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ORIGINAL PAPER

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Evaluation of support materials for the surface immobilization and decoloration of amaranth by *Trametes versicolor*

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Abstract The ability of *Trametes versicolor* ATCC 20869 to colonize several natural and synthetic materials (wheat straw, jute, hemp, maple woodchips, and nylon and polyethylene teraphthalate fibers) and to subsequently decolorize amaranth was evaluated. Jute was found to be the best support material as *T. versicolor* grew well on it without color leaching from the support and without loss of the jute's integrity over a 4 week period. The fungus immobilized on jute, straw and hemp decolorized amaranth (50 mg l^{-1}) at a rate of about 5 mg l^{-1} h^{-1} without glucose being added. When 1 g l^{-1} glucose was added, the dye was degraded more quickly (about 8 mg l^{-1} h^{-1}). Decoloration did not occur in a suspension culture without glucose. As the number of decoloration cycles increased, the rate of decoloration decreased. This rate was restored to its original level after the biomass was incubated in fresh growth medium for 5 days. With all immobilization supports, the toxicity of the medium before and after decoloration was the same or lower.

Introduction

The release of colored effluent into the environment is of growing concern as color is a visible pollutant that is increasingly being regulated in the USA, Canada and Europe. Colored effluents may arise from the textile, dyestuff manufacturing, and pulp and paper industries. Globally, it has been estimated that 10% of the total dyestuff used (Maguire 1992), or about 7×10^5 tonnes per annum (Vaidya and Datye 1982), is released into the environment. Not only is color not aesthetically pleasing, it also impacts the ecosystem by affecting the light penetration and gas solubility in aquatic environments. Furthermore, some synthetic dyes, such as azo dyes, may be carcinogens or mutagens (Zollinger 1991; Spadaro et al. 1992) and, under anaerobic conditions, can be transformed into aryl amines that are potentially more toxic than the parent compounds (Chung and Stevens 1993; Dubrow et al. 1996).

Physical and chemical treatments of textile dyes are expensive and may generate a large volume of sludge (Dubrow et al. 1996), while biological wastewater treatments have low removal efficiencies (Dubrow et al. 1996; Kapdan et al. 2000a). Furthermore, no single conventional method has been found to be effective for all dye classes. Thus, there is a need for the development of new technologies for color removal. Biological treatment is an attractive option as it could be cost-effective and environmentally friendly (Azmi et al. 1998).

White rot fungi produce non-specific, extracellular, ligninolytic enzymes that can degrade a wide range of organopollutants such as polycyclic aromatic hydrocarbons (Barr and Aust 1994; Boyle et al. 1998; Andersson and Henrysson 1996), trinitrotoluenes (Van Aken et al. 1999; Kim and Song 2000), trichloroethylene (Yadav et al. 2000), chlorophenols (Alleman et al. 1995; Grey et al. 1998), polychlorinated biphenyls (Krcmar et al. 1999) and various synthetic dyes (Glenn and Gold 1983; Heinfling et al. 1997; Kapdan et al. 2000b). Textile dye decoloration has been demonstrated with suspension cell culture (Glenn and Gold 1983; Heinfling et al. 1997; Swamy and Ramsay 1999a; Kapdan et al. 2000b), immobilized cells (Yang and Yu 1996; Liao et al. 1997; Pallerla and Chambers 1997; Zhang et al. 1999) and crude enzyme extracts (Ollika et al. 1993; Rodriguez et al. 1999). The major drawback to using an enzyme preparation is that once the enzymes become inactivated, activity decreases. However, with a whole cell culture, the enzymes could be continually replenished. Immobilized cultures tend to have a higher level of activity and are more resilient to environmental perturbations such as pH, or exposure to toxic chemical concentrations than suspension cultures. Immobilization by encapsulation in a matrix such as alginate may be too costly for wastewater treatment while surface immobilization on an inexpensive material such as woodchips is cheaper. In

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this paper, the ability of *Trametes versicolor* to colonize several natural and synthetic materials (wheat straw, jute, hemp, maple woodchips, and nylon and polyethylene teraphthalate fibers) and to subsequently decolorize amaranth as a model azo dye was evaluated. The toxicity of the medium before and after decoloration was also determined.

Materials and methods

Culture maintenance and inoculum preparation

T. versicolor ATCC 20869 was maintained at 4°C on malt agar plates and was used to inoculate 500 ml Erlenmeyer shake flasks containing 200 ml sterilized modified Kirk's medium (Kirk et al. 1978). The inoculum was incubated for 5 days at 30°C and 200 rpm on a rotary shaker (Innova 2000, New Brunswick Scientific, N.J.). The pellets of mycelial biomass were pooled and 10% (volume of biomass/volume of liquid medium) was distributed aseptically to 500 ml shake flasks containing 200 ml modified Kirk's medium and approximately equal volumes of the support materials. The flasks were shaken at 150 rpm at room temperature (23°C) for 5 days to allow colonization. All support materials and culture media had been previously autoclaved for 20 min at 121°C and 15 psi.

Support materials

Wheat straw and peat were purchased from a local gardening center (Kingston, Ont.), and jute twine, nylon fiber (Scotchbrite) and polyethylene teraphthalate fiber were purchased at a local department store (Kingston, Ont.). The hemp materials (core, fiber, and mat) and a composite of 50% hemp and 50% polypropylene were provided by Kenex (Pain Court, Ont.). Maple woodchips were donated by J&A tree service company (Kingston, Ont.). About 315 cm of jute twine with an uncompressed diameter of 3 mm weighing 5.5 g was used in these experiments.

Growth medium

The modified Kirk's medium used for growth and rejuvenation contained, per liter: 10.1 g glucose (Sigma, St. Louis, Mo.), 11.0 g ammonium tartrate, 0.2 g KH_2PO_4 (Anachemia, Montreal, Quebec), 0.05 g $MgSO_4{\cdot}7H_2O$ (BDH, Toronto, Ont.), 0.01 g $CaCl_2{\cdot}2H_2O$ (BDH), 1 µg thiamine (Sigma), and 0.1 ml trace mineral solution. The trace mineral solution consisted of (per liter): 30 g $MgSO_4{\cdot}7H_2O$ (BDH), 10 g NaCl (BDH), 5 g $MnSO_4{\cdot}H_2O$ (Anachemia), 1 g $CoSO_4$ (Fisher, Fair Lawn, N.J.), 1 g $FeSO_4{\cdot}7H_2O$ (Anachemia), 1 g $ZnSO_4{\cdot}7H_2O$ (Fisher), 0.82 g $CaCl_2$ (BDH), 100 mg $CuSO_4{\cdot}5H_2O$ (Anachemia), 100 mg $NaMoO_4{\cdot}2H_2O$ (Fisher), 100 mg H_3BO_3 (Anachemia) and 1 g EDTA (Fisher). The decoloration medium consisted solely of 50–100 mg l^{-1} amaranth (Sigma) and 0–2 g l^{-1} glucose (Sigma).

Colonization and sequential decoloration

After the establishment of a healthy biofilm on the support materials (about 5 days of incubation at room temperature) in 500 ml Erlenmeyer flasks, the growth medium was carefully decanted in the laminar flow hood leaving the support matrix with the attached biofilm in the shake flask. The sequential decoloration began with the addition of 200 ml sterile decoloration medium to the flask. The decoloration sequence for all support materials is the same as for straw (Fig. 1). In the first decoloration, 50 mg l^{-1} amaranth and 1 g l^{-1} glucose were used. Once decolorized, the spent medium was discarded and replaced by fresh medium. After the amaranth was totally decolorized, the medium was replaced with a fresh volume of the same composition. After these two decolorations, the initial dye concentration was doubled to 100 mg l^{-1} and the glucose concentration kept at 1 g l^{-1}. In the next decoloration, the glucose concentration was increased to 2 g l^{-1} and the amaranth kept at 100 mg l^{-1}. Following this decoloration, the biomass was allowed to grow in fresh modified Kirk's medium for 5 days and the sequence repeated starting with the decoloration conditions of day 1. Samples were frozen until analyzed. Amaranth concentration was measured spectrophotometrically at 523 nm (the λ_{max} of amaranth).

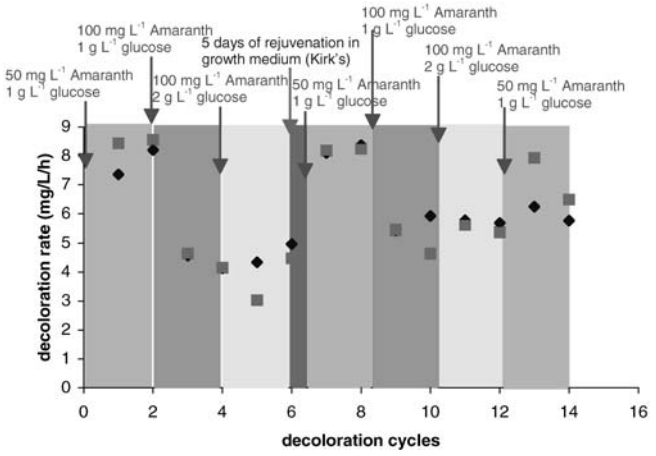


Fig. 1 Rate of amaranth decoloration by *Trametes versicolor* immobilized on wheat straw. For the first decoloration, the growth medium was replaced with a decoloration medium consisting of 50 mg l^{-1} Amaranth and 1 g l^{-1} glucose. At the end of each decoloration, the decolorized medium was replaced with fresh medium. The same sequence of decoloration was used for all support materials. *Squares* and *diamonds* represent two independent experiments performed concurrently

Microtox assay

Frozen samples were brought to room temperature, centrifuged, and the pH of the supernatant adjusted to 6.0 by the addition of 0.5 ml 0.58 M KH_2PO_4 and 70 µl 1.0 M NaOH. Color correction was done at 490 nm. The Microtox acute toxicity assay was performed in a Microtox 500 Analyzer on samples at the start and end of the decoloration cycles according to the test protocols defined by the manufacturer (Azur Environmental, Newark, Del.). From eight serial dilutions, the effective concentration for 20% inhibition of the luminescence of a modified strain of *Vibrio fischeri* (EC_{20}) after 5 min incubation was calculated with the Microtox data analysis program (Microtox Omni Software 1999). A solution of 1 g l^{-1} $ZnSO_4{\cdot}7H_2O$ was used as the positive control and 1 g l^{-1} glucose as the negative control. All values are the average of duplicate experiments in which each sample was analyzed in triplicate.

Results

Amaranth decoloration by *T. versicolor* immobilized on different support materials

Shake flasks containing each support material (wheat straw, hemp fiber, hemp mat, hemp core, jute twine, peat, maple woodchips, nylon fiber, polyethylene teraphthalate fiber or hemp-polypropylene fiber) in modified Kirk's medium were inoculated with *T. versicolor* and incubated for 5 days at 23°C and 250 rpm. The nylon fiber and peat

Table 1 Growth, maximum decoloration achieved in the presence and absence of 1 g l^{-1} glucose and durability of support after 4 weeks of growth when *Trametes versicolor* is immobilized on the support material. Initial amaranth concentration is 50 mg l^{-1}. Relative scale for growth: from +++++ very good to – no growth; for colonization: from +++++ fungus strongly attached to + fungus poorly attached; for durability: from +++++ material intact after 4 weeks to + material destroyed within 1 week

Support	Growth	Colonization	Durability	Maximum rate of decoloration (mg l^{-1} h^{-1})	
				Glucose	No glucose
No support	+++	NA[a]	NA	3.67	0.42
Straw	+++++	+++++	+	8.41	5.62
Jute twine	+++++	+++++	+++++	8.23	5.39
Maple	+++++	++	++	7.94	ND[b]
Peat	+	–	NA	ND	ND
Hemp fibers	+++	+++	+	6.33	4.56
Hemp core	++	+	++	3.31	Very slow
Hemp mat	++	+	+++	3.12	Very slow
Hemp/polypropylene mat	+	+	++++	2.96	No decoloration
Polyethylene teraphthalate fibers	+	+	+++	2.83	No decoloration
Nylon fibers	–	NA	+++++	ND	ND

[a] Not applicable; [b] Not determined

were eliminated at this stage as growth was negligible in their presence. Furthermore, the liquid in the flask containing peat became dark brown in color. After 5 days of growth, the immobilized fungus on the other support materials was subjected to the sequence of decolorations shown in Fig. 1. When no support was provided, the mycelial biomass formed pellets and 50 mg l^{-1} amaranth was decolorized at a rate of about 3.7 mg l^{-1} h^{-1} when 1 g l^{-1} glucose was provided (Table 1). There was good growth on straw and jute with the highest decoloration rate being about 8.4 mg l^{-1} h^{-1}. Poor growth on hemp core, hemp mat and polyethylene teraphthalate fibers resulted in a lower decoloration rate (about 3 mg l^{-1} h^{-1}). Although *T. versicolor* is found on hardwoods such as maple trees in nature, the biomass did not attach well to the maple woodchips. Most of the biomass was suspended in the liquid phase throughout the experiment and was eventually washed out as the spent medium was replaced. Initially, good decoloration activity (7.9 mg l^{-1} h^{-1}) was obtained but this decreased as the biomass levels diminished with each medium replacement. Colonization of maple woodchips may be more successful with no agitation in a reactor such as a trickling filter.

Although the decoloration rate was the most rapid with straw and jute, the straw disintegrated within 2 weeks while the integrity of the jute was maintained over a period of at least 4 weeks. At day 9, the straw was noticeably pulpy. By day 14, there was little visible straw within a large fungal mass. The decoloration rate had also decreased from 8.4 mg l^{-1} h^{-1} in the first decoloration to 4.7 mg l^{-1} h^{-1} at day 14 for the same dye and glucose concentration. Hemp fiber also showed a loss of integrity after 5–7 days.

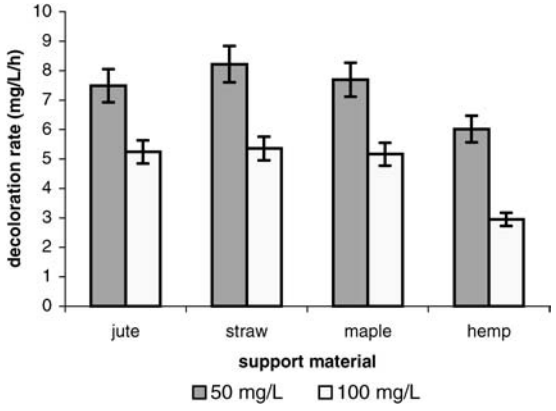


Fig. 2 Effect of dye concentration (50 and 100 mg l^{-1}) on the rate of decoloration at an initial glucose concentration of 1 g l^{-1} when *T. versicolor* was immobilized on various materials. Values are averages of duplicates of four decoloration cycles

Effect of amaranth and glucose concentrations on decoloration

When the initial dye concentration was doubled from 50 to 100 mg l^{-1} with the glucose concentration kept at 1 g l^{-1}, the rate of decoloration decreased in most cases by 27–48% (Fig. 2). When *T. versicolor* was in suspension with no support or was immobilized on a synthetic matrix such as polyethylene teraphthalate fiber, decoloration occurred only when glucose was added (Table 1). However, when glucose was not added to *T. versicolor* immobilized on either jute, straw or hemp fiber, complete decoloration took place in 16–20 h at a rate of 3–3.8 mg l^{-1} h^{-1} at an initial dye concentration of 100 mg l^{-1} (Fig. 3). When 1 g l^{-1} of glucose was added with the dye, higher decoloration rates were obtained with all support materials. Higher glucose concentrations (2 g l^{-1}) did not result in higher rates of decoloration (Fig. 3).

Effect of rejuvenation of biomass on decoloration performance

In all cases, as the number of decoloration cycles increased, the decoloration rate decreased (data not

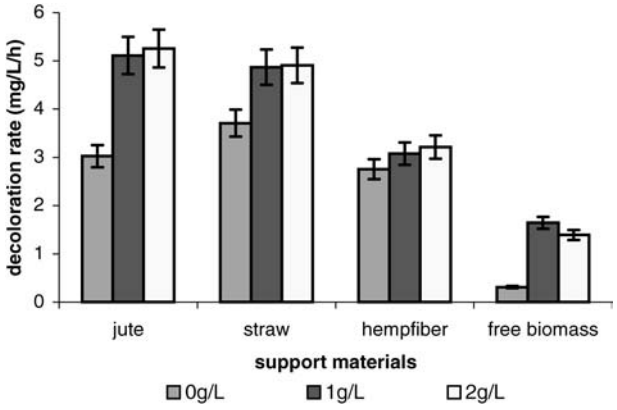


Fig. 3 Effect of glucose concentration (0–2 g l^{-1}) on the rate of decoloration at an initial amaranth concentration of 100 mg l^{-1} when *T. versicolor* was colonized on various materials

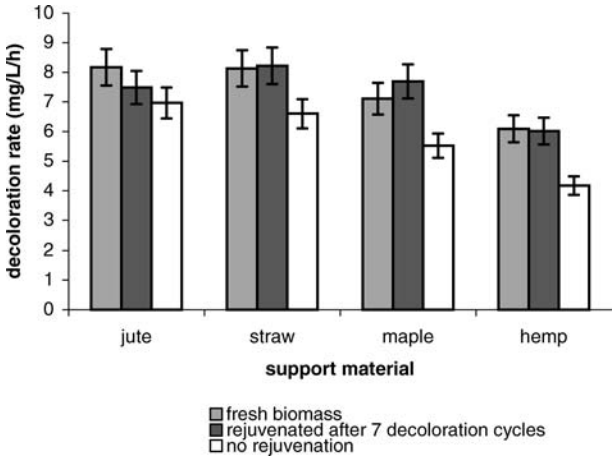
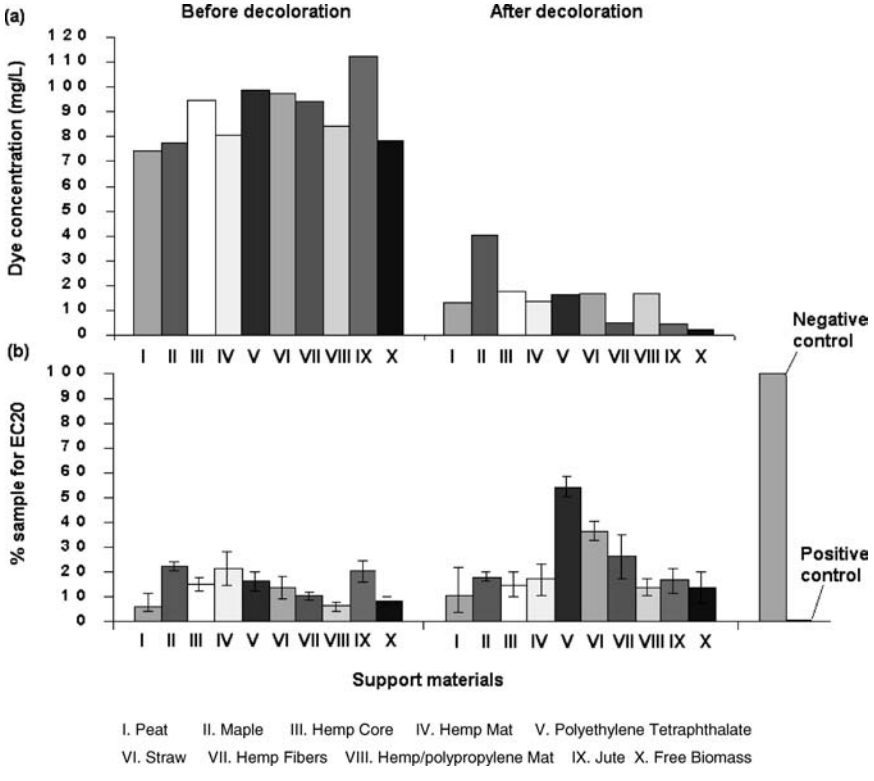


Fig. 4 Effect of the biomass rejuvenation on dye decoloration after seven decoloration cycles

Fig. 5 **a** Amaranth concentration and **b** toxicity of the decoloration medium (1 g l^{-1} glucose and 100 mg l^{-1} amaranth) at the start and end of decoloration. The positive control is 1 g l^{-1} $ZnSO_4 \cdot 7H_2O$ and the negative control is 1 g l^{-1} glucose



shown). In an attempt to restore decoloration efficiency, after seven decoloration cycles the fungus was allowed to grow or rejuvenate by incubating in fresh Kirk's medium for 5 days. In most cases, there was a subsequent increase in the rate of decoloration (Fig. 4) to levels similar to, or even exceeding, that of the first decoloration.

Toxicity of decoloration medium

The toxicity of the decoloration medium before and after decoloration in the presence of the different support materials was evaluated in a separate experiment. After *T. versicolor* colonized the support material in modified Kirk's medium for 3 days, the spent medium was replaced three times with a decoloration medium containing 100 mg l^{-1} amaranth and 1 g l^{-1} glucose only. In the third decoloration, 80–90% of the amaranth had disappeared (Fig. 5a). The medium was not toxic based on the concentration required to reduce 50% of the bacterial luminescence relative to the control (EC_{50}) as the EC_{50} was always greater than 100% (Coleman and Qureshi 1985). To determine whether there was any change in toxicity as a result of decoloration, the dilution required to reduce 20% of the bacterial luminescence relative to the control (EC_{20}) is reported. EC_{20} of the positive control (1 g l^{-1} $ZnSO_4$)

was very low (Fig. 5b) as it took a small amount of it to reduce the bacterial luminescence, indicating that it was quite toxic. However, the negative control (1 g l^{-1} glucose) had no effect on the assay as 100% of this solution did not reduce the bacterial luminescence. In most cases, samples (including jute) had similar toxicities before and after decoloration (Fig. 5b). These results show that, under these experimental conditions, more toxic compounds are not produced during the decoloration process. In a few cases, such as with polyethylene teraphthalate, straw or hemp fiber, the toxicity was actually reduced after decoloration. Results were similar for the first and second decoloration (data not shown).

Discussion

White rot fungi are known to degrade a range of synthetic dyes (Glenn and Gold 1983; Ollika et al. 1993; Heinfling et al. 1997; Rodriguez et al. 1999; Zhang et al. 1999; Kapdan et al. 2000b), and the strain of *T. versicolor* used in this study has previously been shown to decolorize azo dyes such as amaranth, Tropaeolin O, Remazol Black B, and Remazol Brilliant Orange 3R; a reactive anthroquinone dye (Remazol Brilliant Blue R); and a reactive phthalocyanine dye (Reactive Blue 15) (Swamy and Ramsay 1999a). The culture was able to decolorize repeated additions of these dyes and mixtures thereof. Of these dyes, amaranth was chosen as a model azo dye for this work.

Materials of natural (wheat straw, hemp fiber, hemp mat, hemp core, jute twine, peat, and maple woodchips), and synthetic (nylon and polyethylene teraphthalate fibers) origin and a composite fiber of hemp and polypropylene were selected for the immobilization of *T. versicolor*. The natural materials were chosen because they were compostable, and once they are no longer useful as an immobilization support, they could be composted. All selected materials are inexpensive and most of them have been previously used to support biofilms of different microbial cultures (Linko 1988; Zafar et al. 1996; Wolter et al. 1997; Marwaha et al. 1998; Karamanev et al. 1999). Some materials, like the nylon fibers and peat, were inhibitory to growth. Others, such as maple wood chips, jute, wheat straw and hemp fiber, were easily colonized by *T. versicolor*. However, there was poor mycelial attachment to the wood chips, and the fungus quickly degraded the wheat straw and hemp fibers so that they could not serve as long-term immobilization supports. The wheat straw and hemp fibers probably contained a higher proportion of easily accessible and biodegradable plant material such as cellulose and/or hemicellulose than the jute. As the cellulose and/or hemicellulose is/are degraded, the support material would lose its mechanical integrity.

Jute, straw and hemp fiber were also shown to have an advantage in providing a carbon source for decoloration to occur, as reasonable decoloration rates were obtained even if glucose was not added during the decoloration process. Swamy and Ramsay (1999b) have shown that more than 0.13 g l^{-1} glucose was required for decoloration to occur. The carbon for decoloration probably came from the enzymatic degradation of cellulose and/or hemicellulose in the jute, straw and hemp fiber by *T. versicolor*. These results also indicate that carbon substrates in textile dye effluents such as cotton fibers or starch, used as sizing agents, could provide the carbohydrate necessary for the decoloration process and, hence, addition of an exogenous carbon source may not be required. This could help to reduce the cost of the decoloration process.

Overall, jute appears to be the best choice as *T. versicolor* colonized it very readily, the biomass was not easily sloughed off, its integrity was maintained over a long period of time and decoloration rates were high even when glucose was not added.

During the growth phase, ammonium tartrate was used as the nitrogen source. It was omitted during the decoloration step when only glucose and amaranth were provided since decoloration is known to occur under nitrogen-limited conditions (Fenn and Kirk 1981; Swamy and Ramsay 1999b). With each decoloration cycle, the rate of decoloration decreased. This may have been due to an inhibitory metabolite affecting the production or activity of the dye-degrading enzymes, or the limitation of a key nutrient such as a nitrogen source required for the production of new enzymes. Since the spent medium was continually replaced, the accumulation of extracellular inhibitory products is less likely. It is more probable that a key nutrient eventually became limiting. A prolonged period with no nitrogen source, as in this case, may result in a decrease in the production of enzymes necessary for decoloration, and/or in cell death, causing a decline in the decoloration rate over time. The latter is supported by the fact that after fresh growth medium containing all the nutrients was used to "rejuvenate" the culture, decoloration rates were restored to original levels. This was probably the result of new biomass growth with a concomitant increase in enzyme production. Thus, to sustain high rates of decoloration over a long period of time, not only is a carbon source necessary but also small amounts of other nutrients. A key nutrient would be the ammonium concentration where a low level would be needed not only to ensure decoloration but also to sustain a certain level of enzyme production. Minimal nutrient requirements are presently being investigated.

It is promising that under these experimental conditions, more toxic compounds are not produced during the decoloration process, as shown by the Microtox studies. Since jute was the best support material, it is important to note that the toxicity of its decoloration medium was the same before and after decoloration. It is interesting to note that in a few cases, such as with polyethylene teraphthalate, straw or hemp fiber, the toxicity was actually reduced after decoloration. This may be due to sorption of toxic fungal metabolites onto the support material, or perhaps the support material may have induced a different metabolism resulting in less toxic end products.

To summarize, based on the criteria of growth, ease of colonization, durability, and the rate of decoloration, jute was the best support material for the immobilization of

T. versicolor and the decoloration of amaranth. Although a carbon source is necessary for dye decoloration to occur, *T. versicolor* immobilized on jute, straw and hemp fiber decolorized amaranth without the addition of glucose. However, the decoloration rate increased when 1 g l^{-1} glucose was added. As the number of sequential decolorations increased, the rate of decoloration decreased. This rate was restored by "rejuvenating" the biomass in fresh growth medium. The toxicity of the decoloration medium in the various support materials had the same or lower toxicity after decoloration as at the start of decoloration.

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References

Alleman BC, Logan BE, Gilbertson RL (1995) Degradation of pentachlorophenol by fixed films of white rot fungi in rotating tube bioreactors. Water Res 29:61–67

Andersson BE, Henrysson T (1996) Accumulation and degradation of dead-end metabolites during treatment of soil contaminated with polycyclic aromatic hydrocarbons with five strains of white-rot fungi. Appl Microbiol Biotechnol 46:647–652

Azmi W, Sani RK, Banerjee UC (1998) Biodegradation of triphenylmethane dyes. Enzyme Microb Technol 22:185–191

Barr DP, Aust SD (1994) Mechanisms white rot fungi use to degrade pollutants. Environ Sci Technol 28:78A–87A

Boyle D, Wiesner C, Richardson A (1998) Factors affecting the degradation of polyaromatic hydrocarbons in soil by white rot fungi. Soil Biol Biochem 30:873–882

Chung KT, Stevens SE (1993) Decolorization of azo dyes by environmental organisms and helminths. Environ Toxicol Chem 12:2121–2132

Coleman RN, Qureshi AA (1985) Microtox and *Spirillum volutans* tests for assessing toxicity of environmental samples. Bull Environ Contam Toxicol 35:443–451

Dubrow SF, Boardman GD, Michelsen DL (1996) Chemical pretreatment and aerobic-anaerobic degradation of textile dye wastewater. In: Reife A, Freeman HS (eds) Environmental chemistry of dyes and pigments. Wiley, Toronto, pp 75–94

Fenn P, Kirk TK (1981) Relationship of nitrogen to the onset and suppression of ligninolytic activity and secondary metabolism in *Phanerochaete chrysosporium*. Arch Microbiol 130:59–65

Glenn JK, Gold MH (1983) Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl Environ Microbiol 45:1741–1747

Grey R, Hofer C, Schlosser D (1998) Degradation of 2-chlorophenol and formation of 2-chloro-1,4-benzoquinone by mycelia and cell-free crude culture liquids of *Trametes versicolor* in relation to extracellular laccase activity. J Basic Microbiol 38:371–382

Heinfling A, Bergbauer M, Szewzyk U (1997) Biodegradation of azo and phthalocyanine dyes by *Trametes versicolor* and *Bjerkandera adusta*. Appl Microbiol Biotechnol 48:261–266

Kapdan I, Kargi F, McMullan G, Marchant R (2000a) Effect of environmental conditions on biological decolorization of textile dyestuff by *C. versicolor*. Enzyme Microb Technol 26:381–387

Kapdan I, Kargi F, McMullan G, Marchant R (2000b) Comparison of white-rot fungi cultures for decolorization of textile dyestuffs. Bioprocess Eng 22:347–351

Karamanev DG, Ramsay BA, Hassan MC (1999) Design and experimental study of immobilized soil bioreactor for in-well biodegradation of pentachlorophenol in groundwater. Can J Chem Eng 77:936–941

Kim HY, Song HG (2000) Comparison of 2,4,6-trinitrotoluene degradation by seven strains of white rot fungi. Curr Microbiol 41:317–320

Kirk TK, Schultz E, Connors WJ, Lorenz LF, Zeikus JG (1978) Influence of culture parameters of lignin metabolism by *Phanerochaete chrysosporium*. Arch Microbiol 117:177–185

Krcmar P, Kubatova A, Votruba J, Erbanova P, Novotny C, Sasek V (1999) Degradation of polychlorinated biphenyls by extracellular enzymes of *Phanerochaete chrysosporium* produced in a perforated plate bioreactor. World J Microbiol Biotechnol 15:269–276

Liao WL, Tseng DH, Tsai YC, Chang SC (1997) Microbial removal of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. Water Sci Technol 35:255–264

Linko S (1988) Production and characterization of extracellular lignin peroxidase from immobilized *Phanerochaete chrysosporium* in a 10 l bioreactor. Enzyme Microb Technol 10:410–417

Maguire RJ (1992) Occurrence and persistence of dyes in a Canadian river. Water Sci Technol 25:265–270

Marwaha SS, Grover R, Prakash C, Kennedy JF (1998) Continuous biobleaching of black liquor from the pulp and paper industry using an immobilized cell system. J Chem Technol Biotechnol 73:292–296

Microtox Omni Software (1999) Azur Environmental, Newark, Del.

Ollika P, Alhonmaki K, Leppanen V, Glumoff T, Suominen I (1993) Decolorization of azo, triphenylmethane, heterocyclic, and polymeric dyes by lignin peroxidase isoenzymes by *Phanerochaete chrysosporium*. Appl Environ Microbiol 59:4010–4016

Pallerla S, Chambers RP (1997) Characterization of a Ca-alginate immobilized *Trametes versicolor* bioreactor for decolorization and AOX reduction of paper mill effluents. Bioresour Technol 60:1–8

Rodriguez E, Pickard MA, Vazquez-Duhalt R (1999) Industrial dye decolorization by laccases from ligninolytic fungi. Curr Microbiol 38:27–32

Spadaro JT, Gold MH, Ranganathan V (1992) Degradation of azo dyes by the lignin-degrading fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 58:2397–2401

Swamy J, Ramsay J (1999a) The evaluation of white rot fungi in the decoloration of textile dyes. Enzyme Microb Technol 24:130–137

Swamy J, Ramsay J (1999b) Effects of glucose and NH concentrations on sequential dye decoloration by *Trametes versicolor*. Enzyme Microb Technol 25:278–284

Vaidya AA, Datye KV (1982) Environmental pollution during chemical processing of synthetic fibers. Colourage 14:3–10

Van Aken B, Hofrichter M, Scheibner K, Hatakka AI, Naveau H, Agathos SN (1999) Transformation and mineralization of 2,4,6-trinitrotoluene (TNT) by manganese peroxidase from the white-rot basidiomycete *Phlebia radiata* Biodegradation 10:83–91

Wolter M, Zadrazil F, Martens R, Bahadir M (1997) Degradation of eight highly condensed polycyclic aromatic hydrocarbons by *Pleurotus* sp. Florida in solid wheat straw substrate. Appl Microbiol Biotechnol 48:398–404

Yadav JS, Bethea C, Reddy CA (2000) Mineralization of trichloroethylene (TCE) by the white rot fungus *Phanerochaete chrysosporium*. Bull Environ Contam Toxicol 65:28–34

Yang FC, Yu JT (1996) Development of a bioreactor system using an immobilized white rot fungus for decolorization. Bioprocess Eng 15:307–310

Zafar SI, Abdullah N, Iqbal M, Sheeraz Q (1996) Influence of nutrient amendment on the biodegradation of wheat straw during solid state fermentation of *Trametes versicolor*. Int Biodeterior Biodegrad 38:83–87

Zhang FM, Knapp JS, Tapley KN (1999) Development of bioreactor systems for decolorization of Orange II using white rot fungus. Enzyme Microb Technol 24:48–53

Zollinger H (1991) Color chemistry: syntheses, properties and applications of organic dyes and pigments, 2nd edn. VCH, New York