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Evaluation of some white-rot fungi for their potential to decolourise industrial dyes

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Abstract

The ligninolytic white-rot fungi, well known for their capability of breaking diverse phenolic components of lignin and lignin derivatives, have been employed for biodecolourisation of intensely coloured effluents and conventional dyes, but the studies are concentrated on *Phanerochaete chrysosporium* and *Trametes versicolor*. Present study elucidates the role of some lesser studied white-rot fungi in biodecolourisation of industrial dyes. *Dichomitus squalens*, *Daedalea flavida*, *Irpex flavus* and *Polyporus sanguineus* were tested for their potential to decolourise various chromophoric groups of eight dyes, employed in different industries. The fungal-based biocleaning systems have been suffering from drawback of adsorption, thus, in order to overcome this limitation, the cell free enzyme extracts obtained from fungal cultures have been used. *D. squalens* and *I. flavus* were found to be competitive industrial dye decolourisers in comparison to much studied white-rot fungus *P. chrysosporium*.

Keywords: Biodegradation; Industrial dyes; Cell free enzyme extract; Wastewater treatment; White-rot fungi

1. Introduction

The synthetic dyes are widely used for textile dying and other industrial applications. The structural diversity of dyes is attributed to the presence of different chromophoric groups like azo, triphenylmethane and phthalocyanine [1,2]. Such synthetic dyes are released into the environment from textile and various other dyestuff industries in very large quantities on daily basis. The discharge of these coloured effluents into rivers and lakes results into reduced dissolved oxygen concentration, thus creating anoxic conditions and hence killing aerobic organisms. The existing wastewater treatment systems or natural micro flora are unable to completely remove recalcitrant dyes from such effluents [3,4]. Since these dyes are designed to resist degradation, it is not surprising that dye degradation occurs only to a limited extent in activated sludge systems [5]. A few studies reported the dye sorption by white-rot fungal mycelia without real degradation [6], the phenomenon otherwise is scarcely observed in growing cultures. These effects have been seen in non-white-rot fungi, such as *Aspergillus niger*, whose (dead) biomass could be used as an adsorbent [7]. Similarly *Spirogyra* sp. and *Candida tropicalis* (a wild yeast) are found to be good biosorbing/bioacumulating agents for various reactive (azo) dyes [8,9]. Thus it suggests the possibility of using these organisms as part of technical solution in water pollution control. However, disposal of this microbial biomass containing adsorbed dyes itself is a big hurdle in their proposed role in biocleaning of coloured waters. A number of other biotechnological approaches have been suggested, including the use of bacteria or fungi, often in combination with physicochemical processes, for the treatment of such dyes [1,10–14]. Anaerobic degradation of such dyes by bacteria has been reported to be producing carcinogenic and/or mutagenic products [15]. Recently, white-rot fungi and their enzymes have been reported to reduce the toxicity of

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anthraquinonic and triphenylmethane dyes [5,16]. The studies highlight the role of purified laccases in detoxification of malachite green and remazol brilliant blue R. Further the bacterial degradation of these dyes requires their intracellular uptake; whereas fungal systems, especially the white-rot, completely degrade by their extracellular enzymes which are capable of breaking a variety of heterogeneous molecular bonds [16,17]. The better effectivity of fungi for bioremediation of dye-polluted sites is attributed to the penetration ability of their hyphae [18] and their capability to degrade a wide variety of toxic compounds [19]. This potential of ligninolytic fungi could prove useful in biocleaning the industrial dyes and related wastes [20,21]. A little work has been done in the field of fungal degradation of these dyes [22,23]. Previous studies in our lab have shown *Dichomitus squalens*, *Irpex flavus* and *Phlebia* spp. to be better decolourisers of laboratory dyes than the much studied *Phanerochaete chrysosporium* [4,24]. The present study consolidates the role of these white-rot fungi in decolourisation of substitutive industrial dyes at relatively higher concentration, which are preferred for their economy and finishing properties.

2. Experimental

2.1. Material

The industrial dyes disperse, reactive and acid complex were received from Colourtex India Limited (Mumbai, India), Ornet Intermediates Limited (Vatva, Ahmedabad, India) and Rathi Dye Chem Limited (Pune, India), respectively, while rest of the chemicals were procured from Hi Media Chemicals (Mumbai, India).

2.1.1. Microorganisms

Daedalea flavida MTCC 145 and *Polyporus sanguineus* MTCC 137 were obtained from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. *P. chrysosporium* BKM-F-1767 was received as a gift from Prof. T.W. Jeffries, Institute of Microbial and Biochemical Technology, United States Department of Agriculture, Madison USA. *D. squalens* FP-70880-sp was received as gift from Dr. Rita Rentmeester, Forest Products Laboratory, Madison, WI, USA. All the cultures were maintained at 4 °C on yeast glucose agar medium.

2.1.2. Media

The compositions (per litre of distilled water) of the different media used were the following:

Mineral salts broth (MSB): KH_2PO_4 2 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, glucose 10 g, ammonium tartrate 0.2 g, thiamine hydrochloride 10 mg and trace elements solution 10 ml.

Trace elements solution: nitrilotriacetic acid 1.5 g, $MnSO_4 \cdot H_2O$ 0.48 g, NaCl 1 g, $CoSO_4 \cdot 7H_2O$ 10 mg, $FeSO_4 \cdot 7H_2O$ 10 mg, $ZnSO_4 \cdot 7H_2O$ 10 mg, $CuSO_4 \cdot 5H_2O$ 8 mg, H_3BO_3 8 mg and $Na_2MoO_4 \cdot 2H_2O$ 8 mg.

Yeast glucose agar (YGA): glucose 10 g, yeast extract 5 g, peptone 5 g and agar 15 g.

2.2. Methods

2.2.1. Dye decolourisation in nitrogen limited broth medium

The fungi were grown on mineral salts broth. Twenty-five millilitres of MSB taken in a 100-ml flask was autoclaved at 15 psi for 15 min and inoculated with two fungal discs (8 mm diameter) obtained from 6 to 8 days grown cultures on YGA plates and incubated on rotary shaker (200 rpm) at their optimum growth temperature. The cell free enzyme extracts (CFEE) were obtained from fungal cultures grown for different periods viz. 4, 6, 8, 10, 12 and 14 days. The cultures were filtered through Whatman filter paper no. 1, centrifuged (10,000 rpm, 4 °C) and used for dye decolourisation studies. Twenty millilitres of this CFEE obtained from each fungus was taken in sterilized 100 ml conical flasks. To these extracts, filter sterilized stocks of different dyes were added so as to get their final concentration of 30 mg/l. The reaction mixture was incubated at the optimum growth temperature of respective fungi on rotary shaker at 200 rpm. To monitor the decolourisation of dye, duplicate samples from each flask were removed after a period of 0, 1, 2, 3, 5, 24 and 48 h and absorbance was recorded at their respective λ_{max} as follows:

$$\%\ \text{age decolourisation} = \frac{A - B}{A} \times 100$$

where A = initial absorbance at zero hour and B = final absorbance after reaction with CFEE.

2.2.2. Effect of pH on dye decolourisation

To evaluate the effect of pH change on dye decolourisation, which might have occurred during fungal growth, the dyes were incubated in MSB, adjusted to different pH (3–7) without any fungal cultures. However, the dyes showed no change in their absorbance in these abiotic controls. Hence the reported dye decolourisation caused by various fungi is purely biotic and not merely a physical or chemical phenomenon.

3. Results

3.1. Dye decolourisation by fungi

Cell free enzyme extracts obtained at different intervals, when incubated with various dyes resulted in 2–100% of colour loss during 1–48 h of incubation, a greater part of which occurred during first 2–5 h. No further decolourisation occurred during prolonged period of incubation, except in a few cases.

3.1.1. Decolourisation of various industrial dyes by P. chrysosporium

3.1.1.1. Decolourisation of coracryl dyes. The CFEE obtained during different periods of growth of *P. chrysosporium* decolourised the different dyes to variable extent which was

maximum by CFEE obtained on 6th day (Table 1), where the maximum colour loss was recorded in first 2–5 h. However, a moderate to low decolourisation was observed during remaining period of incubation of dyes with CFEE.

For coracryl dyes, a build up in dye decolourisation potential was observed during 4th to 6th day and thereafter the activity declined. Coracryl pink and coracryl violet underwent a 100% decolourisation just in 3–5 h of incubation with CFEE obtained from 6th day grown culture while in coracryl black and coracryl red 75.9 and 63.2% decolourisation occurred in 24 and 5 h, respectively (Table 1).

3.1.1.2. Decolourisation of reactive dyes. The cell free extract obtained from 4 day grown culture decolourised only 6.4% of reactive yellow in 3 h, which increased to 76.3% on 6th day during similar reaction period and thereafter decolourisation potential decreased with increase in fungal growth period. Reactive orange was decolourised to a moderate level of 57.3% in 5 h of reaction when incubated with CFEE of *P. chrysosporium* obtained on 4th day, which increased to 71.8% on 6th day. Thereafter, no decolourisation was observed except on 12th day (7.3% in 3 h). Reactive red was maximally decolourised by using 8th day CFEE, 44.3% in 3 h (Table 1).

3.1.1.3. Decolourisation of rathidol scarlet dye. The 4th day CFEE of *P. chrysosporium* caused a colour loss of 53.6% which increased to a maximum of 76.6% on 6th day (Table 1), followed by a steep decline to 2.8% on 10th day. A slight rise (16.6%) in decolourisation was noticed on 12th day (Table 1).

3.2. Decolourisation of various industrial dyes by D. flavida

The cell free enzyme extracts obtained after different periods of growth of *D. flavida* decolourised the dyes to a moderate level. In most of the cases, the 6th day CFEE caused maximum decolourisation in 3 h. No further colour loss was observed upon prolonged incubation.

3.2.1. Decolourisation of coracryl dyes

The cell free enzyme extracts obtained on 6th day decolourised all the coracryl dyes moderately in 3 h, except coracryl pink, which suffered a colour loss of 52.9% in 5 h (Table 2).

3.2.2. Decolourisation of reactive and rathidol scarlet dyes

The reactive dyes were relatively resistant to decolourisation even up to 48 h of reaction. Reactive red suffered a maximum decolourisation by CFEE of 6th day (39.8% in 3 h). However, reactive orange and reactive yellow, were decolourised only to a low level of 4.8 and 11.7% in 3 h followed by rathidol scarlet (38.9% in 3 h) (Table 2).

3.3. Decolourisation of various industrial dyes by D. squalens

The CFEE obtained during different periods of growth of *D. squalens*, caused colour loss to a variable extent 3.3–100% in 2–3 h of reaction. The 6 day old CFEE caused maximum decolourisation wherein, 50–100% colour loss was observed in first 2 h of reaction.

3.3.1. Decolourisation of coracryl dyes

When incubated with 6th day CFEE, all the coracryl dyes suffered a moderate to high colour loss in just 2 h of incubation wherein, coracryl pink was 100% decolourised. The remaining dyes were 45–76% decolourised in 1 h of reaction (Table 3).

3.3.2. Decolourisation of reactive and rathidol scarlet dyes

The CFEE obtained from 6th day grown cultures appreciably decolourised reactive dyes while removing 76 and 79.9% colour of reactive red and reactive yellow in 1 and 2 h, respectively, except reactive orange which was maximally decolourised (60.2% in 3 h) by 4 day old CFEE (Table 3). The 6th day CFEE efficiently decolourised rathidol scarlet while removing 79% of initial colour in 3 h. Rathidol scarlet was maximally decolourised on 6th day (79% in 3 h).

3.4. Decolourisation of various industrial dyes by I. flavus

3.4.1. Decolourisation of coracryl dyes

The 6th day CFEE of *I. flavus* maximally decolourised coracryl dyes (50.2–100%), just in 2–5 h of reaction. On 4th day, relatively less colour loss was observed. Coracryl pink was

Table 1
Decolourisation of industrial dyes by *P. chrysosporium*

Dyes	Percent dye decolourisation at different periods of fungal growth					
	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Coracryl black	12.6 (3)	75.9 (24)	–	–	21.5 (24)	4 (3)
Coracryl pink	1.4 (2)	100 (3)	1.2 (24)	4.7 (3)	23.8 (5)	6.7 (3)
Coracryl violet	6.6 (3)	100 (5)	51.4 (24)	–	14.8 (5)	–
Coracryl red	–	63.2 (5)	–	–	37 (48)	–
Reactive yellow	6.4 (3)	76.3 (3)	55.3 (5)	–	21.3 (3)	3 (3)
Reactive orange	57.3 (5)	71.8 (5)	–	–	7.3 (3)	–
Reactive red	24 (2)	–	44.3 (3)	–	14.6 (5)	–
Rathidol scarlet	53.6 (5)	76.6 (3)	4.9 (2)	2.8 (2)	16.6 (5)	10.4 (5)

Figures in bracket show the time taken by dye to reach the maximum decolourisation with respective CFEE. (–) No decolourisation even up to 48 h.

Table 2
Decolourisation of industrial dyes by *D. flavida*

Dyes	Percent dye decolourisation at different periods of fungal growth					
	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Coracryl black	–	13.9 (3)	–	–	12 (5)	–
Coracryl pink	0.5 (1)	52.9 (5)	–	–	8 (3)	3 (5)
Coracryl violet	–	26 (3)	19.1 (5)	–	5.1 (3)	–
Coracryl red	–	35.2 (3)	–	–	13.7 (3)	–
Reactive yellow	1 (2)	11.7 (3)	12.1 (3)	–	6.2 (3)	–
Reactive orange	4.7 (5)	4.8 (3)	–	–	6.6 (5)	–
Reactive red	1.8 (2)	39.8 (3)	36.5 (5)	6.7 (3)	15.7 (3)	2.4 (3)
Rathidol scarlet	–	38.9 (3)	2.6 (3)	–	4.6 (3)	11.4 (5)

Figures in bracket show the time taken by dye to reach the maximum decolourisation with respective CFEE. (–) No decolourisation even up to 48 h.

100% decolourised in 3 h while, the remaining dyes suffered a colour loss varying from 50 to 67% in 2–5 h (Table 4).

3.4.2. Decolourisation of reactive and rathidol scarlet dyes

When 6 day old CFEE were tested against reactive and rathidol dyes, a variable decolourisation activity was observed. A moderate to good decolourisation activity was observed for reactive orange and reactive yellow in CFEE of 4th and 8th day, where colour loss of 61 and 58.5% was observed in 2–3 h of reaction, respectively (Table 4). Rathidol scarlet was maximally decolourised (74% in 2 h) by 6th day cell free enzyme extract (Table 4).

3.5. Decolourisation of various industrial dyes by P. sanguineus

3.5.1. Decolourisation of coracryl dyes

The 6th day CFEE obtained from *P. sanguineus* decolourised all the coracryl dyes 36–67% in 3–5 h, except coracryl violet, which was decolourised up to 51% in 24 h by 8th day CFEE (Table 5).

3.5.2. Decolourisation of reactive and rathidol scarlet dyes

Reactive dyes were moderately decolourised by CFEE obtained on different growth periods wherein a variable colour loss of 18.8–59.2% was recorded in 3–5 h of reaction. Reactive red was maximally decolourised by 6th day CFEE wherein, it suffered a colour loss of 59.2% in 5 h. During similar period of reaction, reactive yellow lost 23.4% of its initial colour using 8th day CFEE. However, the extracts obtained on 12th day, removed 18.8 and 38.3% colour of reactive orange and rathidol scarlet in 3 h, respectively (Table 5).

4. Discussion

The release of dye-based effluents into the water bodies is only a small proportion of water pollution, but as a result of their chromophoric groups, these dyes are visible even if they are present in a small quantity. The decolourisation of these wastewaters has acquired immense importance as they pose serious threat to mankind due to their mutagenic, carcinogenic and poisonous nature. There is no simple solution for this problem because the conventional physicochemical methods either are costly or are only partially competent in treating these wastes [25]. The treatment system based on fungi, especially the white-rot, have not been applied extensively due to many factors such as, high costs, longer fungal growth periods [3] and an early inactivation of enzymes [26]. Further, the use of alternate biological means e.g. dead or living mycelia for dye biosorption, itself face disposal problem [27]. It may create more severe pollution problem in terms of their safe disposal of fungal biomass. The aim of this study is to ascertain the role of relatively little explored ligninolytic white-rot fungi and their cell free enzyme extracts in biocleaning of industrial dyes. The results obtained show the differential susceptibility of dyes to fungal decolourisation. In contrast to previous study [28] where the acidic dyes were found to be inhibiting the decolourising ability of *P. chrysosporium*, were

Table 3
Decolourisation of industrial dyes by *D. squalens*

Dyes	Percent dye decolourisation at different periods of fungal growth					
	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Coracryl black	21.7 (3)	69 (1)	–	14.9 (2)	30 (3)	–
Coracryl pink	4.7 (5)	100 (2)	–	–	35 (5)	24 (3)
Coracryl violet	7.7 (5)	76 (1)	37.5 (3)	–	19 (5)	–
Coracryl red	3.3 (3)	45 (1)	–	–	40 (3)	–
Reactive yellow	27.9 (3)	79.9 (2)	55.9 (3)	–	22 (2)	–
Reactive orange	60.2 (3)	50.7 (1)	6.2 (5)	–	19 (3)	–
Reactive red	59.5 (2)	76 (1)	35.4 (3)	–	19 (5)	–
Rathidol scarlet	61.2 (5)	79 (3)	5.1 (1)	25.4 (2)	48 (3)	25 (2)

Figures in bracket show the time taken by dye to reach the maximum decolourisation with respective CFEE. (–) No decolourisation even up to 48 h.

Table 4
Decolourisation of industrial dyes by *I. flavus*

Dyes	Percent dye decolourisation at different periods of fungal growth					
	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Coracryl black	8.3 (5)	67.3 (2)	–	–	34 (3)	–
Coracryl pink	1.6 (2)	100 (5)	11.4 (24)	26.3 (1)	28 (5)	5 (3)
Coracryl violet	22 (5)	58 (5)	58.3 (2)	–	25 (3)	–
Coracryl red	–	50.2 (2)	–	–	23 (24)	–
Reactive yellow	22 (2)	79 (1)	58.5 (3)	–	30 (3)	–
Reactive orange	61 (2)	62.3 (2)	–	–	22 (2)	–
Reactive red	23 (2)	77 (1)	44.7 (3)	–	24 (3)	5 (2)
Rathidol scarlet	57 (3)	73.9 (2)	9.4 (1)	–	39 (2)	30 (2)

Figures in bracket show the time taken by dye to reach the maximum decolourisation with respective CFEE. (–) No decolourisation even up to 48 h.

greatly decolourised by all the fungi tested in the present study. Further the decolourisation was carried out by CFEE obtained from fungi grown for different time intervals wherein, much of the colour removal invariably occurred in the first 5 h which is in agreement with previous studies [24].

The extent of dye decolourisation is dependent on the age of the culture yielding CFEE. The extracts obtained from 6th day grown cultures were enzymatically most active to bring about the maximum decolourisation of all the dyes tested. However, the reactive and rathidol dyes underwent a reasonably sufficient decolourisation by CFEE of most of the fungi even on the 4th day in comparison to coracryl dyes (Table 6). The relatively lower decolourisation of coracryl dyes on 4th day can be attributed to their strong azo bonds. Further the side products formed by the partial degradation of dyes might have inactivated the relatively low amount of enzyme produced by that time. Similar observation was recorded by Yesilada and Ozcan [29] wherein, the residual groups released by partial decolourisation of dye tested inhibited the activity of CFEE. On the other hand the relatively lower decolourisation after 6th day can be attributed to a decline in exogenous enzyme production and accumulation of toxic metabolites formed. Slight increase in decolourisation activity during prolonged incubation of some fungi may be attributed to fungal autolysis and release of cell membrane bound or intracellular enzymes [30]. In agreement with earlier studies, CFEE obtained from different fungi could cause an effective decolourisation of all the dyes tested without the help of any inducer and mediator [17,31].

In the present study, *D. squalens* and *I. flavus* in general, have been found to be better dye decolourisers than the much studied *P. chrysosporium* (Table 6). *D. squalens* caused 100% coracryl pink decolourisation in just 2 h. However, *I. flavus* and *P. chrysosporium* caused the similar colour loss but in 3 h of incubation. The former two white-rot fungi were also better for reactive yellow in terms of time taken and percent decolourisation in comparison to *P. chrysosporium*. In case of reactive red, again similar decolourisation pattern was observed wherein 6th day CFEE of *D. squalens* and *I. flavus* caused near equal decolourisation in just 1 h of incubation. The CFEE obtained from *P. chrysosporium* grown for 8 days, however, removed only 44% of initial colour in 3 h. Though *P. chrysosporium* caused significant decolourisation of coracryl black, reactive orange and rathidol scarlet dyes, yet *D. squalens* and *I. flavus* were competitive in terms of time taken and/or causing near equal percent colour losses.

Coracryl black and red were found to be relatively resistant to decolourisation by cell free enzyme extracts obtained form various tested fungi. This could be attributed either to the strong azo bonds of these dyes or absence of chromophoric groups (like hydroxyl) on these dyes. As these groups have been found to be more susceptible to enzyme action [23], their absence may be held responsible for lower to moderate decolourisation by CFEE. Rest of the dyes were decolourised to appreciable extent by one or all of the tested fungi. Thus the ligninolytic fungal enzyme systems being efficient dye decolourisers could be used as biocleaners of dyes and dye-based effluents emanating from related industries.

Table 5
Decolourisation of industrial dyes by *P. sanguineus*

Dyes	Percent dye decolourisation at different periods of fungal growth					
	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Coracryl black	–	67 (5)	–	–	15.5 (3)	–
Coracryl pink	2 (5)	64.9 (3)	–	–	14.9 (5)	2.3 (5)
Coracryl violet	–	54 (5)	50.9 (24)	–	22.5 (3)	–
Coracryl red	2 (5)	36.5 (5)	–	–	24.8 (3)	–
Reactive yellow	–	23.4 (5)	–	–	19.2 (24)	–
Reactive orange	–	9.3 (2)	–	–	18.8 (3)	–
Reactive red	–	59.2 (5)	10.5 (3)	6.7 (3)	15 (3)	8.3 (5)
Rathidol scarlet	–	13.1 (3)	–	–	38.3 (3)	6.5 (3)

Figures in bracket show the time taken by dye to reach the maximum decolourisation with respective CFEE. (–) No decolourisation even up to 48 h.

Table 6
Comparative decolourisation of industrial dyes by CFEE obtained from 6th day grown fungal cultures

Dyes	Maximum decolourisation (%)				
	P. chrysosporium	*D. flavida*	*D. squalens*	*I. flavus*	*P. sanguineus*
Coracryl black	75.9 (24)	13.9 (3)	69 (1)	67.3 (2)	67 (5)
Coracryl pink	100 (3)	52.9 (5)	100 (2)	100 (3)	64.9 (3)
Coracryl violet	100 (5)	26 (3)	76 (1)	58 (5)	50.9 (24)[b]
Coracryl red	63.2 (5)	35.2 (3)	45 (1)	50.2 (2)	36.5 (5)
Reactive yellow	76.3 (3)	11.7 (3)	79.9 (2)	79 (1)	23.4 (5)[b]
Reactive orange	71.8 (5)	4.8 (3)	60.2 (1)[a]	63.2 (2)	19.3 (2)
Reactive red	44.3 (3)[b]	39.8 (3)	76 (1)	77 (1)	59.2 (5)
Rathidol scarlet	76.6 (3)	39 (3)	79 (3)	73.9 (2)	38.3 (3)[c]

[a] Fourth day.
[b] Eighth day.
[c] Twelfth day.

5. Conclusion

The present study reflects the inherent potential of white-rot fungi in biomanagement of industrial dyes. The lesser explored organisms *D. squalens* and *I. flavus* could emerge as alternative, time and cost effective biocleaning systems thus supporting their potential to be exploited for designing the novel biodegradation and bioremediation processes for coloured industrial effluents. The present work can pave way for providing an alternative cell free enzyme-based biocleaning system which may prove efficient enough in handling dye-based effluents without causing any harm to aquatic ecosystem. However, a lot of work is needed to explore the dye decolourisation potential of WRF at scale up and industrial levels. The study also underlines the importance of exploring the bioremediation potential of relatively little-studied microorganisms so as to expand the pool of existing biocleaning microorganisms.

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