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DECOLORIZATION OF SYNTHETIC TEXTILE DYES BY WHITE ROT FUNGI AND *STREPTOMYCES* SPECIES

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ABSTRACT

Four white rot fungi (*Phanerochaete chrysosporium* NRRL 6359, *Coriolus versicolor* NRRL 6102, *P. chrysosporium* NRRL 6361 and *P. chrysosporium* NRRL 6370) and four *Streptomyces* sp. (*Streptomyces chromofuscus, S. griseoflavus, S. mutabilis* and *S. humidus*) were tested for their ability to produce xylanase, cellulase and peroxidase in a stationary submerged liquid cultures of mineral salts solution supplemented with 0.6 % yeast extract and 3.0 % malt extract for streptomycetes and fungi respectively. Mineral salts solution supplemented with 1% agricultural byproduct and oat meal extract separately were also used. Oat meal extract was the best medium for enzymes production.The highest level of xylanase production was detected on the 9th day by *S. chromofuscus* (40.2 U/ml) in oat meal extract followed by *P. chrysosporium* NRRL 6361 (33.7 U/ml), *P. chrysosporium* NRRL 6370 (17.8 U/ml), *S. griseoflavus* (17.7 U/ml), and *S. humidus* (17.1 U/ml). Peroxidase activities were observed at maximum level with streptomycetes than white rot fungi on the 9th and 12th day. We selected the most active two white rot fungi *P. chrysosporium* NRRL 6361, *P. chrysosporium* NRRL 6370 and three streptomycetes (*S. chromofuscucs, S. griseoflavus* and *S. humidus*) for dyes decolorization. Stationary and submerged liquid cultures with or without synthetic textile dyes were used as substrates for microorganisms. Oat meal extract was the best medium for enzymes production and dyes decolorization. Static liquid culture was the best condition for decolorization than shaking. Culture filterates of *S. chromofuscus* from oat meal extract (Equal volume) decolorized diamine red by 97.7 % at the 10th day followed by *S. griseoflavus* (97.1 %). *P. chrysosporium* NRRL 6370 gave maximum level of "remazol brilliant red" decolorization (69.9 %) at the 7th day, while *S. griseoflavus* gave maximum level of "cibacron blue" decolorization (83.9 %) at 7th day. This study produced a better percentage decolorization through dyes degradation by *Streptomyces* spp. than fungi in a shorter fermentation time with a higher enzyme activities.

Key word: White rot fungi, *Streptomyces* spp., Enzymes, syntheyic textile dyes, Decolorization.

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INTRODUCTION

Dyes found broad applications in various industries for dyeing and printing. Azo dyes, the largest class of synthetic dyes used in the food , pharmaceutices, cosmetics, paper and textiles industries because of their ease of use, cost effectiveness in synthesis, stability and variety of colors compared with natural dyes **(Marmion, 1991).**

Many studies indicated that these dyes are toxic or carcinogenic due to the aromatic amines formation **(Cripps *et al* 1990 and Wong & Yuen, 1996)** while the white rot fungi have been reportted to efficiently degrade azo dyes without the formation of aromatic amines **(Schliephake *et al* 1993 and Chivakula & Renganathan, 1995).**

White rot fungi, a group of lignin degrading basidiomycetes have received considerable attention for their bioremediation potentials. They are able to degrade lignin and other recalcitrant molecules using relatively nonspecific extracellular enzymes **(Kirk & Farrel, 1987 and Paszezynski & Crawford, 1995).**

Decolorization of various dyes by white rot fungi **(Robinson *et al* 2001; Chagas & Durrant, 2001; Yesilada *et al* 2002 and Kasinath *et al* 2003)** and *Streptomyces* species **(Antonopoulos *et al* 2001)** was reported.

Despite the fact streptomycetes are known to produce cellulose-free xylanase and proxidase activity **(Ball & McCarthy, 1988 and Mercer *et al* 1996)**, which are stable and active over a higher pH than the corresponding fungal enzymes **(Sunna and Antranikian, 1997)**, these organisms have not been extensively studied. There is no much information on the decolorization activity of the mutant of white rot fungi (*Phanerochaete chrysosporium* NRRL 6359, *Coriolus versicolor* NRRL 6102 *P. chrysosporium* NRRL 6361 and *P. chrysosporium* NRRL 6370) and *Streptomyces* spp. (*S. chromofuscus, S. griseoflavus, S. mutabilis* and *S. humidus*) and the dyes Diamine Red 8 D200, Remazol Brilliant Red gG D2 (Reactive Dye) and Cibacron blue 3 G-A.

In this study the biotechnological potential of culture supernatant from different types of *Streptomyces* species and white rot fungi (*Phanerochaete chrysosporium*) was evaluated.

In addition, the suitability of this enzymes system for decolorizing three different synthetic dyes has also been determined.

MATERIAL AND METHODS

Dyes

Diamine Red 8 D200 (Hoechst), Remazol Brilliant Red gG D2 (Reactive Dye) (Hoechst) and Cibacron blue 3 G-A (Ciba-Geigy) were used.The dye solutions were added in equal volumes to the medium or fermented cultural filterates (Stock dye concentrated was 200 mg/L equivalent to 100 mg/L final concentration after addition).

Organisms and growth conditions

The microorganisms used in this study were :

1- White-rot fungi, *Phanerochaete chrysosporium* NRRL 6359, *Coriolus versicolor* NRRL 6102, *P. chrysosporium* NRRL 6361 and *P. chrysosporium* NRRL 6370 were obtained

from Department of Agriculture, Agriculture Research Service Peoria, Illinois, U.S.A., known for their delignification. Stock cultures of the white rot fungi were stored at 4°C on slants of yeast extract- malt extract glucose agar for 2-12 weeks.

2- Four cultures belonging to genus *Streptomyces* were isolated from Egyptian soil surrounding Tanta City in the Delta of Nile river, and identified by **Abd El-Nasser (1991).** *Streptomyces* strains (*S. chromofuscus, S. griseoflavus, S. mutabilis* and *S. humidus*) were cultivated routinely and stored at 4°C on a slant of basal starch nitrate agar medium **(I.S.P., 1968).**

In all experiments, spores from stock slants of *Streptomyces* spp. were used as initial inoculum. The initial inocula of fungi were activated on the minerals media incubated on rotary shaker for 24 hr before use.

Liquid static state fermentation media

One millilitre of heavy spore suspensions of the studied microorganisms was used to inoculate Erlenmeyer flasks of 250 ml capacity, each containing 50 ml of the following ingredients (g/L) :

1- The first medium (M_1) contained either 0.6 % (wt/vol) yeast extract in a nitrogen-free, mineral salts solution (g/L: Na_2HPO_4, 5.03; KH_2PO_4, 1.98; NaCl, 0.2; $MgSO_4$ $7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.05; plus 1 ml trace element solution (g/L: Fe SO_4 $7H_2O$, 1.0; $MnSO_4$ $7H_2O$, 0.2; $ZnSO_4$ $7H_2O$, 0.9) pH 6.0-6.5, or 3.0% (wt/vol) malt extract for strains of *Streptomyces* spp and white rot fungi respectively **(Lee *et al* 1991).**

2- The second medium (M_2). The agricultural byproducts (corn cobs and wheat straw) were added to the mineral salts solution. The best concentration of the agricultural byproducts for xylanase production was determined in a previous paper **Abd El-Nasser and Foda (1995).**

3- The third medium (M_3) contained (g/L) yeast extract 6.0 (for streptomycets) or malt extract 30 (for fungi) plus oat spelts xylan, 8; we replace oat splet xylan by 10 g. of oat meal extract, $(NH_4)_2SO_4$, 0.1; NaCl, 0.3; $MgSO_4.7H_2O$, 0.1; $CaCO_3$, 0.02; trace elements solution 1 ml and finally adjusted to pH 7.0-7.6 then sterile at 121°C for 15 min **(Antonopoulos *et al* 2001).**

The inoculated flasks and uninoculated control were incubated at 34°C. Samples of the growth media were taken periodically over the growth period.

At the end of the fermentation period, the content of each flask was filtrated to determine the final pH of the supernatant and immediately analyzed for enzymes production. The crude enzymes produced were applied as culture filterates on the three synthetic types of dyes to determine the percent of decolorization.

The wastes used

Two types of agricultural byproducts were firstly used (wheat straw and corn cobs) as substrates for enzymes and protein production. They were collected from Giza farm. These wastes were al-

lowed to dry in the oven at 70°C for 72 hr. These samples were ground well before use.

Decolorization experiments

Assay

Three types of synthetic textile dyes were added to the first medium (M_1) by equal volume (25 ml each dye + 25 ml media). The inoculated flasks and uninoculated control were incubated on a rotary shaker (180 rpm) at 34 °C. The fermented broths (Culture filterates) of M_2 and M_3 were added to the synthetic dyes in equal volumes and incubated on a rotary shaker or in static incubator at 34°C. The decolorization percentage was examined at different times.

Decolorization of dyes was determined as relative decrease (%) of absorbance for each dye at their absorbance maxima by Spekol Stectrophotometer. The digital pH meter with glass electrode (Knick-646) was used to measure the pH of the media. The dry weight of the mycellia was obtained by filtering cultures through filter paper whatman no. 1 and drying to a constant weight at 65°C.

Detremination of enzyme activity

Xylanase activity was assayed using pure oat spelts xylan (Fluka Biochemika) solution (1% w/v) prepared in potassium phosphate buffer (100 mM, pH 7) as the substrate. Equal volumes (1.0 ml) of culture supernatant and substrate solution were incubated at 40 °C, for 10 min. Then the reaction mixture was placed in an ice bath to stop the reaction. Reaction mixtures were centrifuged at 10,000 g, for 4 min to allow precipitation of the insoluble substrate.

Cellulase activity was assayed according to the above mentioned, procedure but replacing oat spelt xylan solution with carboxymethyl-cellulose (CMC) solution (1% w/v), prepared in potassium phosphate buffer (100 mM, pH 7) as the substrate. Estimation of the concentration of the resulting reducing sugars present in a sample was performed using the dinitrosalicylic acid (DNS) reagent method (**Miller, 1959**). One unit (U) of either xylanase or cellulase activity was defined as the amount of enzyme that released 1 μ mol of reducing sugar (expressed as xylose or glucose equivalent, respectively) per minute per milliliter under the assay conditions described.

Peroxidase activity was assayed using 2,4-dichlorophenol (2,4-DCP) as the substrate (**Antonopoulos *et al* 2001**). The reaction mixture contained equal volumes (0.2 ml) of each of potasssium phosphate buffer (100 mM pH 7.0) 2,4-DCP (25 mM), 4-aminoantipyrine (16 mM), peroxidase-containing sample and H_2O_2 (50mM). The reaction was initiated by the addition of H_2O_2 and the absorbance was determined at 510 nm, after incubation at 53°C, for 1 min. One unit (U) of enzyme activity was defined as the amount required for an increase in absrbance of one unit O.D. per minute.

Protein determination

Protein was determined in the crude enzyme preparation by the method of **Lowry *et al* (1951)**. Protein content was calculated from bovine serum albumin standard curve.

Results are the mean of at least two replicates.

RESULTS AND DISCUSSION

Growth and extracellular enzymes production by selected microorganisms on different media

An estimate of the time taken for microorganisms growth and extracellular enzymes production was given by measuring total released reducing sugars, extracellular protein and by assaying xylanase, cellulase and proxidase activity in culture supernatant.

Experiment-1

The microorganisms firstly were grown on mineral salts medium (M_1) containing 0.6% yeast extract and 3.0% malt extract for streptomycetes and white rot fungi respectively. The results were taken after 5 and 7 days of incubation on static liquid fermentation growth at pH 6.5 and 34°C. From Table (1) white rot fungi proved to be the best for extracellular xylanase formation, total released reducing sugars and protein content than *Streptomyces* spp. *Phanerochaete chrysosporium* NRRL 6370 gave higher extracellular peroxidase activity (0.35 U/ml) than other microorganisms at the 5th day of incubation period and was still stable up to 7 days. *Phanerochaete chrysosporium* NRRL 6359 and *Coriolus versicolor* NRRL 6102 gave high xylanase activity followed by *P. chrysosporium* NRRL 6361 and *P. chrysosporium* NRRL 6370 after 5 days of incubation. Xylanase activity decreased with *Coriolus versicolor* NRRL 6102. Other microorganisms failed to produce xylanase activity up to 7 days. All microorganisms failed to produce cellulase.

Experiment-2

The tested microorganisms were grown on mineral salts medium supplemented with agricultural byproducts (1% wheat straw and corn cobs) as carbon sources for producing reducing sugars, extracellular protein and enzymes. Table 2 show the levels of residual released-reducing sugars, total protein and enzymes formation using two types of agricultural byproducts as substrate. Incubation was carried out at 3, 6 and 9 days at 34°C. All selected microorganisms gave high level of protein content after 3 and 6 days, while increased after 9 days with white rot fungi and decreased with *Streptomyces* spp. In case of wheat straw, reducing sugars decreased with increasing time by white rot fungi while it increased with time by *Streptomyces* spp. at the 6th day and then decreased. But in case of corn cobs reducing sugars increased with increasing time by *Streptomyces* spp. The data concerning the final reducing sugars are the resultant of two processes. The enzymes produced (xylanase and/or cellulase) should hydrolyse the relevant polysaccharides. The presence of final reducing sugars in the culture supernatant and the absence of polysaccharides hydrolysing enzymes might point to adsorption of the enzymes on the insoluble polysaccharides (powdered agricultural residues). Extracellular enzymes (xylanase and peroxidase) were produced by white rot fungi and *Streptomyces* spp. Maximum level of xylanase activity was observed on corn cobs with *P. chrysosporium* NRRL 6370 (12 U/ml) after 6 days, while *Coriolus versicolor* NRRL 6102 and *S. chromofuscus* after 9 days (13.2 U/ml) followed by *S. mutabilis* (8.4 U/ml). Extracellular peroxidase activity

Table 1. Microorganisms grown on mineral salts medium (M1) incubated at 34°C and pH 6.5 for 5 and 7 days.

Organisms	Final total reducing sugars mg/ml	Final total protein mg/ml	Xylanase activity U/ml	Cellulase activity mg/ml	Peroxidase activity U/ml
			5 days		
Phanerochaete chrysosporium NRRL 6359	300	1.2	48.0	0.6	0.15
Coriolus versicolor NRRL 6102	216	1.04	36.0	0.0	0.20
P.chrysosporium NRRL 6361	180	1.12	17.4	0.0	0.20
P.chrysosporium NRRL 6370	240	1.20	16.2	0.0	0.35
Streptomyces chromofuscus	45	0.64	0.0	0.0	0.15
S.griseoflavus	24	0.76	0.0	0.0	0.20
S.mutabilis	48	0.78	0.0	0.0	0.20
S.humidus	18	0.64	1.2	0.0	0.15
			7 days		
Phanerochaete chrysosporium NRRL 6359	260	1.9	0.0	0.0	0.20
Coriolus versicolor NRRL 6102	340	1.8	0.0	0.0	0.15
P. chrysosporium NRRL 6361	310	1.9	0.0	0.0	0.35
P.chrysosporium NRRL 6370	350	2.3	4.4	0.0	0.20
Streptomyces chromofuscus	25	1.1	0.0	0.0	0.20
S.griseoflavus	17	1.2	0.0	0.0	0.20
S.mutabilis	16	1.5	0.0	0.0	0.15
S.humidus	10	1.6	0.0	0.0	0.0

Table 2. Testing micoorganisms for their ability to produce good yeild of enzymes grown on static liquid culture containing agricultural byproducts as a sole carbon source incubated at 34°C and pH 6.5 for different periods.

Organism	Final	Final total protein mg/ml	Final total reducing sugars mg/ml	Xylanase activity U/ml	Cellulase activity U/ml	Peroxidase activity U/ml
			Wheat straw			
			3 days			
P. chrysosporium NRRL 6359	5.50	1.20	450	0.0	0.0	0.10
Coriolus versicolor NRRL 6102	5.70	1.10	420	0.0	1.8	0.20
P. chrysosporium NRRL 6361	5.40	1.32	247	0.0	0.0	0.20
P. chrysosporium NRRL 6370	5.50	1.34	486	0.0	0.0	0.30
S. chromofuscus	7.70	1.44	63	0.0	0.6	0.40
S. griseoflavus	7.90	1.52	45	0.0	1.2	0.35
S. mutabilis	8.00	1.5	36	0.0	0.0	0.60
S. humidus	8.50	1.64	90	0.0	0.0	0.50
			6 days			
P. chrysosporium NRRL 6359	5.31	1.96	153	3.5	0.0	0.50
Coriolus versicolor NRRL 6102	6.04	1.64	99	0.0	0.0	0.40
P. chrysosporium NRRL 6361	5.88	1.80	105	0.0	0.0	0.50
P. chrysosporium NRRL 6370	5.35	1.68	111	0.0	0.0	0.45
S. chromofuscus	8.66	1.88	85.5	6.7	0.0	0.60
S. griseoflavus	8.09	1.92	49.5	4.1	0.0	0.75
S. mutabilis	8.22	1.92	54	0.0	0.0	0.80
S. humidus	8.63	2.20	72	0.0	0.0	0.65
			9 days			
P. chrysosporium NRRL 6359	5.25	1.55	49	0.0	0.0	0.45
Coriolus versicolor NRRL 6102	5.71	1.55	29	0.0	0.0	0.45
P. chrysosporium NRRL 6361	6.71	1.80	38	0.0	0.0	1.00
P. chrysosporium NRRL 6370	5.04	1.55	33	0.0	0.0	0.55
S. chromofuscus	8.63	2.00	36	0.0	0.0	1.50
S. griseoflavus	8.42	2.00	29	0.0	0.0	0.60
S. mutabilis	8.40	2.00	23	0.0	0.0	0.65
S. humidus	8.47	1.60	36	0.0	0.0	0.70

Table 2. Cont

Organism	Final pH	Final total protein mg/ml	Final total reducing sugars mg/ml	Xylanase activity U/ml	Cellulase activity U/ml	Peroxidase activity U/ml
Corn cobs						
3 days						
P. chrysosporium NRRL 6359	6.70	1.10	162	0.0	0.8	0.10
Coriolus versicolor NRRL 6102	6.67	1.10	135	0.0	0.0	0.30
P. chrysosporium NRRL 6361	6.67	1.10	225	5.4	1.0	0.20
P. chrysosporium NRRL 6370	6.13	1.10	234	0.0	0.40	0.60
S. chromofuscus	8.26	1.22	18	6.0	0.0	0.50
S. griseoflavus	7.22	1.48	8	1.2	0.0	0.45
S. mutabilis	7.50	1.64	18	0.9	0.0	0.85
S. humidus	7.30	1.64	5	0.6	0.0	0.10
6 days						
P. chrysosporium NRRL 6359	5.98	1.10	405	8.0	0.0	0.45
Coriolus versicolor NRRL 6102	6.12	1.22	414	8.6	0.4	0.30
P. chrysosporium NRRL 6361	6.18	1.46	270	8.4	0.0	0.50
P. chrysosporium NRRL 6370	4.30	1.18	450	12.0	0.0	0.30
S. chromofuscus	8.63	1.64	45	7.2	0.0	0.30
S. griseoflavus	7.23	1.90	51	0.6	0.6	0.65
S. mutabilis	8.73	1.64	45	1.8	0.0	0.25
S. humidus	8.45	1.48	51	6.6	0.0	0.25
9 days						
P. chrysosporium NRRL 6359	5.86	2.00	165	0.25	0.0	0.25
Coriolus versicolor NRRL 6102	6.11	3.20	183	13.2	0.0	0.25
P. chrysosporium NRRL 6361	6.15	1.26	204	1.8	0.0	0.35
P. chrysosporium NRRL 6370	4.43	2.52	168	0.0		0.10
S. chromofuscus	8.30	0.64	114	13.2	0.0	0.15
S. griseoflavus	8.12	0.40	120	2.7	0.0	0.25
S. mutabilis	8.63	0.38	114	4.2	0.0	0.20
S. humidus	8.84	0.40	108	8.4	0.0	0.20

was observed on corn cobs in a maximum level with *Streptomyces* spp. than white rot fungi after 3 days, then decreased by increasing time. Cellulase activity was not produced with all microorganisms after 9 days on corn cobs. Cellulase activity was detected in a low level with *P. chrysosporium* NRRL 6361 (1.0 U/ml) followed by *P. chrysosporium* NRRL 6359 (0.8 U/ml) after 3 days.

In case of wheat straw, the microorganisms failed to produce extracellular xylanase and cellulase enzymes except, in case of the *S. chromofuscus, S. griseoflavus* and *P. chrysosporium* NRRL 6359 which gave low levels of xylanase activities than corn cobs. Peroxidase activity was produced in a high level with streptomycets at, the 6th day and, the 9th day. *Streptomyces chromofuscus* gave the highest peroxidase (1.5 U/ml) level at, the 9th day of incubation.

Experiment-3

The microorganisms were grown on oat meal extract medium for producing reducing sugars, extracellular protein and enzymes. Our results (as shown in Table 3) shows that, total protein was highly producted with streptomycets than white rot fungi. The highest level for protein content was observed with *S. chromofuscus* at, the 3th day. While the reducing sugars was highly producted with white rot fungi than streptomyctes. The maximum level of reducing sugars was observed with *P. chrysosporium* NRRL 6361 at, the 6th day. Xylanase production was observed at, the 6th day with maximum production by *Coriolus versicolor* NRRL 6102 (14.4 U/ml) followed by *P. chrysosporium* NRRL 6359, *P. chrysosporium* NRRL 6361 and *S. griseoflavus* (7.2 U/ml). While at, the 9th day, the maximum production of xylanase was observed with *S. chromofuscus* (40 U/ml) followed by *P. chrysosporium* NRRL 6361 (33.7 U/ml), *P. chrysosporium* NRRL 6370 (17.8 U/ml), and *S. humidus* (17.1 U/ml) and then decreased at, the 12th day. Cellulase production was observed at, the 3th day with low level by *P. chrysosporium* NRRL 6370 followed by *P. chrysosporium* NRRL 6359 and *P. chrysosporium* NRRL 6361, then disappeared with all microorganisms at the 6th and 12th day. Peroxidase activity was highly detected with streptomycetes than white rot fungi. *Sterptomycyes chromofuscus* gave high peroxidase activity at, the 12th day followed by *S. humidus, S. mutabilis* and *S. griseoflavus.*

Our results seem to be a better for enzymes activites than these obtained by **Antonopoulos *et al* (2001)** who found that the maximal extracellular xylanase activity (11.97 U/ml) at 120 hr (5 days) produced by *S. albus* which remained at the same levels up to 168 hr (7 days) of incubation. Extracellular peroxidase was detected during the growth of *S. albus* with maximal activity of 0.58 U/ml obtained after 72 hr (3 days). They also found that, in contrast to xylanase and peroxidase activity, no cellulase activity was detected at any point throughout *S. albus* growth (168 hr).

This study has shown the suitability of extracellular enzymes production by white rot fungi and *Streptomyces* spp. grown on mineral salts solution supplemented with malt or yeast extract for fungi and streptomycetes respectively, mineral salts solution supplemented with agricultural byproduct waste (wheat straw and corn cobs) or oat meal extract for

Table 3. Testing microorganisms for their ability to produce good yields of enzymes grown on static liquid culture of oat meal extract medium incubated at 34°C and pH 7.0-7.6 at different times.

Organisms	Final pH	Final total protein mg/ml	Final total reducing sugars mg/ml	Xylanase activity U/ml	Cellulase activity U/ml	Peroxidase activity U/ml
			3 days			
P. chrysosporium NRRL 6359	3.93	0.880	378	0.0	0.4	0.40
Coriolus versicolor NRRL 6102	3.51	0.640	540	0.0	0.0	0.50
P. chrysosporium NRRL 6361	3.55	0.580	504	0.0	0.2	0.10
P. chrysosporium NRRL 6370	3.50	0.660	486	0.0	0.2	0.10
S. chromofuscus	7.82	1.720	306	0.0	0.2	0.20
S. griseoflavus	8.63	1.080	279	0.0	0.0	0.30
S. mutabilis	8.71	1.080	243	0.0	0.6	0.25
S. humidus	8.49	1.080	108	0.0	0.6	0.35
			6 days			
P. chrysosporium NRRL 6359	3.34	0.920	282	7.20	0.0	1.10
Coriolus versicolor NRRL 6102	3.48	0.600	102	14.40	0.0	0.20
P. chrysosporium NRRL 6361	3.82	0.720	600	7.20	1.0	0.10
P. chrysosporium NRRL 6370	3.50	0.820	492	0.00	1.4	0.10
S. chromofuscus	9.04	1.040	72	2.40	0.0	0.40
S. griseoflavus	8.86	1.080	87	7.20	0.0	0.45
S. mutabilis	8.74	1.080	60	1.0	0.0	0.25
S. humidus	8.99	1.100	75	1.20	0.0	0.45
			9 days			
P. chrysosporium NRRL 6359	3.65	1.100	270	0.0	0.0	1.05
Coriolus versicolor NRRL 6102	3.24	0.400	360	14.2	1.5	0.45
P. chrysosporium NRRL 6361	3.45	0.680	540	33.7	1.0	0.15
P. chrysosporium NRRL 6370	3.98	0.900	564	17.8	0.2	0.10
S. chromofuscus	8.60	0.160	270	40.2	0.8	0.40
S. griseoflavus	7.83	1.080	180	17.7	0.0	0.40
S. mutabilis	7.80	0.920	108	14.2	0.0	0.40
S. humidus	7.60	1.080	78	17.1	0.0	0.55
			12 days			
P. chrysosporium NRRL 6359	4.44	0.940	270	4.70	0.0	1.0
Coriolus versicolor NRRL 6102	3.13	0.600	90	2.90	0.0	0.10
P. chrysosporium NRRL 6361	3.12	0.640	360	1.20	0.0	0.10
P. chrysosporium NRRL 6370	2.91	0.560	282	7.20	0.0	0.10
S. chromofuscus	9.01	0.820	90	2.90	0.2	0.75
S. griseoflavus	9.16	0.920	84	10.0	0.0	0.50
S. mutabilis	9.07	0.880	54	0.0	0.0	0.55
S. humidus	8.87	0.980	69	2.40	0.0	0.70

application in the decolorization of three different types of synthetic dyes.

Decolorization of synthetic textile dyes by stationary submerged cultures at different times

In liquid culture, the decolorization of the synthetic dye solution could be due to adsorption by the microorganisms biomass or biodegradation. When degradation occurred, there was either complete removal of the major visible light absorbance peak or significant spectral change. Absorbance maxima of Diamine Red 8 D200 was at 550 nm; Remazol Brilliant Red gG D2 was at 500 nm and Cibacron blue 3 G-A was at 620 nm. The structures of the investegated dyes were not found in the **Colour index (1992).**

Dyes decolorization by the testing microoganisms

Experiment-1

Decolorization of the dyes during microbial growth

Four *Streptomyces* spp. and four white rot fungi were tested for their dyes (Diamine red, remazol brilliant red and cibacron blue) decolorization activity grown on mineral salts solution supplemented with 3.0% malt extract or 0.6 % yeast extract for fungi and streptomycetes respectively on a rotary shaker for 9 days. The highest level of decolorization of diamine red 8 D 200 dye was observed with *P. chrysosporium* NRRL 6370 (90 %) followed by *P. chrysosporium* NRRL 6361 (45 %), but all other selected organisms failed to decolorize this dye (Table 4). Microorganisms failed to degrade remazol brilliant red, but 20% decolorization of this dye was observed with *Coriolus versicolor* NRRL 6102 and 10 % with *P. chrysosporium* NRRL 6361 and *P. chrysosporium* NRRL 6370. The highest level of decolorization of cibacron blue dye (75 %) was observed with *P. chrysosporium* NRRL 6370 followed by *P. chrysosporium* NRRL 6361 (73 %) and *S. chromofuscus* (54 %). Also, it is clear that the remazol brilliant red dye was more stable than other two dyes. The decolorization was shown not to be due to any effects of pH on the dyes, since the pH of the cultures did not alter during growth, remaining around 3.1 to 3.5 for fungi and 7.22 to 7.9 for streptomycets. Final pH (high or low) not affecte the decolorization percentage. These results were in agrement with the results obtained by **Chagas and Durrant (2001).**

Experiment-2

Decolorization of the dyes solutions by the culture filtrates under non strerile conditions

The same microorganisms were grown on mineral salts solution supplemented with 1% corn cobs or wheat straw as sole carbon sources on a static fermentation for 9 days (Table 5). Equal volume of the fermented broth was added to equal volume of dye, then incubated in a rotary shaker for 24 hours at 34°C. The highest level for decolorization of diamine red and cibacron blue dye was detected with *S. griseoflavus* (71 % and 50 % respectively). Remazol brilliant red dye was very defficult to degrade. The maximum level for decolorization was observed at 17 % with *P. chrysosporium* NRRL 6359 and *Coriolus versicolor* NRRL 6102.

Table 4. Decolorization (%) of three different synthethic dyes by organisms grown on mineral salts solution supplemented with 3.0% malt extract or 0.6 % yeast extract for fungi and streptomycetes respectively on a rotary shaker for 9 days.

Organism	Diamine red			Remazol Brilliant red			Cibacron blue		
	Final pH	Dry weight mg/ml	%*	Final pH	Dry weight mg/ml	%*	Final pH	Dry weight mg/ml	%*
P. chrysosporium NRRL 6359	3.40	0.80	0	3.10	1.20	0	3.37	1.00	27
Coriolus versicolor NRRL 6102	3.26	1.20	0	3.10	1.20	20	3.26	1.20	11
P. chrysosporium NRRL 6361	3.29	1.40	45	3.43	1.40	10	3.43	1.40	73
P. chrysosporium NRRL 6370	3.16	1.20	90	3.32	1.80	10	3.41	1.60	75
S. chromofuscus	7.22	0.80	0	7.40	1.00	0	7.28	1.8	54
S. griseoflavus	7.84	0.80	0	7.68	1.00	0	7.85	1.20	41
S. mutabilis	7.37	1.20	0	7.39	0.60	0	7.80	1.20	0
S. humidus	7.84	1.40	0	7.90	0.40	0	7.82	1.20	0
Control	–	–	0	–	–	0	–	–	0

* Decolarization

Table 5. Decolorization (%) of three different synthethic dyes by mixing equal volumes of the culture filterates of the fermented broth of selected organisms grown on mineral salts solution supplemented with agricultural byproduct (1% corn cobs) and dye incubated on a rotary shaker for 24 hr at 34°C.

Organisms	Diamine red	Remazol Brilliant red	Cibacron blue
	Decolorization %	Decolorization %	Decolorization %
P. chrysosporium NRRL 6359	30	17	30
Coriolus versicolor NRRL 6102	30	17	33
P. chrysosporium NRRL 6361	30	8	35
P. chrysosporium NRRL 6370	30	8	33
S. chromofuscus	20	8	30
S. griseoflavus	71	8	50
S. mutabilis	53	8	35
S. humidus	40	8	37
Control	0	0	0

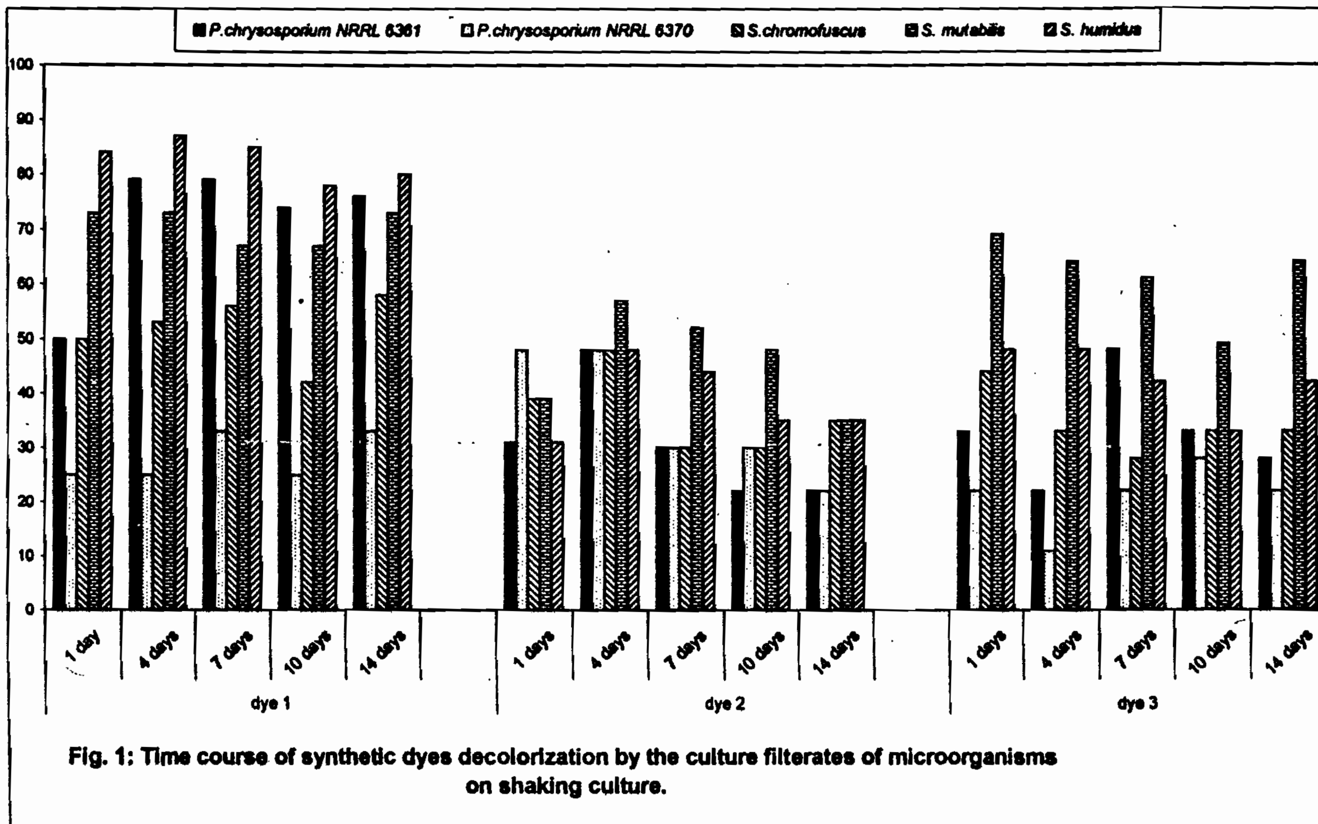

Fig. 1: Time course of synthetic dyes decolorization by the culture filterates of microorganisms on shaking culture.

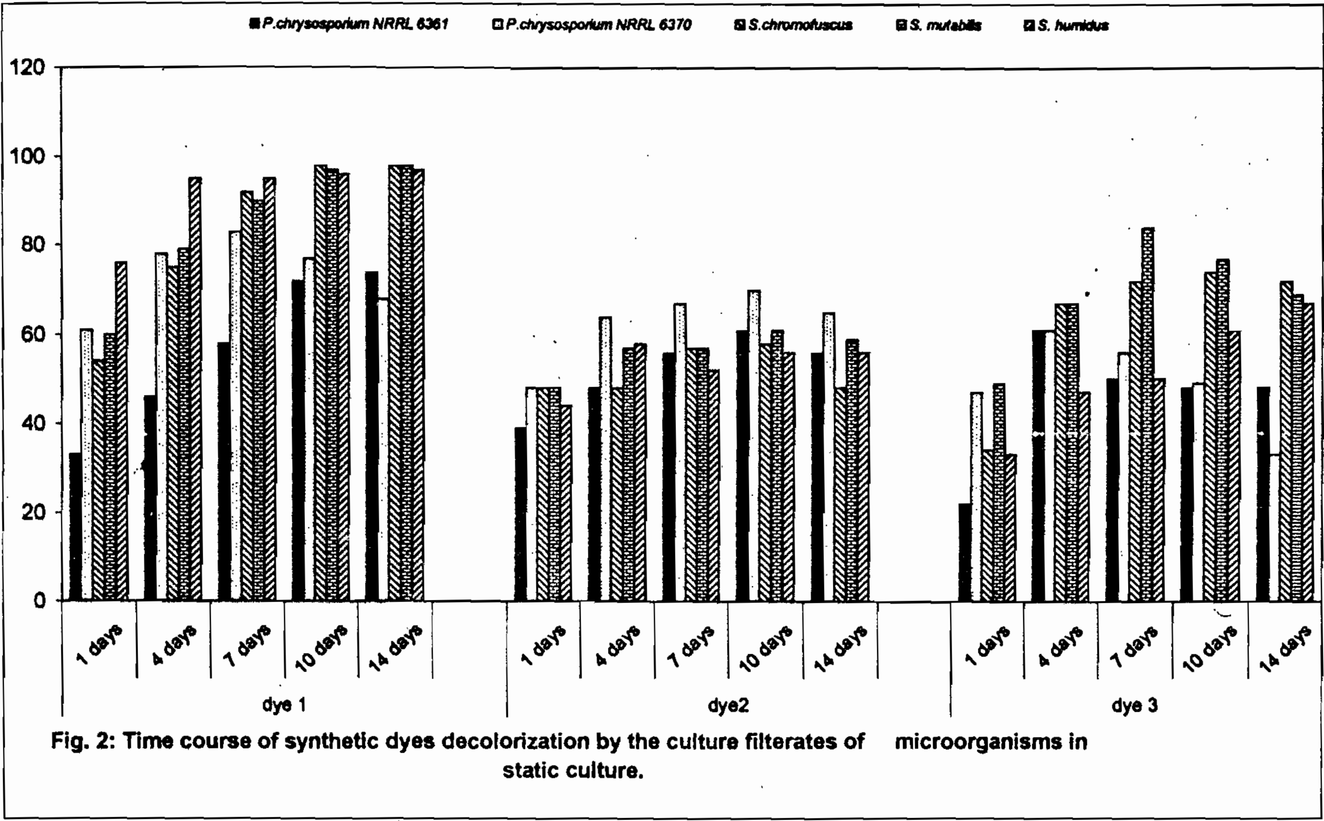

Fig. 2: Time course of synthetic dyes decolorization by the culture filterates of microorganisms in static culture.

These observations indecated that the decolorization process by these two experiments (1 and 2) of decolorization during microbial growth were not suitable.

Time course of decolorization of synthetic textile dyes by culture filtrates under non sterile conditions

From our resultes we observed that, *S. chromofuscus* represented the highest level of xylanase and peroxidase activities at, the 9th day of growth on oat meal extract followed by *P. chrysosporium* NRRL 6361, *P. chrysosporium* NRRL 6370 and *S. humidus*. Therefore, the active enzymes producer must be applied on three different types of synthetic dyes. The enzymes producted were used on the synthetic dyes on shaking and static condition at different periods of time. Decolorization was performed under shaking condition was recorded in Fig. 1. The highest level of decolorization percentage was observed at, the 4th day with diamine red dye (86.5 %) by *S. humidus* culture filterates , remazol brilliant red dye (56.5 %) by *S. griseoflavus* culture filterates and cibacron blue (63.9%) by *S. chromofuscus* culture filterates.

Static decolorization conditions are shown in (Fig. 2), 98.1% decolorization percentage of diamine red dye was observed with *S. chromofuscus* culture filterates at, the 14th day, while *S. humidus* gave 95.4 % decolorization percentage at, the 4th day, and kept constant until, the 7th day then increased to 96.04 % and 96.9 % at 10th day respectively. While *S. chromofuscus* gave maximum level of diamine red decolorization (97.7 %) at, the 10th day followed by *S. griseoflavus* (97.1 %). *P. chrysosporium* NRRL 6370 gave maximum level of remazol brilliant red decolorization percentage (69.6 %) at, the 10th day. *S. griseoflavus* gave high level of cibacron blue decolorization (83.9 %) at, the 7th day. It is also clear that stationary conditions were the best conditions for decolorization than shaking one. **Kaninath *et al* (2003)** stated that, the decolorization of anthraquinone by stationary cultures was slightly more rapid than by the submerged ones after 10 days (100 and 95 %). Our results showed a better percentage of decolorization through dye degradation in a shorter fermentation time with higher enzyme activities.

Our results also indicate that these microorganisms could be used in bioprocesses to remove color from industrial effluents on static conditions. The ability of white rot fungi and streptomycets to degrade a wide variety of environmentally persistent pollutants indicates their potential use in anti-pollution treatments.

However, only a better understanding of the mechanisms used by these microorganisms will allow the development of technologies to apply these organisms to cleaning up aquatic and terrestrial environments.

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إزالة صبغات النسيج الصناعية بواسطة فطريات العفن الأبيض والاستربتوميسيتات

[٤٠]

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تم اختبار أربعة كائنات من العفن الأبيض
(*Phanerochaete chrysosporium* NRRL 6359, *Coriolus versicolor* NRRL 6102, *P. chrysosporium* NRRL6361 and *P. chrysosporium* NRRL 6370).

وأربعة كائنات من الاستربتومسيتات
(*Streptomyces chromofuscus, S. griseoflavus, S. mutabilis* and *S. humidus*).

علي قدرتهم علي إنتاج إنزيم الزيلانيز والسيليولاز و البروكسيديز في ثلاث بيئات أولا بيئة الأملاح المعدنية السائلة الساكنة والمزودة ب ٠.٦% مستخلص الخميرة لاستربتومسيتات و ٣% مستخلص المولت للعفن الأبيض ثانيا بيئة الأملاح المعدنية السائلة الساكنة و المزودة ب ١% من المخلفات الزراعية المطحونة و منها قش القمح و قوالح الذرة ثم ثالثا بيئة مستخلص الشوفان السائلة الساكنة. و من الدراسة وجد أن بيئة مستخلص الشوفان أحسن البيئات المدروسة لإنتاج الإنزيمات عند درجة حرارة ٣٤م°. وقد وجد أن أعلي مستوي لإنزيم الزيلانيز المنتج علي بيئة الشوفان السائلة الساكنة بعد تسعة أيام من فترة التحضين بواسطة ستربتومايسس كروموفوسكس (٤٠,٢ وحدة لكل ملي) يلية فارينوكيت كريزيوسبوريـم ن ررل ٦٣٦١ (٣٣,٧ وحدة لكل ملي) يلية فارينوكيت كريزيوسبوريم ن ررل ٦٣٧٠ (١٧,٨ وحدة لكل ملي) يلية ستربتومايسس جريزيوفلافز و هيوميدز (١٧,٧و ١٧,١ وحدة لكل ملي) علي التوالي.

كذلك وجد أن كائنات الاستربتومسيتات أكفأ من كائنات العفن الأبيض المدروسة لإنتاج إنزيم البروكسيديز ولوحظ أعلي مستوي لإنتاج الإنزيم في اليوم السادس والتاسع.

تم اختيار أكفأ هـذه الكائنات وهم *Phanerochaete chrysosporium* NRRL 6361 and *P. chrysosporium* NRRL 6370

Streptomyces chromofuscus, S. griseoflavus and *S. humidus*.

لمحاولة تطبيقها علي إزالة ثلاث أنواع من صبغات النسيج الصناعية في البيئة السائلة المغمورة المتحركة و الساكنة. وجد أن البيئة الساكنة أفضل من البيئة المتحركة في إزالة صبغات النسيج الصناعية وأن استربتومايسس كروموفوسكس هو أقوي الكائنات لإزالة صبغة دايامين الحمراء بنسبة ٩٧,٧ % يلية استربتومايسس جريزيوفلافز (٩٧,١%) عند اليوم العاشر من فترة التحضين. فارينوكيت كريزيوسبوريم ن ررل ٦٣٧٠ هو أقوي كائن لإزالة صبغة الريمازول بريلينت الحمراء بنسبة ٦٩,٩% عند اليوم السابع بينما استربتومايسس جريزيوفلافز أعطي أعلي مستوي لإزالة صبغة السيباكرون الزرقاء بنسبة ٨٣,٩% عند اليوم السابع من فترة التحضين.

ومن هذه الدراسة نوصي باستخدام هذه الكائنات في معامله مياه صرف مصانع النسيج المحتوية علي الأصباغ قبل صرفها علي خطوط الصرف الصحي.

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