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Synthetic dyes decolourisation by *white-rot fungi*: Development of original microtitre plate method and screening

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Abstract

A microtitre plate-based method was developed for a fast screening of numerous fungal strains for their ability to decolourise textile dyes. In 3 days, this method allowed to estimate significant fungal decolourisation capability by measuring the absorbance decrease on up to ten dyes. More than 325 *white-rot fungi* (WRF) strains belonging to 76 fungal genera were compared with regards to their capability to decolourise five azo and two anthraquinone dyes as well as the dyes mixture. The most recalcitrant dyes belonged to the azo group. Several new species unstudied in the bioremediation field were found to be able to efficiently decolourise all the dyes tested.

Keywords: *White-rot fungi*; Decolourisation; Dyes; Azo; Anthraquinone; Screening; Microtitre plate

1. Introduction

The use of *white-rot fungi* and/or their extracellular enzymes are currently a promising solution as a treatment or as part of a multi-steps treatment of synthetic dyes containing wastewater [1–3].

Phanerochaete chrysosporium was first identified to degrade polymeric synthetic dyes [4]. The range of the dyes decolourised by *P. chrysosporium* was later extended to crystal violet [5] as well as to azo and heterocyclic dyes [6]. However, up to now most works have been performed with only a few different species such as *P. chrysosporium* [7–11], *Bjerkandera* sp. [11,12], *B. adusta* [8], *Trametes versicolor* [9–11,13], and *Pleurotus ostreatus* [9,10,14].

Nevertheless, it has been shown that the *P. chrysosporium* model fungus was not the best decolouriser species [9,15]. Eleven structurally different dyes were tested with regards to their decolourisation by seven isolates of wood-rotting fungi. All dyes were decolourised, at least, by one strain while *P. chrysosporium* was among the least effective of the isolates [9]. Recently 115 fungi from different physio-ecological groups were compared for their capability to decolourise two structurally different dyes (anthraquinone and azo) [15]. It appeared in this work that several strains (*Bjerkandera fumosa*, *Fomitopsis pinicola*, *Geotrichum* sp., *Kuehneromyces mutabilis*, *Phlebia radiata*, *Stropharia rugoso-annulata* and *Tolypocladium* sp.) had a better potential for dyes decolourisation than *P. chrysosporium*, *T. versicolor*, and *B. adusta*.

Previous *white-rot fungi* screening based on the ligninolytic properties have been carried out. De Jong et al. [16] compared the degradation of a polymeric dye (PolyR-478) by 127 strains to estimate the peroxidative activity: the correlation between the polymeric dye decolourisation and the peroxidase activity was pointed out. Pelaez et al. [17] also studied the enzymatic properties of 90 *white-rot fungi* strains.

These studies showed the usefulness to test the capabilities of a wide range of *white-rot fungi* species from various ecosystems in the effort to find more efficient decolourising strains. However the differences in the culture-medium compositions, type and concentration of the dyes, as well as decolourisation measurement render the strains efficiency comparison among those studies impossible. There is an interest to make a large scale screening of decolourising fungi in identical conditions.

The identification of good dyes decolourising species requires a screening method based on the direct measurement of substrate transformation such as colour removal [18]. Since industrial effluents contain a range of different dyes, it would be necessary to perform the screening in presence of several dyes [11]. Useful species should be able to degrade most of those

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dyes as well as a mixture of different ones. In addition, most of those previous laboratory studies used defined culture conditions (limited carbon or nitrogen concentrations), nevertheless, wastewater (and wastewater treatment culture batches) could have a more complex and variable composition. It would be, therefore, useful to find efficient fungal strains less dependent on the culture medium composition.

Previous Petri-dishes-screenings were performed in semi-solid agar medium while industrial wastewaters are liquid by nature: the difference in water activity, oxygenation level could have a non-negligible impact on strains performances.

Therefore we developed a simple miniaturized screening method using microtitre plate that allows to test the decolourisation of a significant number of dyes (up to ten), using a non-defined liquid culture medium such as malt-extract and a reduced incubation time and space.

The fungal strains tested in this work were provided by the "Mycothèque de l'UCL" (MUCL). MUCL belongs to the Belgian Coordinated Collections of Microorganisms (BCCM^{TM}/MUCL). It is hosted in our laboratory and constitutes a wide collection of alive fungi. In this study, 325 *white-rot fungi* strains belonging to 76 fungal genera and 230 species were screened for decolourisation phenotype.

The dyes considered in this study were obtained from an industrial partner confronted with the problem of dye contaminated wastewater treatment; dyes were selected according to their abundance in the textile industry and their high toxicity for environment as well as their recalcitrance to treatment.

2. Materials and methods

2.1. Strains culture

White-rot fungi strains (Table 1) were obtained from the BCCM^{TM}/MUCL collection (Belgium) where they are conserved under cryopreservation (−180 °C). Strains regrowth were performed on 2% malt-extract (Duchefa, Haarlem, The Netherlands) agar medium (MA2) at 25 °C. They were then cultivated on the same medium during 7 days at 25 °C and immediately used for microtiterplate inoculation. Strains (MG) were collected on dead wood from French Guyana tropical forest. These "MG" strains still unidentified were maintained on MA2 medium until being studied in this screening in the same conditions than other MUCL strains.

2.2. Dyes

The dyes used were obtained from the Yorkshire Europe (Tertre, Belgium). Their chemical structures are illustrated in Table 2. They are identified with the following code: NY1, NY7, NY8, IN13 and IN22 which are azo dyes; and NY3 and NY5 which are anthraquinone dyes. Homogeneous mixtures of these seven dyes (Mix 7) or of six dyes (Mix 6: all dyes without NY8) were also tested. Dyes were solubilized in 2% malt-extract liquid medium (ML2) to the concentration of 0.125 g/l (125 ppm). Dyes mixtures (Mix7 and Mix6) were obtained by mixing equal quantities of the different single-dye solutions: final dye concentration of the mixtures was 125 ppm; individual dye concentration was (125/7) ppm and (125/6) ppm for Mix 7 and Mix 6, respectively. Mix7 was usually used except when specified otherwise elsewhere.

2.3. Microtitre plates preparation and inoculation

Decolourisation measurements were carried out in sterile 96 (8 × 12) flat-bottom wells polystyrene microtitre plates (Greiner Labortechnik, Frick-

Table 1
White-rot fungi species studied

Genus	Species	MUCL[a] number	DC_{540} (%)	Devst (%)
Acanthophysium	***bisporum***	**32213**	**58.08**	**23.0**
	cerussatum	32645	12.56	7.3
	lividocaeratum	33688	14.30	4.8
Aleurobotrys	***botryosus***	**32323**	**50.05**	**14.2**
Aleurodiscus	*aurantis*	33921	18.41	13.8
	gabonicus	32433	37.51	25.6
	wakefieldiae	34807	19.64	3.9
Amphinema	*byssoides*	32977	46.30	26.1
Amylostereum	*areolatum*	32874	35.78	25.4
	challetti	32912	44.24	25.0
	laevigatum	33857	10.76	6.8
Armillaria	*gallica*	31339	25.88	19.5
Aspergilus	*niger*	19001	20.42	23.8
Asterostroma	*cervicolor*	38354	33.56	25.8
	laxum	38356	20.83	14.8
	ochroleucum	38358	31.00	24.8
Auricularia	*auricula*	38073	43.75	24.3
	cornea	28966	28.88	15.1
	fuscoscuccinea	28965	21.61	10.9
	polytricha	38067	35.02	22.0
Botryobasidium	*candicans*	33808	18.67	4.6
	sphaericosporum	32749	25.85	13.2
Botryohypochnus	*isabellinus*	33809	21.13	11.3
Calocera	*viscosa*	31690	29.96	22.2
Calumnocystis	*abietina*	33928	35.49	26.1
Chaetomium	*brasiliense*	19261	28.23	11.5
	foecundissimum	4060	7.39	5.6
	globusum var. griseum	39527	20.48	5.3
	pachypodiodes	9586	22.89	14.8
Collybia	*peronata*	20939	29.15	23.2
	reinakeana	38064	46.82	27.7
Coriolopsis	***polyzona***	**38443**	**62.46**	**24.0**
Corticium	*meridioroseum*	34729	23.16	8.4
Cystostereum	***murraï***	**33747**	**61.64**	**21.5**
Daedalea	*quercina*	11661	43.83	16.8
Daedaleopsis	*confragosa*	29566	41.33	24.2
Dichomitus	*leucoplacus*	41472	20.08	16.8
Dichostereum	*durum*	32558	39.51	24.4
	effuscatum	33642	43.31	25.3
	granulosum	33644	33.85	29.6
	peniophoroides	32336	22.23	10.6
	sordulentum	32712	25.84	19.2
	orientale	32644	30.42	22.2
	pallescens	32640	20.18	11.2
	ramulosum	32279	14.98	10.5
	rhodosporum	32191	19.34	20.6
	sordulentum	32167	24.30	19.4
Echinodontium	*tinctorium*	1005	4.98	10.3
Fibulomyces	*septentrionalis*	34891	19.93	11.1
Fomitopsis	*rosea*	40102	17.52	8.3
Fusarium	*annulatum*	8059	30.51	24.8
	concolor	797	20.08	9.4
	incarnatum	38815	24.41	16.7
	oxysporum	1064	22.13	15.9
	reticulatum	19032	19.11	7.3