EXECUTIVE SUMMARY

Contamination of soils, groundwater, sediments, surface water and air with hazardous and toxic chemicals is one of the major problems facing the industrialised world today. Of the various types of industry-generated effluents, those containing organic pollutants such as phenols are generally difficult to remediate. There is a need to develop new technologies that emphasize the destruction of these pollutants rather than their disposal. The bioremediation of phenolic effluents by white rot fungi, and the oxidative enzymes produced by them, has been the topic of much research and is widely reported on in literature. This study focuses on the integration of an optimised process of enzyme production/ phenol degradation by the fungi *Trametes versicolor* and *Trametes pubescens* (Figure (i)) with an Airlift Loop Reactor found suitable for the large-scale fermentation of these organisms.

The broad aim of this project was to develop a reactor-based bioprocess utilising the white rot fungus *Trametes versicolor* for the remediation of phenol-contaminated wastewaters. To this end it was necessary to first investigate the physiology of the organism in terms of laccase production and its response to the presence of phenols in its environment. *T. versicolor* had been reported as an excellent producer of laccase but the successful application of this organism in a bioremediation process required the production of high amounts of this enzyme. The degree of success achieved in the laboratory in the mineralisation/ removal from solution of many major pollutants had so far only been realised in flask culture or in rather laboratory-specific reactor applications. A more practical (in terms of future scale-up), cost effective reactor system that provided a well-oxygenated, yet low shear environment was required.

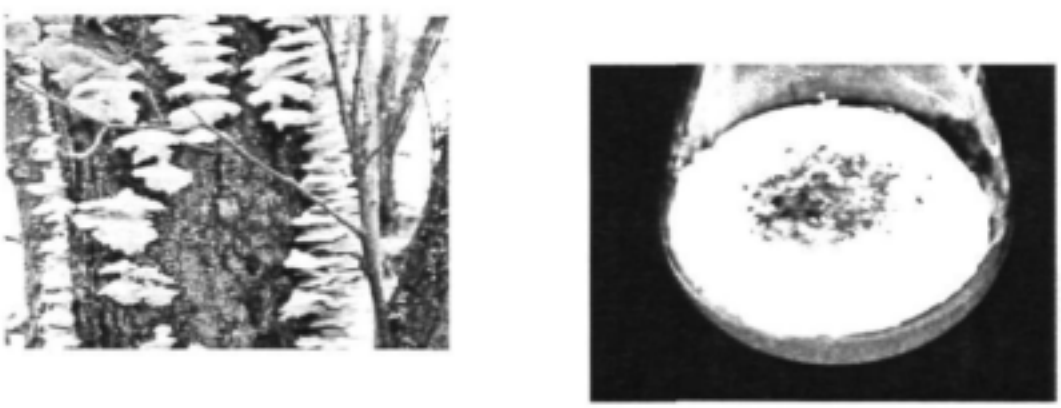
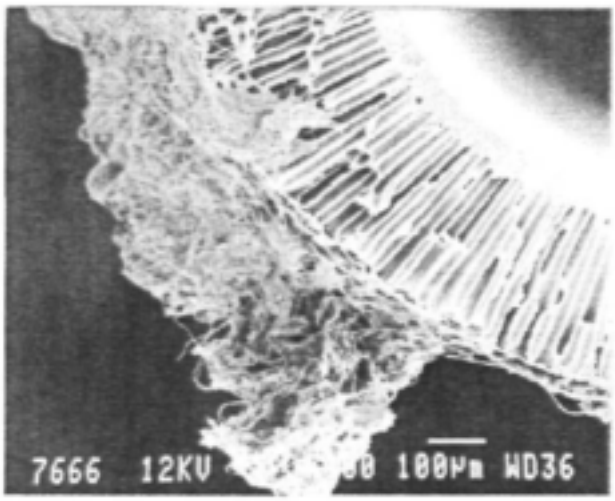
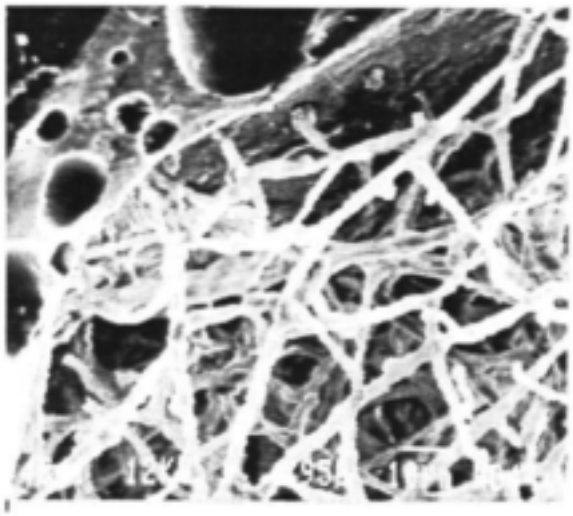


Figure (i) White rot fungal growth *in vivo* and *in vitro*

Using a comparison of the growth media recommended in literature as a basis for further experimentation, a 'nutrient sufficient' medium directed towards rapid biomass accumulation and increased lignolytic enzyme (laccase) production was developed. Enzyme activity was further increased by screening a variety of known inducers and comparing them with additions of small concentrations of stripped gas liquor from a Fischer-Tropsch plant, the target effluent in this study. In this way laccase production was increased by 700 %. The removal from solution of the principal effluent monomers (phenol, *p*-, *m*- and *o*-cresol) was studied, with up to 20 % v/v effluent being removed in flask culture. An investigation was conducted to elucidate the effect of this effluent on the morphology and physiology on the two *Trametes* species, with particular emphasis on laccase production.

Large scale, cost-effective applications of white-rot fungi to continuous treatment of liquid effluent has previously been hindered by the lack of suitable bioreactor systems. A hollow fibre membrane bioreactor and a trickle filter were investigated for suitability as supports for immobilised biofilms of *T. versicolor* and laccase production and pollutant degradation were successfully demonstrated in both reactor configurations. However, the need for a simple, cost effective, yet simple to upscale reactor system led to the investigation and development of an airlift loop reactor (ALR). These reactors have well defined flow patterns, high liquid velocities and yet provide a relatively low shear environment ideal for the growth of *T. pubescens*. The reactor configuration and aeration rate were optimised using recognised chemical engineering principles. Favourable feeding strategies, the effects of inducers/precursors and the timing of effluent addition were investigated. Increased growth (10g/L dry mass) and enzyme production (12000U/L) as well as highly efficient effluent degradation (5% v/v/day) were achieved in the ALR in fermentations over two week periods.





A B

Figure (ii) Fungal biomass immobilsed on synthetic membranes for the membrane bioreactor (A), and Glass foam support for the trickle reactor (B)

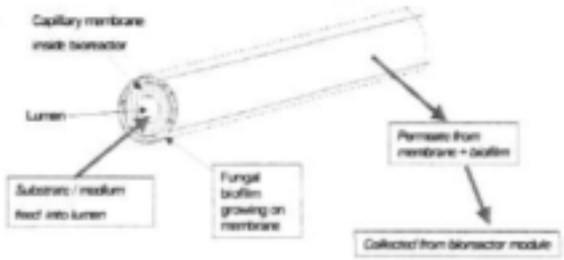


Figure (iii) How capillary membrane bioreactors operate

Figure (iv) Membrane bioreactors in the laboratory.

Figure (v) The trickle bed reactor (with the authors)

In the ALR system, a total pollutant removal of 0.1 g/g biomass/day was achieved in a 3.5L reactor, as compared with 0.5 g/g biomass/day achieved in single dose flask reactions. To our knowledge the concentrations of the phenolic monomers removed by T. versicolor in this system are the highest reported to date. This study demonstrated that in the ALR/*T pubescens* system, the simplicity of design, low cost of operation and high performance that are essential for a successful biological wastewater treatment scheme, can be achieved.

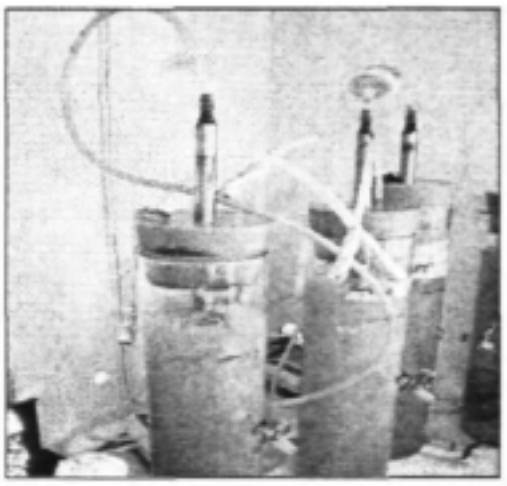


Figure (vi) Airlift reactors in operation

OBJECTIVES

Overall project aim:

Development of a practicable bioremediation process for using the enzymes of *Trametes versicolor* to degrade pollutants in specific industrial wastes, namely chlorinated aromatics and phenolics produced by the pulp-and-paper and petrochemical industry.

Key objectives:

1. Characterisation of the biological conversion by *Trametes versicolor,* of the phenolics, chlorinated phenolics and polyaromatics present in these effluents. This would include characterisation of the roles and activity levels of the enzyme activities involved, and the fate of the organic compounds converted.
2. An optimised fermentation or fed-batch process in which the *Trametes versicolor* successfully degrades these pollutants.
3. A laboratory scale bioreactor involving a pneumatic fermenter linked to a membrane module, with *Trametes versicolor* growing and producing enzymes continuously at steady state with continuous operation of this system for a prolonged time to demonstrate its feasibility.
4. Development of models of the biological reactions in terms of cell growth, enzyme production, biodegradation rates and the operation of the fermenter / membrane module bioreactor for bioremediation
5. Establishment and demonstration of the process in a demonstration plant by scale-up of the laboratory scale bioreactor system, on the basis of models. (However, it was decided at the first Steering Committee meeting of the project that scale-up was outside the scope of the project.)
6. Transfer of technology to relevant industries and reporting on these results.