The removal efficiency of total organic carbon (TOC) by the reactor ranged from 92% (at the beginning) to 97% (after a week and further on). **Fig.6** shows the TOC variation in the effluent during the operation period. The total organic carbon of influent water was around 2000 mg/l, then after the operation period, effluent TOC never exceeded 160mg/l, and shown the average effluent TOC was around 70 mg/l. The supernatant TOC was around 500 mg/l. Major portion of the influent TOC was contributed by the high dose of starch, and the membrane used in this study (pore size 0.4mm) was able to retain a considerable portion of this poorly soluble starch by sieving. In fact, starch was observed to be adsorbed on the fungi attached on the membrane and created a sticky layer on the membrane. Also some amount of starch was observed to settle at the bottom of the reactor. However, there was no gradual accumulation of the settled starch, which indicates its subsequent degradation and assimilation by the fungi.

Degradation and decolorization of dye

The reactor showed stable decolorization throughout its operation period (**Fig. 6**). The concentration of dyestuff as calculated from a calibration curve of 'absorbance versus concentration' revealed nearly complete decolorization (98%). The dye concentration in the supernatant of the reactor indicated 35-50% decolorization by the fungi culture alone under the applied HRT. Degradation and decolorization and of Poly S-119 were also followed by analysis of UV–VIS absorbance scanning before and after the treatment. The UV–VIS spectrum of the effluent of the bioreactor showed a remarkable change after the treatment (**Fig. 7**). The disappearance of the absorbance peak at 472 nm indicated an unequivocal signal of the nearly complete decolorization and the breakdown in the chromophoric group. Besides, the notable diminution of the absorbance peak at 210 nm is related to the cleavage of the aromatic group present in the original structure of the dye.

In this study the dye Poly S-119 itself was soluble enough to pass through the microfiltration membrane (pore size 0.4mm) are used. This, however, was observed to be retained by a

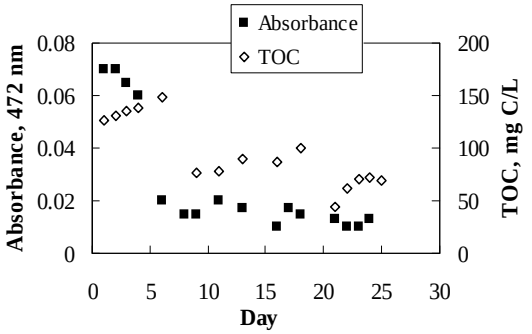


Fig. 6. TOC and color removal by the reactor (Influent TOC » 2000 mg C/L, absorbance=3)

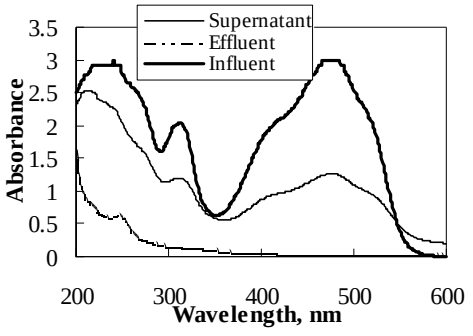


Fig.7. Comparison of UV-visible spectra showing simultaneous decolorization and biodegradation of dye

laboratory Dismic-25 filtering unit (0.45mm) when mixed with starch to make the synthetic wastewater. From this, it might be stated that the membrane first retained the major portion of the dye adsorbed on starch and on the fungi, and then the fungi subsequently degraded the dye. A synergistic effect of the membrane-fungi-starch combination for decolorization is thus projected here.

CONCLUSIONS

In this study, the preliminary agar plate and aqueous batch decoloration investigations revealed the decoloration efficiency of the collected fungi strains (*C. versicolor,* NBRC 9791 and NBRC 30388). The stable TOC and color removal (>95% and 98%, respectively) from the synthetic wastewater by the submerged microfiltration membrane reactor using the white rot fungi (*C. versicolor,* NBRC 9791) presented the system as a promising one. The synergistic effect of starch-fungi-membrane combination on decolorization was of special interest.

Membrane bioreactors are a good solution for textile wastewater treatment because of their many advantages during stable nitrification and denitrification processes. The quality of treated effluent output from the membrane bioreactor is more stable than that achieved by employing other techniques, enabling optimal functioning of the secondary treatment system. According to the authors' knowledge, no attempt has been made until now to use a submerged membrane bioreactor with white-rot fungi culture for decolorization of dye wastewater. Water re-use in textile industry requires appropriate effective treatment process which enables acceptable water quality. Further, capital and maintenance cost have to be considered carefully by implementing a treatment process. Our results show that MBR with spiral wound model is a technique of choice for water re-use.

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REFERENCES

1. Fu Y, Viraraghavan T. Fungal decolorization of dye wastewaters: a review. *Bioresour. Technol.*, 2001; **79**, 251-262.
2. Zhang J, Chuan H C, Zhou J, and Fane A G. Factors affecting the Membrane Performance in Submerged Membrane Bioreactors. *Journal of Membrane Science*, 2006; **284**, 54–66.
3. Asgher M, Bhatti H N, Ashraf M, Legge R L. Recent developments in bioremediation of industrial pollutants by white rot fungi and their enzyme system, *Biodegradation*. 2008; **19**, 771–783.
4. Asgher Muhammad, Azim Naseema, Bhatti Haq Nawaz. Decolorization of practical textile industry effluents by white rot fungus *Coriolus versicolor* IBL-04, *Biochemical Engineering Journal*. 2009; **47**, 61–65
5. Palmieri G, Cennamo G, Sannia G. Remazol Brilliant Blue R decolourisation by the fungus Pleurotus ostreatus and its oxidative enzymatic system, *Enzyme and Microbial Technology*. 2005; **36**, 17–24.
6. Lopez M J, Guisado G, Vargas Garcia M C, Estrella F S, Moreno J. Decolourization of industrial dyes by ligninolytic microorganisms isolated from compositing environment, *Enzyme and Microbial Technology*. 2006; **40** 42–4532.
7. Azbar N, Yonar T, Kestioglu K. Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fiber dyeing effluent, *Chemosphere*. 2004; **55**, 35-43.
8. Yun M A, Yeon K M, Park J S, Lee C H, Chun J, Lim D J. Characterization of biofilm structure and its effect on membrane permeability in MBR for dye wastewater treatment." *Water Res.*, 2006; **40**, 45-52.
9. Oliver J, Hao, Hyunook Kim, Pen-Chi Chiang. Decolorization of Wastewater, *Critical Reviews in Environmental Science and Technology,* 2000; **30**: 4, 449-505.
10. Levin L, Papinutti L, Forchiassin F. Evaluation of Argentinean white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes, *Bioresource Technology*. 2004; **94**, 169–176.
11. Shu H Y, Chang M C, Hsieh W P. Remedy of dye manufacturing process effluent by UV/ H2O2 process, *Journal of Hazardous Materials*, 2006; **128**, 60- 66.
12. Alatona I A, Balcioglub I A, Bahnemann D W. Advanced oxidation of a reactive dye bath effluent: comparison of O3, H_2O_2/UV-C and

TiO2/UV-A processes, *Water Res.*, 2002 **36**, 1143-1154.

13. Asgher M, Shah S A H , Ali M , Legge R L. Decolorization of some reactive dyes by white rot fungi isolated in Pakistan, *World Journal of Microbiology and Biotechnology.* 2006; **22**, 89–93.
14. Meng F, Chae S R, Drews A, Kraume M, Shin H S, Yang F. Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material, *Water Research,* 2009; **43**, 1489-1512.
15. Brik M, Schoeberl P, Chamam B, Braun R, Fuchs W. Process Biochemistry: Advances treatment of textile wastewater towards reuse using membrane bioreactor, *Process biochem.,* 2006; **41**(8), 1751-1757.
16. Susla M, Novotny C, Srobodora K. The implication of Dichomitus squalens laccase isoenzymes in dye decolourization by immobilized fungal cultures, *Bioresource Technology.* 2007; **98**, 2109–2115.
17. Tavaker M, Svobodova K, Kuplenk J, Novotny C, Pavko. Biodegradation of Azo Dye RO16 in different reactors by immobilized Irpex lacteus, *Acta Chimica Slovakia.* 2006; **53,** 338–343.
18. Chander M, Arora D S. Evaluation of some white-rot fungi for their potential to decolourize industrial dyes, *Dyes and Pigments.* 2007; **72**, 192–198.
19. Revankar M S, Lele S S. Synthetic dye decolorization by white rot fungus, *Ganoderma sp*., WR-1, *Bioresource Technology.* 2007; **98**, 775–780.
20. Hamedaani H R., Sakurai A, Sakakibara M. Decolorization of synthetic dyes by a new manganese peroxidase producing white rot fungus, *Dyes and Pigments.* 2007; **72**, 157–162.
21. Bhatti H N, Akram N, Asgher M. Optimization of culture conditions for enhanced decolorization of Cibacron Red FN-2BL by *Schyzophullum commune* IBL-6, *Applied Biochemistry and Biotechnology.* 2008; **149**, 255–264.
22. Hai FI, Yamamoto K, Fukushi K. Development of a submerged membrane fungi reactor for textile wastewater treatment, *Desalination.* 2006; **192**, 315–322.
23. Kang I.-J, Lee C-H, Kim K-J. Characteristics of microfiltration membranes in a membrane coupled sequencing batch reactor system. *Water Research.* 2003; **37**, 1192–1197
24. Mielgo I, Moreira M T, Feijoo G, Lema J M. A packed-bed fungal bioreactor for the continuous decolourisation of azo-dyes (Orange II). *J. Biotechnol.* 2001; **89**, 99-106
25. Lopez C, Mielgo I, Moreira M T, Feijoo G, Lema J M. Enzymatic membrane reactors for biodegradation of recalcitrant compounds. Application to dye decolourisation. *J. Biotechnol.* 2002; **99**, 249-257
26. Fujita M, Era A, Ike M, Soda S, Miyata N, Hirao, T. Decolorization of Heat-Treatment Liquor of waste sludge by a bioreactor using polyurethane foam-immobilized white rot fungus equippedwith an ultra-membrane filtration unit. *J. Bioscience and bioengineering.* 2000; **90**(4), 387-394.
27. Anja Drews. Membrane fouling in membrane bioreactors characterization, contradictions, cause and cures. *Journal of Membrane Science.* 2010; **363;** 1–28
28. Kapdan I K, Kargi F. Simultaneous biodegradation and adsorption of textile dyestuff in an activated sludge unit. *Process Biochemistry.* 2002; **37**, 973-981.
29. American Public Health Association 21th edition, (2005) Standard Methods for the Examination of Water & Wastewater.
30. Moghaddam M R A, Satoh H, Mino T. Performance of coarse pore filtration activated sludge system. *Wat. Sci. Technol.,* 2002; **64**(11-12), 71-76.
31. Rozzi, A; Malpei, F; Bianchi, R; Mattioli, D. Pilot-scale membrane bioreactor and reverse osmosis studies for direct reuse of secondary textile effluent, *Water Sci. Technol.,* 2000; **4;** 189-195.
32. Lee J-W, Choi S-P., Thiruvenkatachari R, Shim W G, Moon, H. Submerged microfiltration membrane coupled with alum coagulation/ powdered activated carbon adsorption for complete decolorization of reactive dyes', *Water Research.* 2006; **40**(3) 435–444.
33. Santos R, Dixit A, Guha S. Sequential batch culture studies for the decolorization of reactive dye by *Coriolus versicolor*, *Bioresource Technology*. 2006; **97**, 396–400.
34. Jin Y L, Lee W N, Lee C H, Chang I S, Huang X, Swaminathan T. Effect of DO Concentration on biofilm structure and membrane filterability in submerged membrane bioreactor. *Water Res.,* 2006; **40**(15), 2829-2836.