ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Capacity of white-rot fungi *Perreniporia tephropora* for decolourization of different dyes in solid medium

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Manuscript Info

Manuscript History:

Received: 13 January 2014
Final Accepted: February 2014
Published Online: March 2014

Key words:
Perreniporia tephropora, Phanerochaete chrysosporium, ligninolytic enzymes, discoloration, dyes.

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Abstract

The aim of this study is to evaluate the capacity to degrade synthetic dyes (Orange G, Reactive blue 4 and Methyl Orange) used in textile industry by white-rot fungus *Perreniporia tephropora* isolated from Gabonese forest. The isolate was able to decolorize different dyes in wide range (up to 0.3 g/L), at temperature 30°C in solid culture media. The results revealed that the discoloration dyes was important after 6 days and this fungus presented an optimum growth temperature between 30°C and 35°C. This fungus on solid culture media produces different enzymatic activities like laccase, phenoloxidase, and peroxidases, which represented their important role in biotransformation.

1-Introduction

Water is central to all the socio-economic processes; it is our responsibility to do everything to keep it clean. Unfortunately, lots of water is polluted due to industrial activities; the textile industry, for example, is among the most important polluter. Currently, with population increase in the world, the use of chemicals is increasing too. These chemicals discharged into rivers, pollute waters. The textile industry currently uses large amounts of synthetic dyes find into the surrounding waters.

Because of their timeliness of production, their chemical stability and their wide variety of colours, synthetic dyes are used in different domains such as cosmetic, pharmaceutical, food, etc. However, the most important problem is that they are very difficult to degradation.

In the rivers, the synthetic dyes cause ecological imbalances that affect many plant and animal species including the man who is in the food chain [1]. Some allergy problems are among the diseases caused by water pollution by dyes [1]. The fight against this type of pollution has become a major political and economic stake in protecting the environment [2].

World production of dyes is estimated over 800,000 tons per year. Approximately 280,000 tons of textile dyes are discharged each year into rivers [3]. The synthetic dyes belong to different chemical groups (basic azo acid, anthraquinone, triphenylmethane etc.). Among these families, azo dyes are the most widely used (60 to 70% of world production per year).

Several techniques have been used for the removal of dye wastewater [4, 5]. Treatment processes physicochemical (adsorption, ion exchange, membrane filtration, coagulation/flocculation, precipitation and ozonation). However, these techniques are costly and energy consuming. Moreover, during these processes of elimination of toxic products, dyes can be generated and discharged in the environment through water. These difficulties have led to seek more promising degradation pathways such as biodegradation of dyes to replace or supplement the current treatment [6, 7, 8]. These methods have the advantage of being cheaper, cleaner and efficient according to the selected strain.

Indeed, the use of white rot fungi basidiomycetes is one of alternative methods as these are provided with ligninolytic enzymes able to degrade some of the synthetic molecules and recalcitrant compounds as wood lignin [9, 10, 11, 12, 13]. In the industrial and biotechnological processes, ligninolytic enzymes have shown the potential of a

wide variety of specific organic and inorganic materials [14]. In the detoxification of industrial effluents [15, 16], the applications of these enzymes have been shown in the textile and petrochemical industry in the processes of bleaching and delignification of pulp and paper [17] in the food industry by removing phenolic compounds from beer and wine [18]. Capacity ligninolytic eliminate xenobiotics and produce polymer products enzymes were also shown. All these actions are due to extracellular enzymes such as laccases (EC 1.10.3.2), lignin peroxidase (EC 1.11.10.14) and manganese peroxidase (EC1.11.1.13) [19]. Recently, part of the scientific community is interested to search the new strains of fungi [11] producing ligninolytic enzymes with high efficiency in the degradation of recalcitrant chemical molecules to protect water and the environment. It is in this context that our study. In previous work, we have shown the effectiveness of other fungal strains isolated in Gabon decolourization and degradation of synthetic dyes [20, 21].

This study also aims to assess the ability of the strain *Perreniporia tephropora* MUCL 47500 to degrade three recalcitrant synthetic dyes, Orange G, Reactive blue 4 and Methyl Orange after optimizing culture parameters to maximize decolourization on solid medium.

2-Material and methods

2.1-Organism and culture conditions

Pure culture of fungus *Perreniporia tephropora* MUCL 47500 was obtained from Mycothèque de Louvain-la-Neuve, Belgium. Fungus was conditioned and replicated in tubes containing the medium malt-agar at 25°C. These tubes were then preserved and maintained at 4°C before use. The composition of the solid growth medium used in this study is same that is used by Atteke et al. 2013. The pH was adjusted to 5.5 with hydrochloric acid (0. 5 N) before autoclaving (at 121°C for 15 min). The culture medium was transferred into 4 Erlenmeyer flasks (250 mL each) and each reagent (enzymatic revelation) or dye, depending on its final concentration was added. The mixture was shaken, then about 15 mL were distributed in a Petri dish (diameter 8.5 mm); each test was replicated four times.

2.2-Dyes and chemicals

The three dyes: Reactive blue 4 (RB4), Methyl Orange (MO) and Orange G (OG) were obtained from Sigma-Aldrich (Sigma-Aldrich Chimie sarl, Saint Quentin Fallavier, France); agar powder and malt extract from Acros Organics (Fisher Scientific SAS, Strasbourg, France); ABTS (acid, 2, 2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid), TFA (trifluoroacetic acid) and ethyl acetate were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). Methanol for HPLC was obtained from Carlo Erba Réactifs (Carlo Erba Reactifs-SDS, Val de Reuil, France). Glucose was obtained from Prolabo (VWR International S.A.S, Strasbourg, France). All minerals to culture media were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

2.3-Growth and decolourization experiments

The optimum temperature of growth was evaluated using different temperatures (15, 22, 30, 35, 40 and 45°C) on solid culture media by measured the growth diameters. Dyes decolourization on solid culture medium was conducted with 0.3 g/L of each dye (RB4, OG and MO), pH=5.5 and temperature 30°C. Various controls were also prepared: some Petri dishes with fungus alone and others with dye alone. One square portion of agar (1 cm of diameter) containing the fungus was placed at the centre of each Petri dish. The fungal growth diameters and the halos of decolourization were measured every day during one week (30°C and humidity 75%). Different ratios and rates (discoloration or inhibition) were determined as described by Sánchez-López et al., 2005. All tests were conducted four times (averages are presented with the corresponding standard deviations).

2.4-Enzymatic activities

On solid culture media, enzymatic activities (laccase, phenoloxydase, tyrosinase and peroxidase) were obtained by the methods that were used by Atteke et al., 2013.

Laccase activity: The method described by Mishra et al. [23] has been slightly modified to characterize the presence of laccase activity on the solid medium. The solid culture medium contained 0.01% (w/v) of guaiacol (orthomethoxy phenol). It was autoclaved (at 121°C for 15 min) and 5×5 mm2 of agar containing the fungus was placed at the centre of a Petri dish after cooling. After a few days, a red coloration appeared around the colony, indicating the presence of laccase.

Phenoloxidase activity: As stated previously, a piece of agar with the fungus was put in a Petri dish containing the solid culture medium and 0.5% of gallic acid was added. The appearance of a brown area around the colony after 3 days indicates the presence of polyphenol oxidases [24].

Peroxidase activity: Peroxidase activity was evaluated by the method described by Pointing [24] using ABTS (0.1%) and 0.5% H_2O_2.

3-Resultats and discussion

3.1a- Optimal growth temperature of *Perreniporia tephropora*

To determine the optimum growth temperature of *Perreniporia tephropora*, the fungus was set to grow on solid medium, at different temperatures (15°C to 45°C) with a standard pH for six days. A fungal growth (mycelium) is observed at temperatures ranging from 22-40 °C from the third day. For the same temperatures (22-40°C), growth will increase continuously until about 8.4 cm on the sixth day. For the temperatures of 15°C and 45°C on the sixth day , a small increase in growth (approximately 2cm) compared to other temperatures was found. The mycelia growth is very high between 30 and 40°C on the fifth day (about 7.3 cm of colony growth) and sixth day (about 8.4 cm of colony growth). The optimum growth temperature of *Perreniporia tephropora* can be between 30°C and 35°C the sixth day on solid culture medium (Figure 1) . This range of temperatures is similar to that we determined for the optimal growth of *Pycnoporus sanguineus* was 35°C [20] .

3.2- Optimization of dyes decolourization

. The incubation of the fungus (*Perreniporia tephropora*) in the solid culture medium containing each one dye (RB4, OG or MO), shows a very high discoloration temperatures of 30 °C and 35 °C. At 30 ° C, discoloration is 100% for RB4, OG for 96% and 91% for MO. At 35 °C, 93%, 90% and 88% are the percentages of discoloration dyes, respectively for RB4, OG and MO. The low percentages of discoloration were observed at temperatures of 15 and 45 °C. 30 °C corresponds to the temperature at which the dye or some, of the percentage discoloration is highest. It can be inferred that 30 ° C may be the optimal temperature for discoloration dyes.

In the presence of the fungus, the discoloration is very high; it is almost 100% to the lowest concentrations, 0.05 g / L, 0.1 g / L and 0.2 g / L with different dyes. At the next concentration of 0.3 g / L, the discoloration remains high, over 90% for the three dyes still before decreasing the highest concentration (0.5 g / L, 1.5 g / L). Concentration 0.3 g / L can be considered optimal concentration of discoloration of the three dyes with *Perreniporia tephropora*. It may be observed with concentrations above 0.3 g / L, the efficiency of the discoloration dye decreases by this fungus. In the literature the concentrations usually used is below 0.3g / L [28, 29].

3.3-Decolourization experiments

In Figure 4, the comparison of images of the Petri dishes corresponding to controls (no fungus) with those prepared under the same conditions with fungus, clearly shows the efficiency of this strain on the discoloration of the three dyes (MO, OG and RB4 respectively) at a concentration of dye 0.3 g / L. In 5 days, the growth of mycelium has completely covered the solid culture medium, with areas of decolourization.

We also tested the relative ability of discoloration and growth on solid culture medium containing 0.3 g / L of each dye (OG, OM or RB4) (Table 2) fungi *Phanerochaete chrysosporium* and *Perreniporia tephropora* which is used as control. Both fungi grow rapidly on solid support in the presence of each dye. However, *Perreniporia tephropora* seems more effective to discolor dyes compare to *Phanerochaete chrysosporium*. One can also note a positive correlation between radial growth and the rate of decolouration especially regarding *Perreniporia tephropora*.

As observed, the dyes used in this study are discoloured by the fungus *Perreniporia tephropora*. The efficacy of the growth and the efficiency of the decolourization of the fungus tested (determined based on its capacity discoloration) in a solid medium are presented in Table 2. The rate of decolourization of Methyl Orange is greater than that of the OG and RB4. Furthermore, the inhibition rates are very low in the presence of different dyes (3.7% OG, 6.1% to 8.6% and MO for RB4).

These two fungi produce different ligninolytic enzyme activities on solid culture medium (Table 3). As generally described in the literature, *Phanerochaete chrysosporium* [25] does not produce laccase, unlike *Perreniporia tephropora*. The highest ligninolytic activities such as laccase, peroxidase and phenoloxidase produced by *Perreniporia tephropora* as seen in Figure 5, indicates a high enzymatic activity and thus a high ability to degrade dyes and xenobiotics.

In this study, the influence of different concentrations of dyes and discoloration temperature was studied to evaluate the optimum conditions for maximum decolourization at pH 4.8. 0.3g / L and 30 °C were considered optimal conditions decolourization. It is in these conditions, that a high level of ligninolytic activities (Figure 5) has been shown as discoloration of three dyes tested.

For some other fungi, they are capable of degrading dyes and may contain ligninolytic enzymes. It was shown that *Hexagonia apiaria* and *Pycnoporus sanguineus* had peroxidase and laccase activities when grown on solid and liquid media and removed of textile dyes effluent [20; 21]. The enzyme activities of *Perreniporia tephropora* were evaluated by us.

In the present study, dyes might be degraded by the production of extracellular enzymes as well as adsorption of dyes by the mycelium of *Perreniporia tephropora* during a growth in culture medium containing each dye. We observed low coloration of the mycelium in the presence of RB4 and MO indicating the possible adsorption. Decolourization of dye is related to the process of extracellular oxidases, particularly Laccases and peroxidases (MnP and LiP), all of which are involved in lignin degradation, have been reported to decolourize dyes. In the present study, the degradation and decolourization of different dyes appeared to be due to the production of extracellular enzymes by this fungus in the dye containing medium and adsorption process. It is quite clear that the change in colour might be due to the biochemical (metabolic) reactions of *Perreniporia tephropora* .

Our fungus (*Perreniporia tephropora*) is a candidate with a potential on the degradation and decolourization of synthetic dyes polluting industrial effluents discharged into nature. Its ability to degrade a wide range many industrial dyes and biotechnological processes molecules should be tested to increase the number of strains tendered in bioremediation.

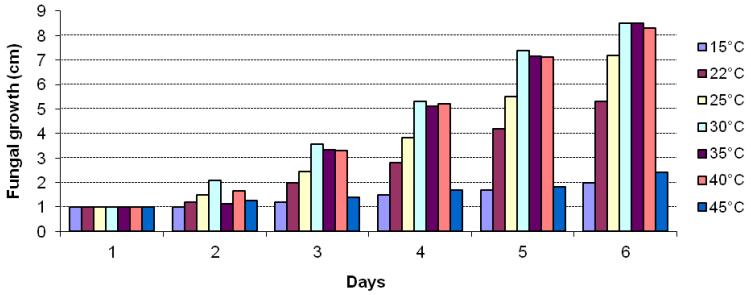


Figure 1: Effect of temperature on growth of *Perreniporia tephropora* during a week

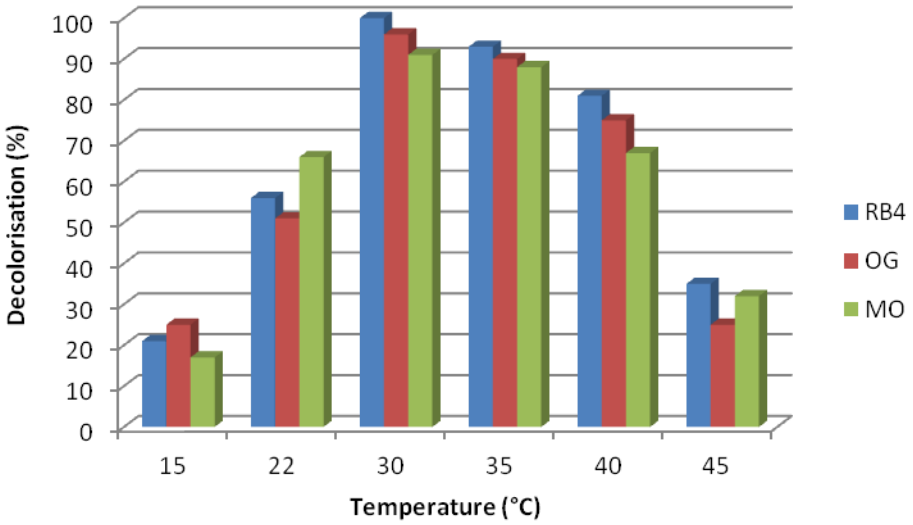


Figure 2: Decolourization of synthetic dyes by *Perreniporia tephropora* at different temperatures on solid culture medium during six day. The averages are on four tests.

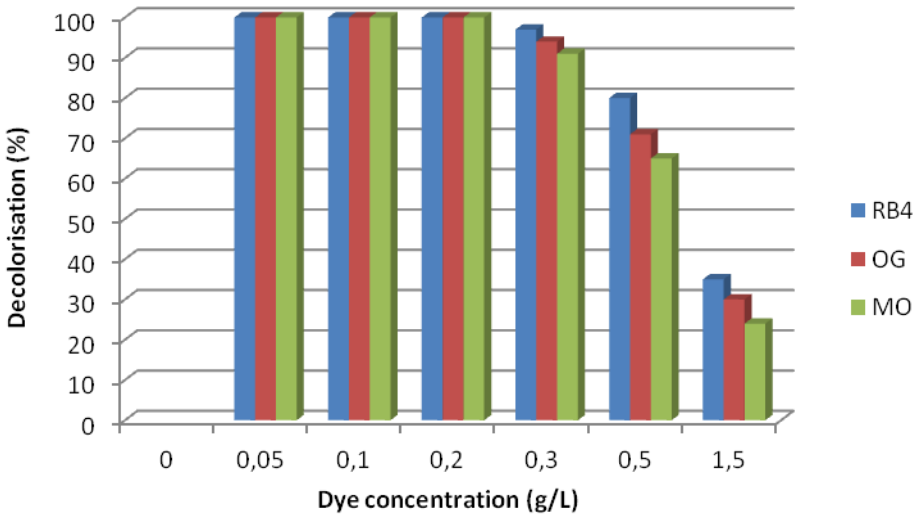
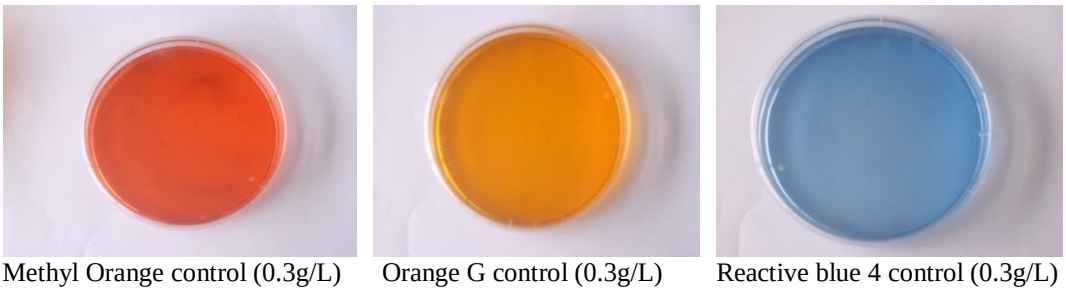
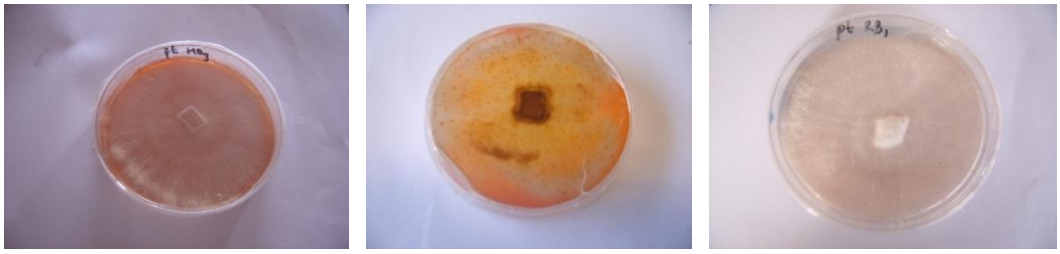


Figure 3: Decolourization of synthetic dyes by *Perreniporia tephropora* at different concentrations on solid culture medium during six days. The averages are on four tests.



Methyl Orange control (0.3g/L) | Orange G control (0.3g/L) | Reactive blue 4 control (0.3g/L)



Methyl Orange decolourization on solid medium | Orange G decolourization on solid medium | Reactive blue 4 decolourization on solid medium

Figure 4: Screening of dyes degrading by *Perreniporia tephropora*

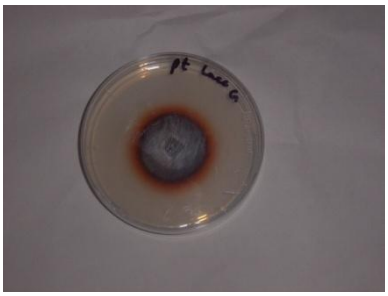
Table 1: Fungal growth on solid medium containing dyes after six days

Fungal species	Orange G		Methyl Orange		Reactive Blue 4	
	Decolourization scale [a]	Radial growth[b]	Decolourization scale [a]	Radial growth[b]	Decolourization scale [a]	Radial growth[b]
Perreniporia tephropora	+++	+++++	+++	+++++	+++	+++++
Phanerochaete chrysosporium	+	+++++	+	+++++	+	+++++

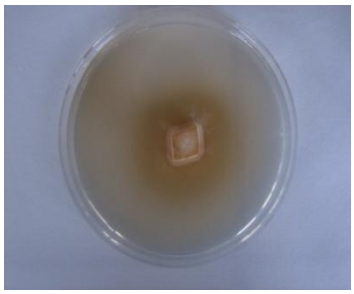
[a]Decolourization scale measured on the 6th day of cultivation on solid culture medium containing 0.3g/L of each dye: + diameter of the discoloured zone 0–20 mm, ++ zone diameter 21–30 mm, +++ zone diameter 31–50 mm, ++++ zone diameter, 51–70 mm, +++++ zone diameter 71–90 mm.
[b]Radial growth rate measured on the 6th day of cultivation on solid culture medium containing 0.3g/L of each dye: + diameter of the mycelia colony 0–20 mm, ++ colony diameter 21–30 mm, +++ colony diameter 31–50 mm, ++++ colony diameter 51–70 mm, +++++ colony diameter 71–90 mm.

Table 2: Decolourization of dyes (0.3g/L) on solid medium by white rot fungus after six days

Fungus	Orange G			Methyl Orange			Reactive blue 4		
	Decolourization rate (cm/jour)	Inhibition rate (%)	Growth rate (cm/jour)	Decolourization rate (cm/jour)	Inhibition rate (%)	Growth rate (cm/jour)	Decolourization rate (cm/jour)	Inhibition rate (%)	Growth rate (cm/jour)
Perreniporia tephropora	0.64	3.7	1.56	0.72	6.1	1.52	0.68	8.6	1.48



Observation of red colour around a colony with guaiacol indicates laccase activity



Observation of the brown zone around a colony indicates phenoloxidase activity

Figure 5: Screening for ligninolytic enzymes of *Perreniporia tephropora* on solid media

Tableau 3: Qualitative enzymes production of fungi

Fungal species	Family	Code	Laccase (Guaiacol)	Phenoloxydase (Gallic acid)	Laccase (ABTS)	Peroxydase	Tyrosinase
Perreniporia tephropora	Polyporaceae	47500	++++	++++	++++	++	+++
Phanerochaete chrysosporium	Corticiaceae	MARD 78	-	+++	-	+++	-

4-Conclusion

Our results show that dyes (MO, OG and RB4) are decoloured quickly by fungus. *Perreniporia tephropora* has a good potential for the dye decolourization than strains such as *Phanerochaete chrysosporium*. The optimum parameters of the temperature and the concentration of the dyes were determined at 30°C and 0.3 g/L respectively

for the three dyes. These conditions were then used for further experiments such as the degradation reactions of dyes by ligninolytic enzymes systems (laccase, peroxidase and phenoloxidases). A dye concentration increasing from 0.05 to 0.3g / L, for the three dyes, the decolourization is greater than 90%. This fungus grows very well on solid culture medium at an optimal temperature of 35°C, but the better temperature of dyes decolourization is 30°C.
The fungus *Perreniporia tephropora* appear to be promising in terms of their biotechnological application.

5- Acknowledgements

Mounguengui Steeve, on behalf of the other authors, is grateful to the International Tropical Timber Organization (ITTO) for the financial assistance (award of Research Fellowship N°003/10A). The authors would like to express their sincere thanks to Dr Cony Decock (Université Catholique de Louvain, Belgium) for sending of the fungus.

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