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Membrane-Coupled Fungi Reactor— An Innovative Approach to Bioremediation of Hazardous Dye Wastewater

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Owing to the inherent shortcomings of conventional biological dye effluent treatment processes, researchers have proposed diverse intriguing approaches that await practical implementation. This study demonstrates the feasibility of an innovative membrane-coupled fungi reactor. Preliminary batch tests revealed the noteworthy role of biosorption along with biodegradation in decoloration, and also confirmed excellent decoloration even in the presence of hardly biodegradable polyvinyl alcohol besides recalcitrant dye in the wastewater. Conversely, the continuous reactor achieved stable 97% total organic carbon (TOC) and 99% color removal with a hydraulic retention time (HRT) of 15 h. A marked decrease in the UV absorbance of the membrane permeate, and the detection of short-chain aliphatic acids in the permeate provided evidence of the subsequent biodegradation of the aromatic group following the breakdown of the color-imparting chromophoric group of the dye.

1. Introduction

Large amounts of dyes are produced annually and applied to 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 more than 7×10^5 tons, 15% of which is lost during dyeing. Residual dyes and other auxiliary chemical reagents used for processing simultaneously impose a massive load on wastewater treatment systems, eventually leading to poor color and COD removal performance. Conversely, the presence of even trace concentrations of dyes in effluent is highly visible and the release of such colored wastewater into the ecosystem is a significant source of esthetic pollution, eutrophication and perturbations (due to toxicity and persistence) in aquatic life.[1]

Although several physicochemical decolorization techniques have been reported (*e.g.*,

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adsorption, chemical transformation, incineration, photocatalysis, ozonation or membrane separation), none, however, has appeared as a panacea because of their high cost, low efficiency and inapplicability to a wide variety of dyes. Biodegradation is an environmentally friendly and cost-competitive alternative; however, conventional aerobic treatments have been proved ineffective, whereas 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 shown in Table 1.[2–17] At the present stage of development, the available reports, however, serve rather as 'preliminary proof of concept', and barriers against their practical implementation prevail. For instance, although the literature is replete with reports demonstrating the excellent capacity of white-rot fungi to degrade recalcitrant dye effluent, to date, their application in large-scale waste treatment has been impeded by the lack of bioreactor systems that can sustain the steady production of high levels of enzymes for a prolonged period together with the controlled growth of fungi.

On the basis of a comprehensive state of the art review of the potential bioremediation approaches to hazardous dye wastewater, in this study, we propose a submerged membrane bioreactor (MBR) containing white-rot fungi and accordingly demonstrate its feasibility through batch tests and bench-scale continuous flow reactor investigations.

Table 1
Bioremediation approaches to dye wastewater at a glance.

Category	Example	Selected Reference
Conventional biological processes	• Activated sludge, fluidized biofilm, different fixed film systems or a combination thereof	2–5
Stand-alone or sequential application of innovative biological processes	• Anaerobic decoloration followed by aerobic mineralization	6
	• Integration of textile production with wastewater treatment *e.g.*, two-phase anaerobic treatment wherein an acidic phase bioreactor is also shared for textile production.	7
	• Activated sludge pretreatment (to reduce organic nitrogen) before fungi decoloration	8
	• Fungi pretreatment before anaerobic treatment	9
	• Combined fungi (biofilm) and activated sludge culture	10
	• Activated algae reactor (with mixed population of algae and bacteria)	11
Hybrid systems having biological process as core	• Chemical coagulation step preceded by or antecedent to or even concomitant with biological treatment	3,12
	• Direct addition of activated carbon in biological reactor yielding enhanced microbial degradation and bioregeneration of adsorbent	13
	• Biological activated carbon bed	14,15
	• Partial breakdown by advanced oxidation process prior to biological mineralization	16
	• Membrane filtration of biologically treated wastewater	17
	• Membrane bioreactor, MBR	**This study**

2. Materials and Methods

The white-rot fungus *Coriolus versicolor*, NBRC 9791, obtained from the Nite Biological Resource Center (NBRC), Japan, was used for this study. The feasibility of the proposed MBR system was studied under controlled temperature and pH of 29±1°C and 4.5±0.2, respectively, in a specially designed reactor (Fig. 1) using a synthetic wastewater (TOC = 2 g/L) containing dye (100 mg/L; Poly S119), starch (two common components in actual textile wastewater) and other nutrients. Details regarding the media have been documented elsewhere.[18] The system was first inoculated with fungi grown for two weeks in 1-L Erlenmeyer flasks each containing 500 ml of the culture media. During the preliminary trials the mixed liquor suspended solid (MLSS) concentration increased up to 10 g/L, whereas in the course of the final trial, it increased from 10 g/L to 55 g/L in 50 days in the absence of any sludge withdrawal. 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 the 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). Conical flasks (500 ml) containing 250 ml of culture media (with a specific amount of dye) were each aseptically inoculated with four pieces (approximately 1 cm^2) cut from actively growing cultures on an agar plate and incubated at the optimum growth temperature of 28°C under aerobic conditions (natural air diffusion through the foam stopper of the flask) on a shaker at a speed of 90 rpm for a 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 the total organic carbon (TOC) concentration and absorbance at the peak wavelength of the dye (472 and 520 nm for Poly S119 and Poly R478 dye, respectively), whereas high-performance liquid chromatog-

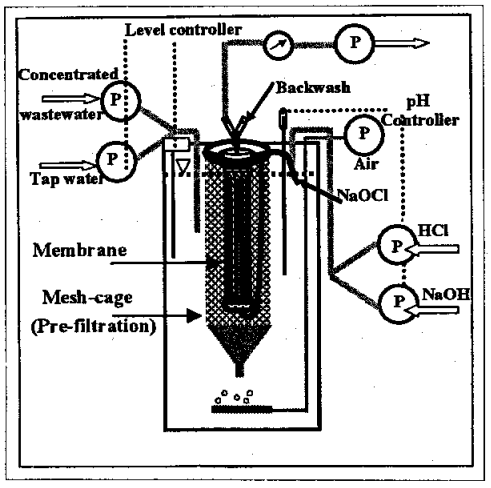

Fig. 1. Schematic of bench scale bioreactor system.

raphy using UV (HPLC-UV) detecting short-chain volatile fatty acids (VFAs) provided insight into the degree of biodegradation. For the HPLC analysis, 0.025% (v/v) H_2SO_4 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 the method of Paszczynski *et al.*[19] by monitoring the OD468 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]

3. Results

In line with the available reports, preliminary batch test explorations with the collected strain (*C. versicolor*, NBRC 9791) revealed reasonable color and TOC removal performance (almost complete and 66%, respectively, within three weeks) concurrent with a detectable level of the expression of an extracellular enzyme (Fig. 2). In the course of the depletion of nutrients, the enzyme profile exhibited a progressively increasing trend.

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

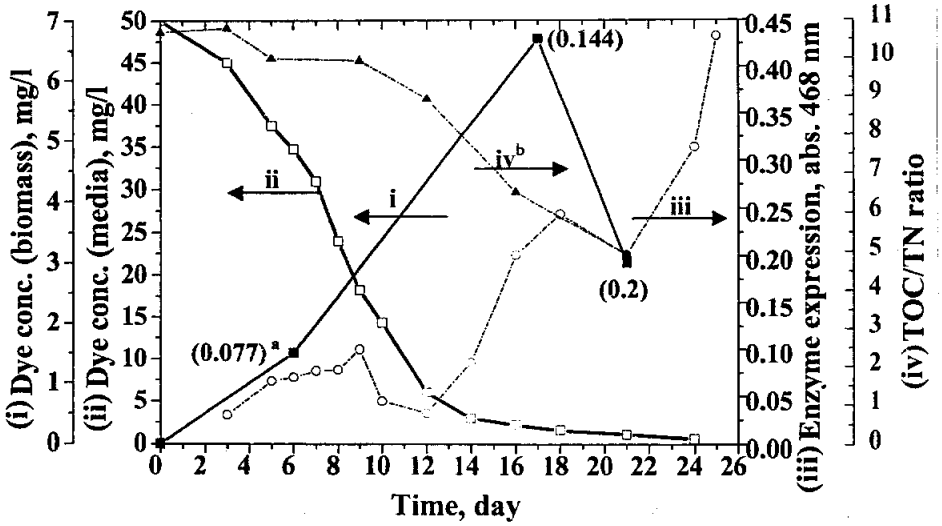

Fig. 2. Decoloration and enzyme profile in course of nutrient depletion (Batch test).
[a]Values within parentheses indicate corresponding dry biomass wt. (gm).
[b]Initial TOC/TN=2000/187; Final (3 weeks) TOC/TN=680/138 (mg/L).

The probable inhibition of dye decoloration in the presence of polyvinyl alcohol (PVA), another hardly biodegradable common pollutant in textile wastewater, was also assessed by adding PVA in the amount of 500 mg/L in accordance with its actual concentration in such wastewater. The decoloration in the 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, although the media containing only the 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).

Conversely, the continuous reactor achieved approximately 97% TOC and 99% color removal from the synthetic wastewater (TOC = 2 g/L; Dye = 100 mg/L) for a prolonged period of observation under an applied HRT and an average flux of 15 h and 0.011 m/d, respectively (Table 3). A marked decrease in the absorbance of the membrane permeate in the UV range (Fig. 3) confirmed the subsequent biodegradation of the aromatic group following the breakdown of the color-imparting chromophoric group of the dye. The detection of only short-chain aliphatic acids, such as acetic acid, in the permeate by HPLC-UV analysis provided further evidence of considerable dye mineralization.

In addition to achieving excellent stable pollutant removal, membrane fouling (an inevitable consequence of interactions between the membrane and the mixed liquor) was successfully precluded by placing a bundle of hollow fibers within a prefiltration assembly so as to prevent the direct deposition of sludge onto it, together with periodic high-pressure back-washing and low-dose chemical backflushing (100 ml chlorinated permeate, 3000 mg Cl/L per m^2 of membrane surface, every 3rd day) (Fig. 4). Following slight fluctuations during the preliminary trials with the proposed fouling-mitigation strategy, the transmembrane pressure (TMP) remained stable at approximately 4 kpa for an extended period of operation under a progressively increasing MLSS concentration.

Table 2
Dye* and TOC removal** in presence of polyvinyl alcohol, (PVA)***.

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

*Poly R 478 (0.1 g/L); **Batch test lasting for 3 weeks; ***0.5 g/L.

Table 3
Dye and TOC removal in continuous fungal MBR.

	Concentration, mg/L		Removal, %
	Influent	Permeate*	
Dye	100	1–3	97–99
TOC	2000	40–60	97–98

*Membrane permeate was devoid of long-chain aromatic and aliphatic acids and only contained acetic acid at an average concentration of 140 mg/L.

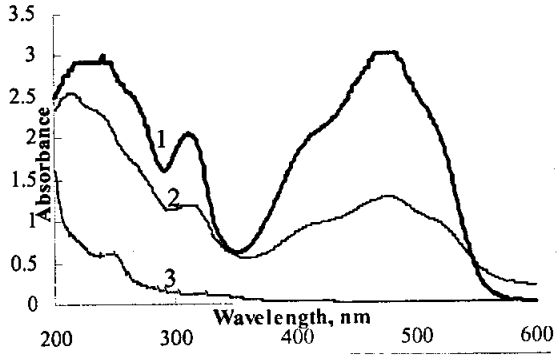

Fig. 3. Typical observed changes in UV-VIS spectra indicating dye mineralization. (1) Influent, (2) supernatant, (3) permeate.

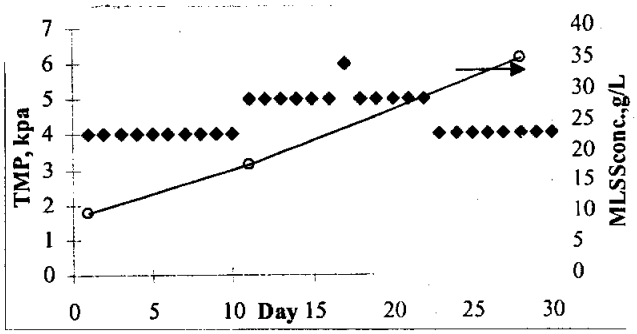

Fig. 4. Mitigation of membrane fouling in proposed MBR: Time course of variation in transmembrane pressure along with increase in MLSS concentration.

4. Discussion

Biological treatment of hazardous dye wastewater is a cost-competitive and ecofriendly alternative to conventional chemical treatment. 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 or to utilize special dye-degrading microbes or to integrate textile production with wastewater treatment, as summarized in Table 1, have been documented in the 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 during the course of the depletion of nutrients, the enzyme was detectable from the first couple of days following inoculation, and considerable 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 the necessity of nutrient limitation (usually N) and the presence of an easily degradable carbon source for extracellular enzyme secretion by white-rot fungi, most of the studies (*e.g.*, ref. 21) tend to utilize a media with an impractically high TOC/TN ratio (*e.g.*, ≈100), so that nitrogen is completely exhausted within the first few days and an earlier onset of the peak enzymatic secretion, which enables a faster decoloration, is obtained. Such an approach, however, is of little practical significance keeping in mind that the operation of a continuous bioreactor necessitates the maintenance of active biomass, which, in turn, requires an appropriate TOC/TN ratio in the 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, partial inhibition of dye decoloration efficiency in the presence of the hardly biodegradable compound PVA was observed (Table 2). It may however be stated that, although inhibition of dye decoloration in the presence of PVA was evident, its extent was not severe, and, keeping in mind that batch tests were preformed only with limited amounts of biomass, the performance of the proposed bioreactor (MBR) can be expected to be better. In addition, as MBR offers the advantages of the maintenance of high biomass and long sludge retention time, SRT (independent of HRT), perhaps a slightly reduced removal rate may be acceptable.

In this study, the MBR system was considered to be capable of coping with impedances in the application of white-rot fungi to large-scale industrial wastewater treatment, such as the rather slow fungal degradation, the loss of extracellular enzymes and mediators with discharged water, and the 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 result 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, the probable enhancement of fungal decoloration by tuning the process parameters should be explored. Also, an assessment of performance with a more representative wastewater containing a mixture of dyes of different structures along with other auxiliary chemicals would reveal the potential of the proposed system.

5. Conclusion

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

(i) Preliminary batch tests revealed the noteworthy role of biosorption along with biodegradation in decoloration and also confirmed stability of the process during application with wastewater containing complex matrices of pollutants (dye along with PVA).

(ii) The stable decoloration in the MBR was accompanied by a marked level of dye mineralization.

(iii) Membrane fouling, the major impedance against the widespread use of the MBR technology, was successfully prevented by employing cost-effective periodic chemical cleaning.

The proposed innovative approach shows great potential in terms of developing a sustainable system for treating hazardous dye wastewater.

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