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DECOLOURISATION OF AN ARTIFICIAL TEXTILE EFFLUENT BY *Phanerochaete chrysosporium*

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Summary
Phanerochaete chrysosporium decolourised 6 out of 9 synthetic textile dyes tested in the presence of glucose. 3 textile dyes were decolourised in the absence of a primary carbon source. Decolourisation of an artificial textile effluent was complete after 7 days, however, the role of lignin peroxidase was unclear.

Introduction

At present over 100,000 synthetic dyes are available and large quantities of these compounds are released into the environment in industrial effluents each year (Meyer, 1981). Many synthetic dyes, for example the azo dyes, are resistant to microbial degradation under conditions normally found in wastewater treatment plants (Michaels & Lewis, 1986; Pagga & Brown, 1986; Shaul *et al.*, 1991). Such recalcitrance is desirable in a commercial textile dye under typical usage conditions (Seshadri *et al.*, 1994). The metabolism of pure dyes has been extensively studied but little has been published on biological treatments of industrial dye effluents (Zhou & Zimmermann, 1993). Filamentous fungi have been used to remove colour from textile and dyestuff industry wastewater by adsorption (Mou *et al.*, 1991), whilst a strain of *Pycnoporus cinnabarinus* has been found to decolourise pigment plant effluent (Schlephake *et al.*, 1993). Reactive dye effluents have been treated by both actinomycetes and an immobilized *Pseudomonas* strain (Zhou & Zimmermann, 1993). In this study we show the rapid decolourisation of an artificial textile effluent, and some of its component dyes, by *Phanerochaete chrysosporium*.

Materials and Methods

Chemicals

Chemicals all of highest available purity were obtained from Sigma Chemical Co., (Poole, UK) with the exception of the textile dyes which were a gift from Fruit of the Loom International (Buncrana, Rep. Ireland). The dyes obtained are widely used by this textile company: Cibacron Red C-2G , Cibacron Orange CG (both reactive dyes); Remazol Navy Blue GG, Remazol Red RB, Remazol Blue B, Remazol Black B (all diazo dyes); Remazol Golden Yellow RNL (azo dye); Disperse Navy D2GR (Disperse dye); Remazol Turquoise Blue G133 (phthalocyanine dye). A stock solution of artificial textile effluent, consisting

of an equal proportion of each textile dye dissolved in distilled water, was made to a final dye concentration of 100 g l^{-1}.

Media and culture conditions

Phanerochaete chrysosporium MUCC19343 (ATCC 24725) was maintained on 2% malt extract agar plates at room temperature. The isolate was routinely incubated at 37°C(150 rpm) in the liquid culture medium described by Kirk *et al.*, (1978) except that 0.1% (v/v) Tween 80 was added. Where indicated textile dyes (0.5 g/litre) were also added.

Plate studies

Solidified culture medium was made by adding 1.2%(w/v) Bacto Agar (Difco). Stocks of plates containing each of the above listed textile dyes (0.5 g/litre) were prepared either with or without glucose (10 g/litre). Triplicate plates were inoculated centrally with colony growth and plate decolourisation monitored over a 14 day period. Uninoculated plates served as controls. Plates were incubated at 37°C.

Analytical methods

The utilisation of glucose was measured using a Sigma D-glucose Kit. Decolourisation of artificial textile effluent was monitored in culture supernatants using a scanning spectrophotometer (Shimadzu UV-2101PC). Lignin peroxidase activity was measured using UV spectroscopy to quantify veratraldehyde production from veratryl alcohol as described by Tien and Kirk (1984). One unit (U) was defined as 1μmol of veratryl alcohol oxidised in 1 min, and activities were reported as U l^{-1}.

Results and Discussion

Plate studies were carried out to assess the potential for the use of *P. chrysosporium* in the biotreatment of wastewater from a textile plant (Table 1). Visual comparisons of plates indicated that mycelial growth was much more dense and extensive in the presence of glucose than in its absence. 6 out of 9 dyes were decolourised in the presence of glucose, whilst in its absence decolourisation of only 3 dyes was observed (Table 1). These results suggest that whilst a primary carbon source, such as glucose, is essential for extensive dye decolourisation, certain dyes may be metabolised as sole carbon and energy sources by *P. chrysosporium*. Despite extensive data on dye decolourisation by strains of *P. chrysosporium* none have previously been reported to utilise these compounds as growth substrates. At present it is unclear why these 3 dyes are decolourised whilst structurally similar dyes such as Remazol Navy Blue GG are not.

Lignin peroxidases produced by *P. chrysosporium* have been implicated in the decolourisation of many dyes (Spadaro *et al.* 1992; Ollikka *et al.*, 1993). A maximum lignin peroxidase activity of 100 U l^{-1} was produced by our strain of *P. chrysosporium* MUCC 19343 after 14 days (Figure 1). Glucose was not depleted until day 16. In the presence of artificial textile effluent (0.5 gl^{-1}) dye decolourisation commenced at day 5 and

was complete by day 7 (Figure 2). Effluent decolourisation shows both a significant decrease in colour intensity and a shift towards the UV region as reported by Schliephake *et al.* (1993). Previously it has been suggested that some decolourisation can result from dye binding to fungal mycelium (Cripps *et al.*, 1990). This phenomenon accounts for only a small percentage of colour removal observed in these experiments (data not shown).

DYE	DECOLOURISATION[A]	
	Glucose	No Glucose
Cibacron Red C-2G	-	-
Remazol Navy Blue GG	+	-
Remazol Red RB	+	+
Cibacron Orange CG	-	-
Remazol Golden Yellow RNL	+	-
Disperse Navy D2GR	-	-
Remazol Blue B	+	+
Remazol Turquoise Blue G133	+	-
Remazol Black B	+	+

[A] No decolourisation was observed with uninoculated controls

TABLE 1: Decolourisation of textile dyes by *Phanerochaete chrysosporium*

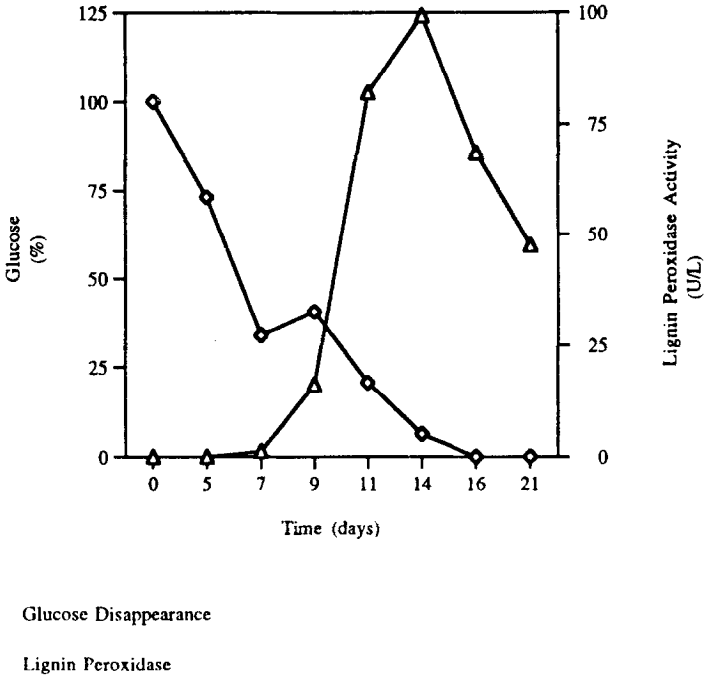

Figure 1: Lignin peroxidase activity and glucose utilisation by *P. chrysosporium*.

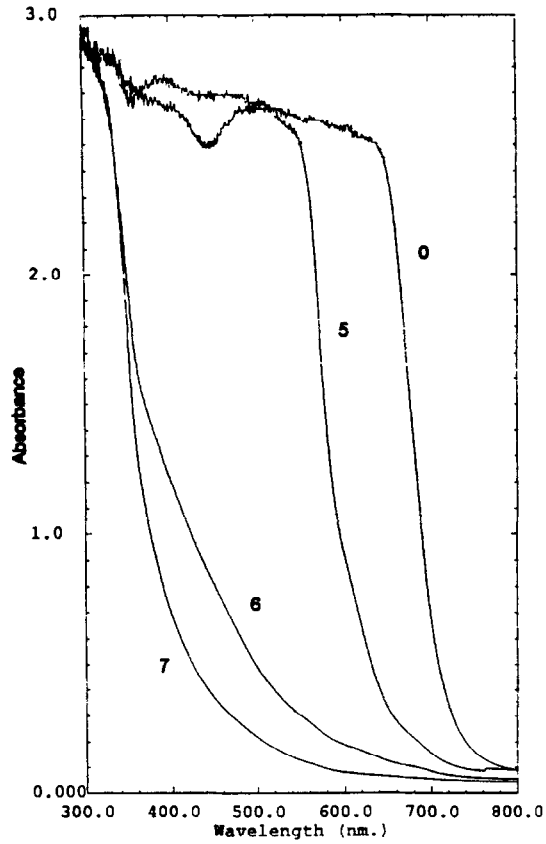

Figure 2: Decolourisation of artificial textile effluent by *P. chrysosporium.* Samples were taken at day 0, 5, 6, and 7.

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