Improving the bioremediation of phenolic wastewaters by *Trametes versicolor*

Daniel Ryan, Winston Leukes and Stephanie Burton

Abstract

The successful bioremediation of a phenolic wastewater by *Trametes versicolor* was found to be dependent on a range of factors including: fungal growth, culture age and activity and enzyme (laccase) production. These aspects were enhanced by the optimisation of the growth medium used and time of addition of the pollutant to the fungal cultures. Different media containing 'high' (20 g/L), 'low' (2 g/L) and 'sufficient' (10 g/L) concentrations of carbon and nitrogen sources were investigated. The medium containing both glucose and peptone at 10 g/L resulted in the highest Growth Related Productivity (the product of specific yield and μ) of laccase (1.46 Units of laccase activity)/gram biomass/day and was used in all further experiments. The use of the guaiacol as an inducer further increased laccase activity 780% without inhibiting growth; similarly the phenolic effluent studied boosted activity almost 5 times. The timing of the addition of the phenolic effluent was found to have important consequences in its removal and at least 8 days of prior growth was required. Under these conditions, 0.125 g phenol/g biomass and 0.231 g *o*-cresol/g biomass were removed from solution per day.

1. Introduction

Phenolic compounds are ubiquitous in aqueous streams of industrial processes including: petroleum refining, coking and coal conversion, chemical plants, foundries and pulp-and-paper plants (Van Schie and Young, 1998 and Aitken et al., 1994). The presence of these compounds in drinking and irrigation water represents a health and environmental hazard (Alberti and Klibanov, 1981). Although conventional methods for dephenolisation do exist, they are often expensive, deliver incomplete purification, can form hazardous byproducts and are often applicable to only to a limited concentration range (Boman et al., 1988). The challenge to biotechnology is to generate efficient, cost effective and environmentally safe bioremediation methods to replace existing technologies as well as to provide unique solutions for the remediation of contaminated waste streams.

White-rot fungi (WRF) are among the few groups of microorganisms capable of completely degrading polymers of phenolic origin, including lignin. The random nature of the structure of lignin requires its degradation to function non-specifically; consequently other compounds of aromatic structure, including many xenobiotic compounds, are also susceptible to degradation by lignolytic enzymes, and it is this property that confers their bioremediation potential on these organisms (Davis and Burns, 1990). *Trametes versicolor* is an excellent model for these activities with proven bioremediation capacity.

The first steps in generating an efficient fungal bioremediation process are the identification of a suitable microorganism and the optimisation of the culture conditions necessary for its rapid growth and the production of the relevant enzymes. Often, aspects such as growth medium development are overlooked in favour of over-engineered, conventional, historically established formulations. This work aimed to develop a simple, cost effective medium for the growth of *T. versicolor*, its production of laccase and the optimisation of aspects pertaining to its use in a bioremediation process, such as pollutant removal, culture age and the maintaining of microbial activity.

2. Methods

2.1. Chemicals

2,2′-Azinobis-(3-ethylbenzthiozoline sulfonic acid) (ABTS) was obtained from Boehringer Mannheim (Germany). *m*-, *o*- and *p*-Cresol were obtained from Aldrich Chemical Company. Phenol and acetonitrile (HPLC-grade) were obtained from BDH Laboratory Supplies (England) and the fluorescein diacetate (FDA) from Sigma.

2.2. Strain preservation

A slant culture of *T. versicolor* (PPRI #3845) was obtained from the Plant Protection Research Institute (South Africa) and was maintained on 2% malt extract agar slants. These were subcultured every 60 days.

2.3. Liquid culture

Trametes Defined Medium (TDM) (Addleman and Archibald, 1993) was used initially as the medium for the production of biomass and enzyme, with the glutamine replaced by peptone for cost reasons. Since their exclusion had no observable effects, the medium was further simplified by the omission of the dimethyl succinate and sodium and calcium chloride. The glucose (carbon source) and peptone (nitrogen source) concentrations were varied between 20 g/L (high) and 2 g/L (low).

2.4. Inoculation

To acclimatise the cultures to growth in liquid media, pre-inoculum flasks were prepared. In each, a *T. versicolor* plate culture was diced into 5 mm × 5 mm blocks and added aseptically to 400 mL of growth medium in a 2 L flask. These flasks were incubated at 28 °C in an orbital shaker at 200 rpm. After 4 days of incubation, the contents of these flasks were homogenised in a sterile Sorvall bench top homogeniser. This was used as the inoculum for the production flasks.

2.5. Flask culture

All flask experiments were conducted in 250 mL Ehrlenmeyer flasks containing 45 mL autoclaved growth medium. These were inoculated with 5 mL of the pre-inoculum mixture and incubated at 28 °C. For biomass measurements, the contents of the flasks were filtered through pre-dried and weighed Whatman #1 filter papers using a vacuum pump. The filter papers containing biomass were dried for 24 h at 70 °C and then re-weighed. The dry mass of the mycelium was calculated by subtracting the initial weight of the paper from the final.

2.6. Determination of laccase enzyme activity

Laccase activities were determined by monitoring the oxidation of 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as the substrate (Wolfenden and Willson, 1982). The reaction mixture contained 2.5 mL 0.1 M sodium acetate buffer, 0.33 mL 5 mM ABTS and 0.17 mL sample (Roy-Arcand and Archibald, 1991). Oxidation of ABTS was measured by determining the increase in absorbance of the mixture on a Shimadzu UV-160 spectrophotometer at 420 nm ($\varepsilon = 36\,000\ M^{-1}$). One unit (U) of enzyme activity was defined as the amount of enzyme required to oxidise 1 μmol of ABTS per mL per min.

To determine the error associated with this technique 3 samples were run 10 times each. A variance of less than 5% was found and in light of the high degree of confidence associated with this assay, all subsequent samples were done in duplicate.

2.7. Comparison of growth media

Media were compared in terms of biomass yield ($Y_{x/s}$), product yield ($Y_{p/s}$), specific yield ($Y_{p/x}$) and specific growth rate (μ). No appropriate term was found in literature that correlated the productivity of a microorganism with growth and to this end we defined the lump parameter 'Growth Related Productivity (GRP) where

$$\mathrm{GRP} = (Y_{p/x})(\mu) \qquad \text{with units: U/g/d}$$

$$\frac{\text{units of laccase (U)}}{\text{biomass (g)}} \cdot \frac{1}{\text{time (d)}}$$

where x is the biomass, p is the product (laccase) and s is the substrate (glucose).

2.8. Induction of enzyme activity

The four inducer compounds tested: ethanol, veratryl alcohol, 2,6-xylidine and guaiacol, were aseptically added to the flasks after 7 days growth of *T. versicolor* to final concentration of 1 mM. One milliliter samples were drawn aseptically and assayed for laccase activity. The activity of the control samples (containing no inducer) was subtracted from the rest to calculate the increase in activity.

2.9. Effect of culture age on growth and enzyme production after effluent addition

The effluent used in this study was obtained from the 'stripped gas liquor' (SGL) stream of a local coal gasification plant. The effluent contained negligible amounts of settled solids and high concentrations of phenol (82.80 mM) and *p*-cresol (24.999 mM), *m*-cresol (25.80 mM) and *o*-cresol (77.03 mM). A number of flasks were inoculated as above and left to grow for 4 days after which SGL was added to the flasks at 2.5%, 5%, 10% and 20% v/v concentrations. Samples were taken every day for 4 days, assayed for laccase activity, and the remainder frozen for degradation studies. After four days, the flasks were removed from the incubator and the biomass was filtered off, dried and weighed. At this time, effluent was added to a second set of flasks, now 8 days old, and the process repeated, and similarly for a third set. This resulted in different effluent concentrations being added to cultures of different ages: 4 days (set 1), 8 days (set 2), and 12 days (set 3) and in different stages of growth. No effluent was added to the control flasks.

2.10. Measurement of total microbial activity

Fluorescein diacetate (FDA) (Sigma) was dissolved in analytical grade acetone and stored as a stock solution (2 mg/ml) at −20 °C. FDA hydrolysis was measured as absorbance increase at 490 nm (A_{490}). Biomass samples were obtained on filter paper discs as described above, but were not dried. The samples were added to sterile 50 ml sodium phosphate buffer (60 mM, pH 7.6) along with FDA (final concentration, 10 μg/ml) and the mixture incubated at 24 °C on a rotary shaker. The buffering capacity was sufficient to maintain the pH at 7.6 for the duration of the experiments (Schnurer and Rosswall, 1982). Samples (1.5 mL) were drawn from each flask and centrifuged for 4 min at 5000 rpm to remove any debris. Absorbance (490 nm) values were determined from the supernatant of these samples on a Shimadzu UV160 spectrophotometer. Experiments were run over 3 h with readings taken every 30 min to determine the rates of FDA hydrolysis.

2.11. Effluent bioremediation studies

SGL was added to 250 mL flasks containing growing cultures of *T. versicolor* as described in Section 2.9. Samples were aseptically drawn from the flasks, centrifuged and then filtered to remove any cell debris. The removal/conversion of the effluent components was monitored by HPLC (Merck LaChrom), utilising a Waters S5 (5 μm), C_{18} reverse phase column (250 × 4.6 mm) with a mobile phase of water:acetonitrile 85:15 containing 1.66% w/v β-cyclodextrin, to separate the cresol isomers.

3. Results and discussion

3.1. Growth medium optimisation

Fungal growth is significantly influenced by the carbon and nitrogen concentrations in the growth medium used (Wu et al., 2005). The first parameters to be established in this work were the carbon (glucose) and nitrogen (peptone) requirements of the *T. versicolor* strain used. This was investigated by varying the growth medium concentrations between 20 g/L (high) and 2 g/L (low). An intermediate or 'glucose sufficient' third option, 10 g/L, was also tested. As would be expected, the cultures with the highest concentration of readily available glucose grew fastest and achieved the highest biomass accumulation. The medium containing 20 g/L of glucose produced 11.6 g/L of dry mycelial mass after 7 days (Fig. 1). However, in terms of enzyme activity and biomass accumulation, the nitrogen- and glucose-sufficient cultures were the most productive (Table 1). This finding was in agreement with those of Kaal et al. (1995) who demonstrated that among five common white-rot fungi tested by them (*T. versicolor* not included), higher biomass and lignolytic enzyme activities were observed in response to N (organic)-sufficient conditions. Fungal biomass production in flask cultures was slower in nitrogen-deficient cultures and their long-term viability was reduced compared to nitrogen-sufficient ones (Alleman, 1991). Induction of lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase has been shown to occur at the level of transcription with nitrogen being an important factor in regulating these lignolytic enzymes in WRF (Collins and Dobson, 1997).

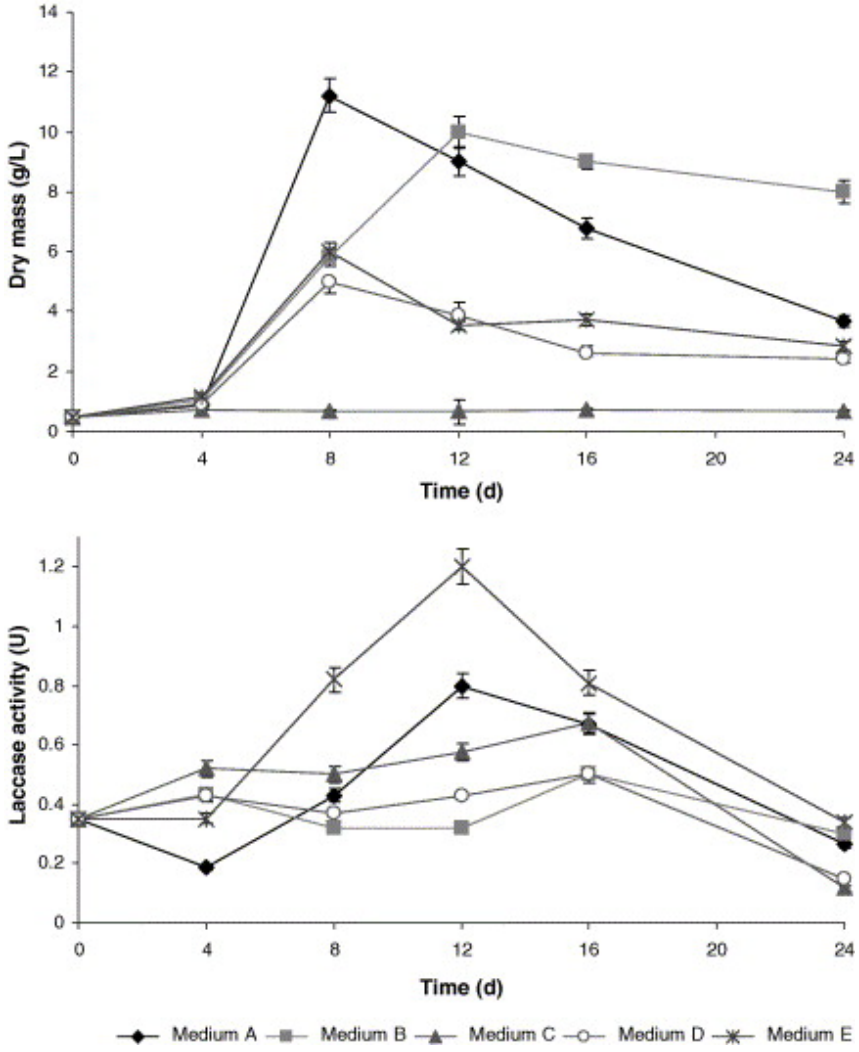

Fig. 1. The increase in biomass (A) and laccase (B) activity over time for cultures of *T. versicolor* grown in TDM with different glucose (C) and peptone (N) concentrations: 20 g/L C:20 g/L N (A), 20 g/L C:2 g/L N (B), 2 g/L C:2 g/L N (C), 2 g/L C:20 g/L N (D) and 10 g/L C:10 g/L N (E).

Table 1.
Table showing the process parameters for the different growth media: 20 g/L C:20 g/L N (A), 20 g/L C:2 g/L N (B), 2 g/L C:2 g/L N (C), 2 g/L C:20 g/L N (D) and 10 g/L C:10 g/L N (E)

Process parameter	Growth medium				
	A	B	C	D	E
Biomass yield ($Y_{x/s}$) (g/g)	0.032	0.036	0.005	0.125	0.023
Product yield ($Y_{p/s}$) (U/g)	0.034	0.029	0.350	0.215	0.150
Specific yield ($Y_{p/x}$) (U/g)	1.000	0.020	6.960	0.412	4.780
μ (per day)	0.388	0.305	0.041	0.286	0.305
GRP (U/g/day)	0.388	0.006	0.285	0.117	1.458

The GRP term is the product of specific yield and μ.

In this flask culture investigation, the glucose concentration in all the flasks was reduced to almost zero by day 12. This coincided with the maximal enzyme production for media A and E, and at least 80% of maximum for the other media. Biomass yield $Y_{x/s}$ (grams of biomass produced per gram of glucose) but not overall biomass accumulation, was highest in medium D (0.125 g/g). Product yield $Y_{p/s}$ (units of laccase produced per gram of glucose) was highest for medium C (0.35 U/g), and the overall amount of enzyme produced was far higher in E (1.2 U/mL) compared to 0.67 U/mL for C (Fig. 1). Specific yield $Y_{p/x}$ (units of laccase produced per gram of biomass) was highest in medium C (6.96 U/g) and E (4.78 U/g). The high value obtained for medium C was explained by the low biomass titre of this medium as well as the induction effect of nutrient limitation on laccase production. The lack of biomass however made medium C of limited value in process terms. Medium E, on the other hand, produced an adequate amount of biomass (3.7 g/L) and the highest enzyme activity (1.2 U/mL). As expected, the specific growth rate (μ) was highest in the cultures with highest initial carbon (glucose) concentrations: A, B, and E. The results from the growth indicators monitored (Table 1) indicated that nutrient regime E (10 g/L glucose and peptone) was the best of those tested. Growth Related Productivity (GRP), the product of specific yields and μ, was 3.75 times higher in medium E than the next best, medium A. This term was defined here as it was necessary to relate laccase production with growth. Specific yield did not give an idea of growth rate or total biomass accumulation, which made it difficult to predict total enzyme production in a fermentation. GRP provided an indication of overall or total enzyme yield, for example: medium C had a very high specific yield (6.96 U/g) but this was misleading because there was limited fungal growth and low total laccase production. On the basis of its fungal growth, enzyme production and cost benefits, it was concluded that medium E (10 g/L glucose, 10 g/L peptone) was the best possible formulation in the range tested. Recent researchers (Pazarlıoglu et al., 2005 and Tekere et al., 2005) have also used growth media containing 10 g/L glucose but gave no indication as to why this concentration was chosen.

3.2. Effect of inducers on growth and enzyme production

Laccase expression has been a topic of some research interest and laccase genes from a number of lignolytic fungi, including *T. versicolor*, have previously been cloned and characterised (Jonsson et al., 1995). It has been suggested that genes encoding various isozymes are differentially expressed, with some being constitutive and others being inducible (Bollag and Leonowicz, 1984). In an experiment to test the efficacy of four known laccase inducers, it was found that the presence of all the compounds did, to some extent, increase laccase enzyme activity in this strain of *T. versicolor* (Fig. 2A). Guaiacol, a lignin monomer, was the most effective, resulting in an increase of 780% in enzyme activity. This was not entirely surprising, as it most closely resembles the natural substrates of the lignolytic enzyme system and would have been the most easily recognisable to the fungus. The decrease in activity after day 3 was attributed to the removal/polymerisation of the inducer compounds and the subsequent discontinuation of their affect. This could be overcome in a process situation by monitoring the laccase activity and adding more of the inducer compound as required.

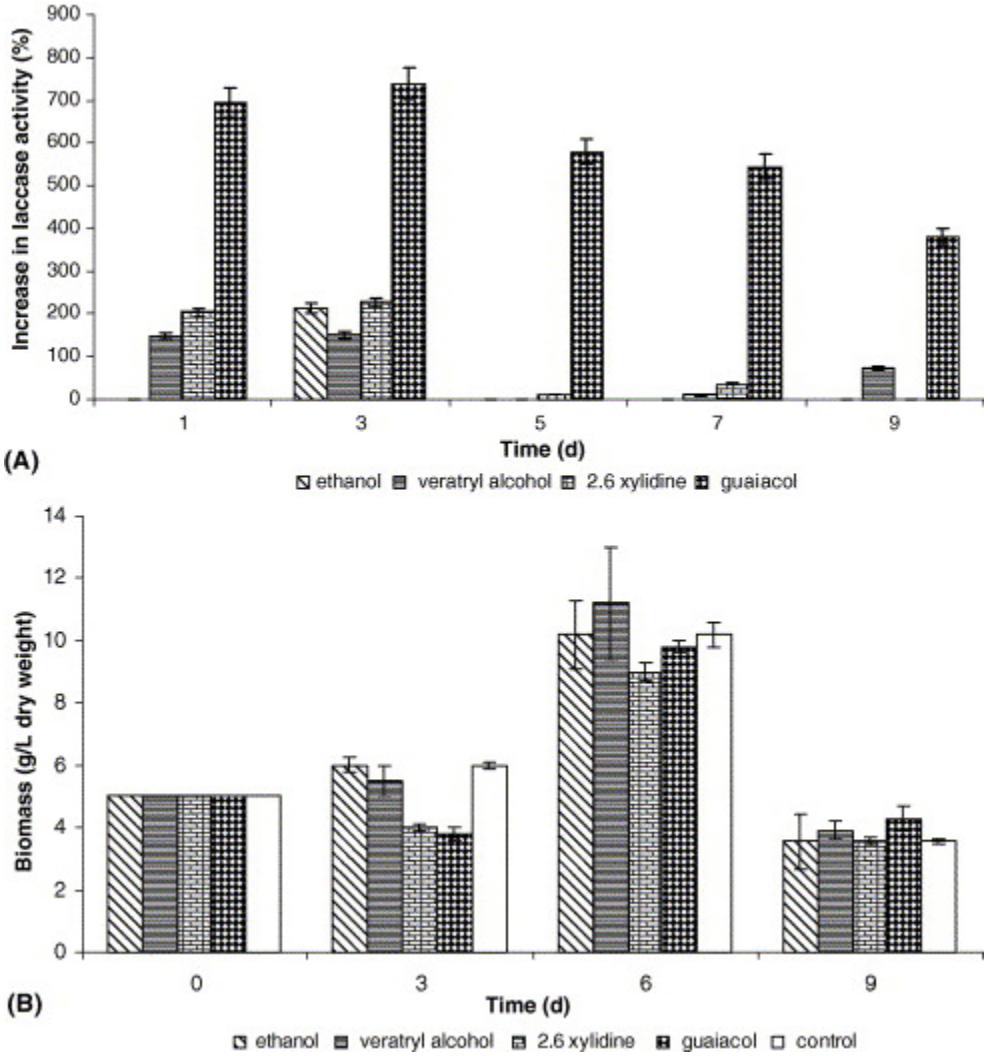

Fig. 2. Effect of different inducers on laccase production (A) and growth (B) in flask cultures of *T. versicolor.*

One function of laccases is to detoxify highly reactive aromatic compounds by polymerising them (Thurston, 1994). This is further verified by the fact that the increase in laccase production coincides in many instances, as well as in this one, with the formation of dark precipitates that may represent laccase-polymerised forms of the inducer. Coll et al. (1993) found that the *lcc* genes of *T. versicolor*

were also activated by aromatic compounds. The results obtained in this study agreed with the available knowledge on the induction effect of aromatic inducers on *T. versicolor*.
Aromatic inducer compounds such as phenol and cresol are potentially toxic to fungi. However, the low concentrations (1 mM) used here did not have a marked toxic effect on culture growth, as indicated by the similarity of the biomass accumulation of induced cultures relative to that of the control (Fig. 2B).
The use of these inducers would increase enzyme production in *T. versicolor*, but in any industrial application the cost could be prohibitive. An obvious solution would be to identify an inexpensive, readily available source of inducer. Therefore, an experiment to trial the laccase induction effect of two industrial effluents was conducted. It was reasonable to assume that the effluent from a pulp and paper mill would contain guaiacol and other similar lignin monomers, so this was tested. Similarly, the phenolic effluent stream (SGL) from a Fischer–Tropsch process contains the benzene ring compounds found in lignin such as phenol and the isomers of cresol. The effluents were filter sterilised and added to *T. versicolor* flask cultures after 7 days growth to final concentrations of 1% and 2% v/v. The presence of both concentrations of both effluents resulted in increased laccase production over the growth-medium-only control. The 1% pulp-and-paper effluent affected the greatest increase in laccase activity (670%). This increase was short lived however, and the guaiacol disappeared rapidly from solution along with its induction effect. Two percent of SGL produced a longer lasting induction effect over 8 days (Fig. 3) and on this basis was selected for further research. Similar increase in laccase yields have been observed with the use of lignocellulosic agricultural by-products as inducers in submerged cultures of *T. versicolor* (Lorenzo et al., 2002), but these had to be added as supplementary to treatment.

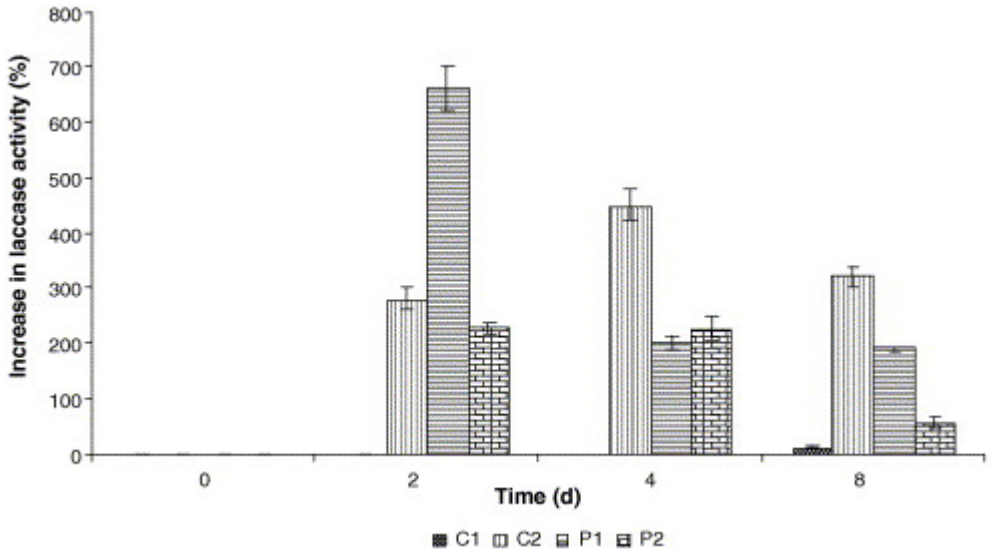

Fig. 3. The effect of industrial effluents, as inducers, on laccase enzyme activity of *T. versicolor* (1% v/v Cresylic (C1); 2% v/v Cresylic (C2); 1% v/v Pulp mill (P1); 2% v/v Pulp mill (P2)) percentage increase is relative to control flasks that contained no inducers. All data points are means of triplicate samples; errors encountered are less than 5%, and are thus not shown.

3.3. Effect of time of addition of phenolic effluent on laccase production by *T. versicolor*
The addition of even low concentrations of effluent to flask cultures prior to inoculation severely inhibited growth of *T. versicolor* and it was decided to assess the optimum culture age at which it would be most advantageous to add effluent to the fungal cultures. The addition of varied concentrations of phenolic effluent to flask cultures at different stages of growth showed that the time of addition played an important role in the resultant effect of that addition. At all effluent concentrations, the 12-day-old cultures were better able to tolerate the effluent monomers addition, as indicated by their producing more degradative enzymes, up to 3.8 U/mL compared to a maximum of

0.65 U/mL for the 4-day-old (Set 1) and 2.9 U/mL for the 8-day-old (Set 2) cultures (Fig. 4). Furthermore, increasing the pollutant concentration in the 12-day-old cultures (Set 3) resulted in increased enzyme production; whereas the opposite effect was observed in Set 1 and only low concentrations (1.825 mM) had a positive effect on Set 2.

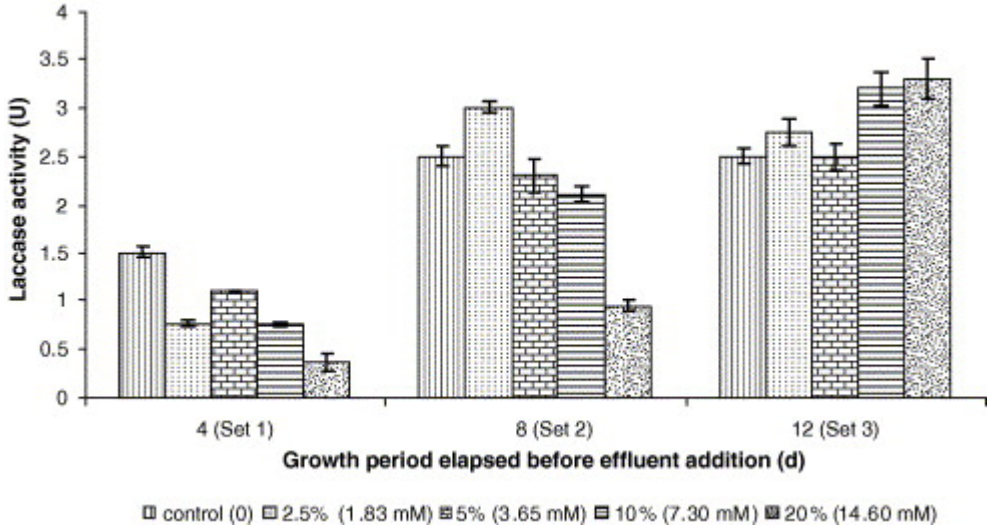

Fig. 4. The effect of concentration and time of addition of SGL on laccase enzyme production. Flasks were allowed 4 days (Set 1) 8 days (Set 2) and 12 days (Set 3) growth prior to effluent addition. The control contained zero effluent.

Peak laccase production of this strain in glucose sufficient medium (E) coincided with glucose depletion (results not shown) and fitted the model of lignolytic enzyme production as a result of nutrient limitation. The control cultures, to which no pollutant was added, generated the highest laccase activities after pollutant addition in Set 1, but the lowest in Set 3. The flasks with the highest phenol concentration (14.6 mM) produced the lowest amounts of enzymes in Set 1, but the highest in Set 3. The control samples had a maximum laccase activity of 2.45 U/mL while a 14.6 mM addition of phenol resulted in an activity of 3.8 U/mL, both in Set 3 (Fig. 4). Therefore, although nutrient limitation did increase laccase production in *T. versicolor*, it was not as an effective inducer as the aromatic compounds tested here.

The toxic effect of cresols and chlorophenols has been measured by the growth retardation and the detoxification of these compounds by white rot fungi (WRF) by the removal of toxicity associated inhibition (Bollag et al., 1988). Pollutant addition resulted in an increased lag phase in fungal growth and complete inhibition of growth at concentrations above 2 mM. Laccase was reported to be 'unequivocally responsible' for removing *p*-cresol, *o*-cresol and 2,6-dimethylphenol solution and the detoxification of the medium allowed fungal growth to take place (Bollag et al., 1988). In the present study, results from the dry mass determinations (Fig. 5) showed that allowing more time for growth before adding toxic compounds reduced the negative effects of these compounds on the physiological state of the *T. versicolor* cultures. The cultures in Set 1 deteriorated markedly, in terms of biomass, at pollutant concentrations above 7 mM, while the 8- and 12-day-old cultures actually showed increased biomass accumulation after pollutant addition. This was due possibly to a combination of the fact that the effluent provided an additional carbon source and that the older cultures were able to detoxify their environment more quickly by removing the pollutants, and in doing so were better able to continue normal growth. The fact that there was no available glucose in the culture fluid of any of the (triplicate) flasks when the effluent was added to Set 3 and the highest concentrations of effluent supported the highest biomass concentrations is further evidence that *T. versicolor* used the phenols provided as an additional carbon source. Although this argument contradicts previous research (Bollag et al., 1988 and Hammel, 1995) it was difficult to draw another conclusion from these results.

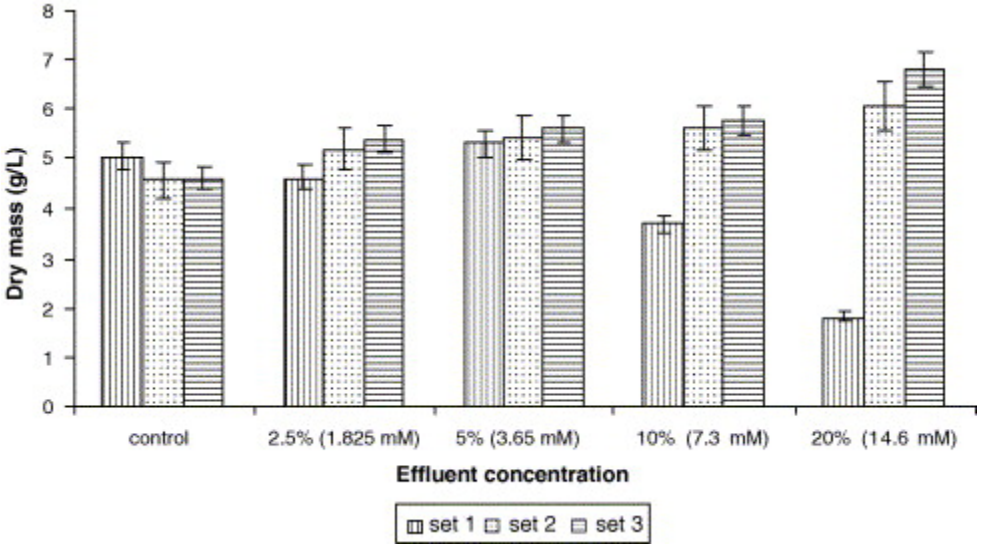

Fig. 5. The effect of time of addition of different concentrations of cresylic effluents on biomass accumulation, where Set 1 was allowed 4 days prior growth, Set 2, 8 days and Set 3, 12 days

Phenol is not readily biodegradable and has been reported to be toxic or growth inhibitory to most types of microorganisms, even to those species that have the metabolic capacity of using it as a growth substrate (Annachhatre and Gheewala, 1996). The ability of *T. versicolor* to grow in the presence of up to nearly 15 mM phenol was further evidence of its bioremediation potential. These results highlighted the importance of allowing cultures sufficient growth time to develop the systems necessary for dealing with environmental challenges before exposure.

3.4. Changes in microbial activity of *T. versicolor* cultures exposed to aromatic compounds

The comparative microbial activity of the cultures exposed to different effluent concentrations was investigated by the spectrophotometric determination of the hydrolysis of fluorescein diacetate (FDA) to fluorescein. FDA is a useful substrate for determining the overall activity of decomposer organisms because it is hydrolysed by a number of different growth related enzymes and a good correlation between FDA hydrolysis and respiration has been found (Schnurer and Rosswall, 1982). The total microbial activity of control cultures of *T. versicolor* was linearly correlated ($R^2 = 0.99$) with biomass accumulation during the exponential growth phase of the fungus. This is not surprising, as one would expect almost the entire culture to be uniformly active during this period.

The proportion of metabolically active biomass after effluent addition was measured as a ratio of microbial activity (as measured by FDA hydrolysis) to total biomass for cultures of different ages. This ratio was highest in Set 1 (4-day-old cultures) at low (2.5% v/v) effluent concentrations (0.31 ODU/g at 1.87 mM phenol) but decreased to less than half at phenol concentrations above 7 mM (10% v/v). Since increasing the concentrations of phenol in the medium also decreased the biomass of the exposed Set 1 cultures, the decrease in activity was even more marked. Cultures at an early stage of development were not able to survive the adverse conditions resulting from high effluent (phenolic) concentrations. The 8-day-old cultures of Set 2 exposed to 20% v/v effluent (14.6 mM phenol) maintained a level of activity similar to that of the zero effluent control (0.22 ODU/g) and were almost twice as active as those of Set 1 (0.12 ODU/g) and Set 3 (0.13 ODU/g) at the same effluent concentration. This could be attributed to the fact that they were still producing the enzymes associated with trophophasic growth and enough laccase to remove the phenols thus avert any negative effects they might have had. The Set 3 cultures were more active than Set 1 at effluent concentration above 5%, but did not cope as well as the Set 2 cultures over the range tested. After 12 days of growth before the effluent addition, these cultures had entered idiophasic growth and their general metabolic activity had decreased. However, they still produced enough laccase to tolerate the addition of the effluent; illustrated by the fact that they were able to begin another period of growth (Fig. 4), sustained possibly by the phenolic carbon being made bio-available. This speculation was

supported by the greatest increase in biomass observed for the cultures that had the most effluent added to them (Fig. 5). Time of addition was critical in the bioremediation of a phenolic effluent, (and possibly therefore any potentially toxic substance). A minimum of 8 days growth (flask culture) was required before addition of phenols to cultures of *T. versicolor* to best utilise the potential of this organism.

3.5. Removal of cresylic effluent components from culture medium

In order for the fungal system developed in this study to be applied in bioremediation, it would need to both survive and affect the removal the pollutant monomers from the effluent at concentrations much higher than those already shown to be effective inducers of laccase activity.

To quantify the removal of effluent phenolic monomers from solution, different concentrations of the effluent were added to flask cultures of *T. versicolor* and samples were taken over time for HPLC analysis. The removal of the effluent monomers from solution in flask cultures with up to 20% v/v (14 mM) concentration of effluent was successfully completed after 12 h contact time. The phenol proved to be more recalcitrant than the cresol (*o*, *m* and *p*) isomers. This was unexpected as Shuttleworth and Bollag (1986) showed that the ability of the laccase to remove cresol was dependent on the position of the methyl group with *meta*-substituted cresol being the least oxidised and *para*-cresol the most.

To our knowledge the concentrations of the phenolic monomers removed by *T. versicolor* in this system are the highest reported to date (Table 2). Although the removal of single doses of the target effluent monomers from flasks does not constitute an efficient bioremediation process; the knowledge generated in this work has proved crucial to the development of a fed-batch airlift reactor system (Ryan et al., 2005).

Table 2.
Removal of effluent (20% v/v) monomers from solution by flask cultures of *T. versicolor*

Effluent monomer	Initial concentration (mM)	Residual after 1 day (mM)	Removal rate (g/g biomass/day)
Phenol	13.80 (±0.07)	5.99[a] (±0.02)	0.125
p-Cresol	4.17 (±0.02)	0.0[a]	0.075
m-Cresol	4.30 (±0.02)	0.0[a]	0.077
o-Cresol	12.84 (±0.05)	0.04[a] (±0.01)	0.231

[a] Removal completed by day 2.

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