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Microbial biodegradation of recalcitrant synthetic dyes from textile-enriched wastewater by *Fusarium oxysporum*

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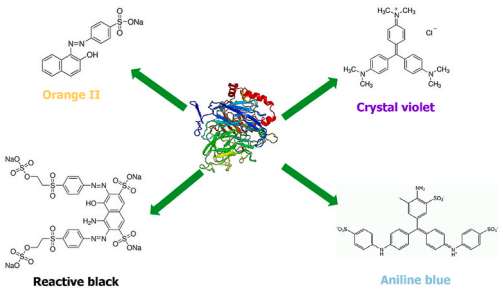
HIGHLIGHTS

- Biodegradation of recalcitrant synthetic dyes increased up to 4.46 folds.
- RBBR boosted the biodegradation up to 97.06% for aniline blue.
- 1-HBT was the best mediator for crystal violet biodegradation.
- Biodegradation method could remediate different classes of dye up to 10^4 mg/L.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study reported the improvement of biological treatment for the removal of recalcitrant dyes including aniline blue, reactive black 5, orange II, and crystal violet in contaminated water. The biodegradation efficiency of *Fusarium oxysporum* was significantly enhanced by the addition of mediators and by adjusting the biomass density and nutrient composition. A supplementation of 1% glucose in culture medium improved the biodegradation efficiency of aniline blue, reactive black 5, orange II, and crystal violet by 2.24, 1.51, 4.46, and 2.1 folds, respectively. Meanwhile, the addition of mediators to culture medium significantly increased the

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Fusarium oxysporum
Waste water treatment

percentages of total removal for aniline blue, reactive black 5, orange II, and crystal violet, reaching 86.07%, 68.29%, 76.35%, and 95.3%, respectively. Interestingly, the fungal culture supplemented with 1% remazol brilliant blue R boosted the biodegradation up to 97.06%, 89.86%, 91.38%, and 86.67% for aniline blue, reactive black 5, orange II, and crystal violet, respectively. Under optimal culture conditions, the fungal culture could degrade these synthetic dyes concentration up to 10^4 mg/L. The present study demonstrated that different recalcitrant dye types can be efficiently degraded using microorganism such as *F. oxysporum*.

1. Introduction

In recent years, the rapid industrialization and modernization has led to serious environmental pollution caused by the massive discharge of toxic wastewater containing heavy metals, radionuclides as well as various organic pollutants (Novikau and Lujaniene, 2022). The dyeing industry is one of the largest contributors to the economies of developing countries. While the colors are the main attraction of any textile, the pollutants generated from the textile and dyeing industries have become an alarming problem. The main challenges facing these industries are the indiscriminate use of synthetic dyes and the inefficiency of the dyeing process (Mahmoud et al., 2017). It is estimated that 100,000 different types of synthetic dyes and pigments are currently in use at the industrial scale, with their yearly consumption reaching 700,000 tons (Adar, 2021) and most of them being toxic and carcinogenic (Kurade et al., 2017). Approximately 10–15% of the dyes used in textiles and other industries are discharged into wastewater (Edison et al., 2016), and this leads to the widespread of water pollution that consequently affects the environment, ecology, agriculture, aquaculture and public health. Thus, cost-effective and high sustainability treatments strategies for synthetic dyes contaminated wastewater are demanded for a green economic-social development.

By the standard of the conventional wastewater treatment methods, dyes are considered to be the most difficult components to treat (Ayele et al., 2021). Several physical and chemical methods including membrane filtration, adsorption, ion exchange, ozonization, coagulation-flocculation, and oxidation have been used to treat dye-containing wastewater (Sellami et al., 2022). Recent advanced technique based on electrospun nanofibers has been proposed as promising method for both cationic and ionic dyes contaminated wastewater treatment (Huong et al., 2020; Pakalapati et al., 2020; Sarkodie et al., 2023). Meanwhile, biological treatment is considered as less expensive and environmental friendly for removal not only synthetic dyes but also other harmful organic pollutants (Yaseen and Scholz, 2019). Degradation of dyes by biological methods can be performed by biosorption, enzymatic degradation, or a combination of both (Oke and Mohan, 2022). Biosorption treatment is the process by which the contaminants are concentrated and bound to the surface structure of the cells. The biosorption of dyes by microorganisms occurs due to strong attractive forces between different functional groups such as carboxyl, hydroxyl, phosphate, amino, and other polar groups on cells membrane. Yeast, bacteria, algae and fungi have been reported to participate in biosorption for dyeing removal (Bharathi et al., 2022). Enzymatic degradation of synthetic dyes has numerous advantages such as high efficiency, less secondary contamination, less energy consumption and eco-friendly with nature (Bharathi et al., 2022). Basically, enzymes used in dyes degradation consist of oxidative enzymes including manganese peroxidases, lignin peroxidases, laccase, dye peroxidases and reductive enzymes such as azoreductases. The oxidative enzymes act by oxidizing the phenolic groups of substrates, while the reductive enzymes catalyze to the azo backbone linkage (Ngo and Tischler, 2022). Among the discovered enzymes, laccases are considered as the most effective enzyme because it catalyzes a wide range substrates, with no additional conditions required for the reaction, and it can be highly expressed in various organisms, especially in fungi (Viswanath et al., 2014).

Fusarium oxysporum is a fungus naturally found in soil (Chen et al., 2014). The genome of *F. oxysporum* consists numerous laccase-like encoding genes (Kwiatos et al., 2015). Thus, this fungus has been explored for synthetic dye degradation with promising applicability in the treatment of textile wastewater. El-Fakharany isolated a *F. oxysporum* strain having good capability for degradation of malachite green, congo red, and methyl orange with degree of 98%, 95%, and 87.6%, respectively (El-Fakharany et al., 2016). Furthermore, *F. oxysporum* has efficiently degraded over 95% of bromothymol blue, methyl orange, remazol brilliant blue R (RBBR), indigo carmine (Huy et al., 2020). The fungus also shows as promising candidate for bioremediation of blue 21, orange 16, yellow 160, blue 16, glycoconjugate azo dye (Abd El-Zaher, 2010; Porri et al., 2011). Recombinant *F. oxysporum* laccase expressed in *Pichia pastoris* exhibits degradation ability toward various synthetic dyes (Huy et al., 2021). The degradation of synthetic dyes by the fungus has also been applied into the detoxification of the dyes such as malachite green, congo red, and glycoconjugate azo dye (Abd El-Zaher, 2010; Porri et al., 2011; Thoa et al., 2022). However, some synthetic dyes strongly resist to biodegradation by fungus or laccase (Huy et al., 2020, 2021). Thus, overcoming the low degradation efficiency of these recalcitrant synthetic dyes could significantly improve the application of fungus and laccase in the treatment of textile-enriched waste water. In this present study, a biological treatment has been developed to overcome the low degradation efficiency on recalcitrant synthetic dyes including aniline blue, reactive black 5, orange II, crystal violet.

2. Materials and method

2.1. Microorganism and chemical

F. oxysporum HUIB02 used in this study was kept as a stock storing at −40 °C at Institute of Biotechnology, Hue University. The fungus was activated by culturing in petri disk containing PDA medium (20 g/L dextrose, 200 g/L potato extract, and 10 g/L agar) at 30 °C for 3 day. The synthetic dyes including triphenylmethane dye (aniline blue), azo dyes (reactive black 5, orange II) and triarylmethane dye (crystal violet) were purchased from Sigma. Laccase mediators including syringaldehyde (SA), vanillin (VA), and 1-hydroxybenzotriazole (1-HBT) were also purchased from Sigma. Chemicals used in preparation of the basal medium (BSM) are 0.2 g/L KH_2PO_4, 0.05 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $CaCl_2{\cdot}2H_2O$, 0.08 g/L $CuSO_4{\cdot}5H_2O$, 0.05 g/L $Na_2MoO_4{\cdot}2H_2O$, 0.033 g/L $MnSO_4{\cdot}H_2O$, 0.043 g/L $ZnSO_4{\cdot}7H_2O$, and 0.05 g/L $FeSO_4{\cdot}7H_2O$; all of these chemicals were of analytical or biological culture grade.

2.2. Cells biomass culture

F. oxysporum HUIB02 mycelium was obtained by inoculating this fungus strain into 50 mL PD culture media consisting of 20 g/L dextrose and 200 g/L potato extract. The culture was then incubated for 3 days at 30 °C and 180 rpm. Next, the fungal biomass was collected by centrifugation at 5000 rpm for 10 min, washed three times in distilled water and used for further investigation.

2.3. Degradation of synthetic dyes

For the experiments involving dye degradation, the fungal biomass in pre-determined weight was transferred to 50 mL of basal minimal medium with triphenylmethane dye (aniline blue), azo dyes (reactive

black 5, orange II) and triarylmethane dye (crystal violet). The culture was maintained at 30 °C and shaken at 180 rpm for 15 days. Samples were collected every three days and the mycelium was then removed by centrifugation at 5000 rpm for 10 min and the supernatant was filtered through a 0.22 μm cellulose membrane. The concentration of synthetic dyes in the supernatant was quantified spectrometrically by using specific wavelength for anilin blue (599 nm), reactive black 5 (597 nm), orange II (485 nm) and crystal violet (589 nm). The dye degradation efficiency was determined using Equation (1):

$$\text{Degradation efficiency } (\%) = \frac{\textit{Initial absorbance} - \textit{Final absorbance}}{\textit{Initial absorbance}} \times 100\% \quad (1)$$

2.4. Effect of treatment conditions

Dye degradation efficiency by *F. oxysporum* HUIB02 was evaluated under different culture conditions. To evaluate the effect of initial fungal biomass, the fungal culture was transferred to BSM medium with initial weight of 1 g, 3 g, and 5 g, respectively. Synthesis dyes were added into medium at final concentration of 10^2 mg/L. Then, the optimal initial fungal biomass was used to examine the effect of glucose supplementation at concentration of 1%, 5%, and 10%. The culture condition was performed as mentioned in Section 2.3 above. The dye removal efficiency was evaluated for every three days by spectrophotometric measurement. Meanwhile, the effects of biodegradation in the present of mediators were conducted with different mediators including SA, VA, and 1-HBT at a final concentration of 1 mM, 3 mM and 5 mM. The role of RBBR as laccase inducer was evaluated at concentrations of 0.01%, 0.03% and 0.05%; *F. oxysporum* HUIB02 was pre-cultured with RBBR for 3 days before the addition of synthetic dyes. Biodegradation processes were conducted for 15 days. Samples were collected every three days and the degradation efficiency was evaluated by spectrophotometric measurement of maximum absorbance.

2.5. Limitation of synthetic dyes removal

The optimal culture conditions including initial fungal biomass, glucose concentration and inducers were chosen to be investigated for understanding the limitation of synthetic dyes removal by *F. oxysporum* HUIB02. Synthetic dyes was added into medium culture with concentration from 10^2 to 10^4 mg/L. The culture was incubated for 15 days as mentioned in Section 2.3 above. Treatment efficiency was recorded by measuring the concentration of residual synthetic dyes in medium culture.

2.6. Data analysis

Experimental data of three replicates were statically analyzed by one-way ANOVA using Duncan's *t*-test with $p < 0.05$ by SPSS software (version 16.0).

3. Results

3.1. Effect of fungal biomass on synthetic dye degradation

Table 1 shows the efficiency of synthetic dyes degradation using different initial concentrations of fungal biomass. The ranges of dye removal efficiency are 10.89–35.52%, 9.69–34.67%, 2.87–10.98%, and 0.93–15.45% for aniline blue, reactive black 5, orange II, and crystal violet, respectively. Increasing initial fungal biomass to 5% significantly enhanced the removal of reactive black 5 and crystal violet. However, the initial fungal biomass concentration did not greatly exhibit any difference to the degradation efficiency of aniline blue. In particular, all 4 synthetic dyes removal efficiencies dramatically increased from Day 3 to Day 12 of culture period. Then, extending the culture time to Day 15 did not significantly enhance the degradation. Based on the results, initial fungal biomass of 5% was selected for further investigation.

Table 1
Effect of initial fungal biomass on synthetic dyes degradation.

Synthetic dyes	Fungal biomass (g)	Dye degradation efficiency (%)				
		Culture time (days)				
		3	6	9	12	15
Aniline blue	1	10.89^{a}	16.51^{a}	26.41^{b}	31.03^{bc}	31.45^{bc}
	3	11.73^{a}	13.04^{a}	29.55^{bc}	32.5^{bc}	33.56^{c}
	5	11.77^{a}	16.22^{a}	32.83^{bc}	33.06^{bc}	35.52^{c}
Reactive black 5	1	9.69^{a}	12.26^{ab}	19.50^{cde}	21.79^{ef}	22.33^{ef}
	3	14.92^{bc}	15.72^{bcd}	19.46^{cde}	23.37^{ef}	24.33^{ef}
	5	20.42^{de}	23.29^{bc}	25.74^{f}	31.88^{g}	34.67^{g}
Orange II	1	2.87^{a}	2.79^{a}	5.68^{b}	5.89^{bc}	8.69^{de}
	3	3.07^{a}	3.98^{a}	7.04^{bc}	8.79^{e}	9.57^{ef}
	5	4.21^{a}	7.16^{bc}	7.27^{cd}	10.44^{f}	10.98^{f}
Crystal violet	1	1.54^{ab}	2.41^{abc}	3.09^{bc}	5.16^{de}	4.76^{de}
	3	2.8^{bc}	3.68^{cd}	5.68^{ef}	6.87^{f}	9.86^{g}
	5	0.93^{a}	9.16^{g}	10.13^{g}	13.52^{h}	15.45^{i}

Data were expressed as the mean of three-replicating and were analyzed by one-way ANOVA with $p < 0.05$. The letters representing in each synthetics dye group indicate a significant difference.

3.2. Effect of glucose supplementation

Glucose is an essential nutrient providing energy for organisms. There have been reports showing that laccase genes are inhibited by the presence of glucose, thus, reducing the synthetic dyes degradation (Revankar and Lele, 2006; Strong, 2011; Yang et al., 2017). However, a previous report suggested that glucose greatly enhanced laccase activity as well as synthetic dyes removal by the *F. oxysporum* (Huy et al., 2020). Thus, glucose supplementation at concentration ranging from 1% to 10% was carried out to evaluate the effect of glucose supplementation on the removal of synthetic dyes by the fungal culture. The results showed that glucose supplementation significantly improved synthetic dyes removal. The dye removal efficiency rapidly increased from Day 3 to Day 12 of culture period, but it dropped on Day 15. The improvement in degradation degree was dependent on each dye. The maximum removal increased from 35.52% to 79.61%, 34.67%–52.48%, 10.98%–49.02%, and 15.45%–32.5% for aniline blue, reactive black 5, orange II, and crystal violet, respectively. The highest degradation efficiencies for all dyes were observed in fungal culture supplemented with glucose at 1% concentration, as shown in Table 2. However, it is worth nothing that the increase in glucose concentration over 1% was due to the increasing in sugar supplementation that reduced synthetic dyes degradation efficiency. Glucose supplementation at concentration of 1% enhanced orange II degradation more than 4 folds compared with the control (no glucose addition), while the degradation of aniline blue increased >2 folds. On the other hand, the lowest degradation improvement was observed on crystal violet and reactive black 5 samples, with the significant factor of 1.24 and 2.45 folds, respectively.

3.3. Effect of mediators

Mediators are chemical substances that are continuously oxidized by laccase (Christopher et al., 2014). Mediators are known to help the binding between laccase and substrate as binding electron carrier, resulting in increasing enzyme activity and improvement in synthetic dyes degradation (Ancona-Escalante et al., 2018). Moreover, the presence of synthetic dye such as RBBR enhanced extracellular laccase production, leading to a significantly high efficiency of synthetic dyes degradation (Huy et al., 2020). Thus, the present study aimed to investigate the effect of mediators including SA, Vanillin, and 1-HBT as well as RBBR as inducer.

Table 3 shows the degradation efficiency of aniline blue, reactive

Table 2
Effect of glucose concentration on dyes degradation.

Synthetic dyes	Glucose concentration (%)	Dye degradation efficiency (%)				
		Culture time (days)				
		3	6	9	12	15
Aniline blue	1	41.45^{ab}	56.18^{cd}	67.98^{de}	75.92^{e}	79.61^{e}
	5	36.38^{a}	53.25^{bc}	66.85^{de}	67.7^{de}	68.25^{de}
	10	34.80^{a}	46.45^{abc}	50.67^{bc}	52.09^{bc}	55.55^{cd}
Reactive black 5	1	12.61^{a}	18.91^{ab}	42.33^{fg}	50.32^{gh}	52.48^{h}
	5	22.77^{ab}	28.53^{abc}	$36.18d^{ef}$	41.93^{fg}	41.55^{fg}
	10	26.23^{abc}	31.03^{cde}	35.74^{def}	38.98^{ef}	41.68^{fg}
Orange II	1	15.07^{b}	22.54^{cd}	27.86^{de}	43.28^{hi}	49.02^{i}
	5	8.45^{a}	18.39^{bc}	31.87^{ef}	35.32^{fg}	38.94^{gh}
	10	14.89^{b}	15.68^{b}	17.84^{bc}	23.15^{cd}	22.72^{cd}
Crystal violet	1	4.91^{ab}	7.05^{ab}	12.8^{c}	29.62^{ef}	32.49^{f}
	5	9.4^{bc}	12.73^{c}	14.78^{c}	25.77^{de}	30.87^{ef}
	10	3.41^{a}	4.42^{ab}	14.62^{c}	23.24^{d}	26.2^{de}

Data were expressed as the mean of three-replicating and were analyzed by one-way ANOVA with $p < 0.05$. The letters representing in each synthetics dye group indicate a significant difference.

Table 3
Effects of mediators and inducer on synthetic dyes degradation.

Synthetic dyes	Mediator	Dye degradation efficiency (%)				
		Culture time (days)				
		3	6	9	12	15
Anilin blue	Vanilin	64.39^{cdef}	67.57^{cdefg}	73.08^{efg}	84.94^{hik}	86.07^{ikl}
	1-HOBT	45.75^{a}	50.43^{ab}	63.14^{cdef}	63.36^{cdef}	67.11^{cdefg}
	Syringaldehyde	58.21^{bcd}	60.61^{bcde}	63.93^{cdefg}	68.71^{defg}	74.01^{fgh}
	Remazol B. Blue	55.60^{ab}	64.13^{cdef}	77.69^{ghik}	91.31^{kl}	97.06^{l}
Reactive black 5	Vanilin	25.51^{ab}	35.63^{bcde}	40.59^{cdef}	47.54^{efd}	57.46^{gh}
	1-HOBT	39.78^{bcdef}	45.00^{cdefg}	46.09^{defg}	57.07^{gh}	68.29^{h}
	Syringaldehyde	12.52^{a}	14.82^{a}	32.50^{bcd}	48.14^{efg}	57.25^{gh}
	Remazol B. Blue	30.55^{bc}	37.57^{bcde}	53.19^{fg}	84.28^{i}	89.86^{i}
Orange II	Vanilin	45.03^{a}	56.56^{bcd}	59.84^{ef}	63.70^{ef}	76.53^{h}
	1-HOBT	43.69^{a}	48.19^{ab}	61.5ef	66.27^{efg}	74.10^{gh}
	Syringaldehyde	51.59^{abc}	60.82^{cde}	70.05^{fgh}	74.16^{gh}	76.35^{h}
	Remazol B. Blue	59.87^{cd}	63.23^{ef}	73.09^{gh}	87.10^{i}	91.38^{i}
Crystal violet	Vanilin	47.16^{ab}	53.02^{bcd}	58.98^{cde}	75.51^{fg}	91.53^{i}
	1-HOBT	50.57^{bc}	59.01^{cde}	67.46^{b}	88.20^{hi}	95.30^{i}
	Syringaldehyde	36.68^{a}	45.19^{ab}	60.39^{cde}	64.27^{def}	70.29^{efg}
	Remazol B. Blue	43.97^{ab}	46.42^{ab}	66.23^{ef}	79.33^{gh}	86.67^{hi}

Data were expressed as the mean of three-replicating and were analyzed by one-way ANOVA with $p < 0.05$. The letters representing in each synthetics dye group indicate a significant difference.

black 5, orange II, and crystal violet during 15 days treatment. In particularly, mediators and inducer significantly improved synthetic dyes degradation. Maximum degradation was observed after 12 days of culture period. Among the tested mediators, vanillin found to maximize the degradation of aniline blue and orange II, with degradation efficiency of 86.07%, and 76.53%, respectively. Meanwhile, 1-HOBT was the best mediator for reactive black 5 and crystal violet degradation, with improvement of 68.29% and 95.3%, respectively. Syringaldehyde also significantly increased the fourth synthetic dyes degradation compared to the control (no mediator addition) system. Interestingly, the presence of RBBR enhanced all dyes degradation, resulting in the maximal efficiency of 97.06%, 89.86%, 91.38%, and 86.67% for aniline blue, reactive black 5, orange II, and crystal violet, respectively. RBBR was the best supporter for aniline blue, reactive black 5, and orange II degradation in comparison to other mediators. The impact of RBBR on the biodegradation of crystal violet was lesser, as the degradation efficiency is lower than that of vanilin and 1-HBT.

3.4. Effect of mediator concentrations

Based on the results of the influences of mediators and inducer on synthetic dyes degradation, different concentrations of RBBR and 1-HOBT were chosen to examine dyes removal efficiency. RBBR concentrations were in the range of 0.01%–1%, while vanillin concentrations were varied between 1 mM to 5 mM. The data are presented in Table 4 and Table 5.

The results indicated that the increasing RBBR concentration in medium culture did not enhance synthetic dyes removal. The degradation yield of aniline blue dropped to 84.7% and 75.56% at the concentration of 5×10^2 and 10^3 mg/L, respectively compared to that of 97.06% removal at the concentration of 10^2 mg/L. Similar observation occurred on reactive black 5 and orange II degradation where the maximum removal reduced from 95.26% to 59.99% and 91.38%–73.26%, respectively. However, the pre-cultured with RBBR still resulted in a higher synthetic dyes removal compared to that of the control (no RBBR supplementation), especially for reactive black 5 and orange II.

Crystal violet removal reached the maximum (>95%) under the treatment supplemented with 1 mM of 1-HOB. However, the efficiency reduced to 83.85% and 80.02% when 1-HOB concentration increased to 3 mM and 5 mM, respectively. Those negative effects indicated that the high concentration of mediators and inducer may have prevented the binding between enzyme and substrate due to large accumulation of intermediate degradation substances that inhibit enzyme action.

Table 4
Effect of RBBR concentration on synthetic dyes degradation efficiency.

Synthetic dyes	Inducer concentration (mg/L)	Dye degradation efficiency (%)				
		Culture time (days)				
		3	6	9	12	15
Aniline blue	10^2	55.60^{ab}	64.13^{bc}	77.69^{def}	91.31^{gh}	97.06^{h}
	5×10^2	47.09^{a}	64.04^{bc}	66.50^{ef}	80.32^{efg}	84.70^{fg}
	10^3	47.66^{a}	50.25^{a}	70.02^{cde}	72.98^{cdef}	75.56^{cdef}
Reactive black 5	10^2	52.25^{abc}	59.65^{cd}	80.42^{ef}	85.96^{fg}	95.26^{g}
	5×10^2	45.55^{ab}	50.91^{abc}	54.49^{abc}	$64,5^{cd}$	69.21^{de}
	10^3	43.49^{a}	51.39^{abc}	57.27^{bcd}	60.07^{cd}	59.99^{cd}
Orange II	10^2	59.87^{a}	63.23^{ab}	73.09^{bc}	87.10^{d}	91.38^{d}
	5×10^2	58.01^{a}	61.31^{ab}	66.58^{abc}	72.47^{bc}	76.15^{c}
	10^3	55.61^{a}	59.65^{a}	62.62^{ab}	67.77^{abc}	73.26^{bc}

Data were expressed as the mean of three-replicating and were analyzed by one-way ANOVA with $p < 0.05$. The letters representing in each synthetics dye group indicate a significant difference.

Table 5
Effect of 1-hydroxybenzotriazole concentration on crystal violet degradation.

Synthetic dyes	1-HBT concentration (mM)	Dye degradation efficiency (%)				
		Culture time (days)				
		3	6	9	12	15
Crystal violet	1	50.57^{ab}	59.01^{abcd}	67.46^{cde}	88.20^{hi}	95.30^{i}
	3	56.11^{abc}	$62.39b^{cde}$	66.56^{cde}	73.76^{efg}	83.85^{gh}
	5	48.65^{a}	52.96^{ab}	61.43^{bcd}	69.28^{def}	80.02^{fgh}

Data were expressed as the mean of three-replicating and were analyzed by one-way ANOVA with $p < 0.05$. The letters representing in each synthetics dye group indicate a significant difference.

3.5. Limitation of synthetic dyes removal

To determine the synthetic dyes removal capacity under the best culture conditions, each dye was added to medium culture to final concentration up to 10^4 mg/L. Aniline blue treatment showed that the fungus removed >90% of dye (at concentration of 10^3 mg/L) after 15 days of culture period, whereas the removal slightly reduced to 87.02% at dye concentration of 10^4 mg/L. The fungus removed about 80% of 10^3 mg/L of reactive black 5, orange II, and crystal violet after 15 days of culture period. The degradation significantly decreased at the concentration of 10^4 mg/L but still achieved ≥65% removal percentage, as shown in Table 6. The dye removal was the highest when the dye concentration was 10^2 mg/L. Although the fungus exhibited a lower degradation degree at higher concentration of dye, the total accumulated dye degradation was higher than that the treament of low concentration of dye. The limitation in dye removal may be related to the increasing accumulation of intermediate substances resulted from enzyme reaction, leading to inhibition of enzyme activity. Fig. 1 shows the changes of synthetic dye colors before and after the biodegradation process corresponding to each dye concentration.

4. Discussion

F. oxysporum has been reported for its strong ability to degrade various synthetic dyes such as bromothymol blue, remazol brilliant blue R, methyl orange, indigo carmine, and malachite green. However, crystal violet, aniline blue, reactive black 5, and orange II are highly resisted to biodegradation (Huy et al., 2020). The degree of synthetic dyes degradation typically increases when biomass concentration increases, and this phenomenon is due to extension of binding site for dyes absorption or a high amount of extracellular enzymes that are required for its degradation (Bankole et al., 2018; Almeida and Corso, 2019). Typically, synthetic dyes degradation accumulates the intermediate substances that are toxic to the cells, leading to the reduction in

Table 6
Degradation capacity of recalcitrant synthetic dyes.

Synthetic dyes	Dye concentration (mg/L)	Dye degradation efficiency (%)				
		Culture time (days)				
		3	6	9	12	15
Aniline blue	10^2	55.60^{ab}	64.13^{bc}	77.69^{de}	91.31^{f}	97.06^{f}
	10^3	44.21^{a}	46.01^{a}	62.71^{bc}	79.02^{de}	90.54^{ef}
	10^4	50.19^{i}	63.14^{bc}	67.51^{bcd}	69.66^{cd}	87.02^{def}
Reactive black 5	10^2	30.55^{b}	37.57^{bc}	53.19^{def}	84.28^{i}	89.86^{i}
	10^3	28.35^{b}	42.25^{bcd}	45.64^{def}	69.59^{gh}	78.00^{hi}
	10^4	13.55^{a}	31.92^{bc}	39.99^{bcd}	57.95^{efg}	65.77^{fgh}
Orange II	10^2	59.87^{de}	63.23^{e}	73.09^{f}	87.10^{gh}	91.38^{h}
	10^3	41.55^{ab}	58.62^{de}	61.62^{e}	79.46^{fd}	87.96^{gh}
	10^4	36.97^{a}	49.27^{bc}	59.49^{de}	62.54^{e}	77.57^{fg}
Crystal violet	10^2	50.57^{cd}	59.01^{de}	67.46^{ef}	88.20^{gh}	95.30^{h}
	10^3	30.20^{a}	37.49^{ab}	52.49^{cd}	69.19^{ef}	80.60^{gh}
	10^4	35.19^{ab}	44.67^{bc}	46.51^{bc}	51.05^{cd}	72.35^{fg}

Data were expressed as the mean of three-replicating and were analyzed by one-way ANOVA with $p < 0.05$. The letters representing in each synthetics dye group indicate a significant difference.

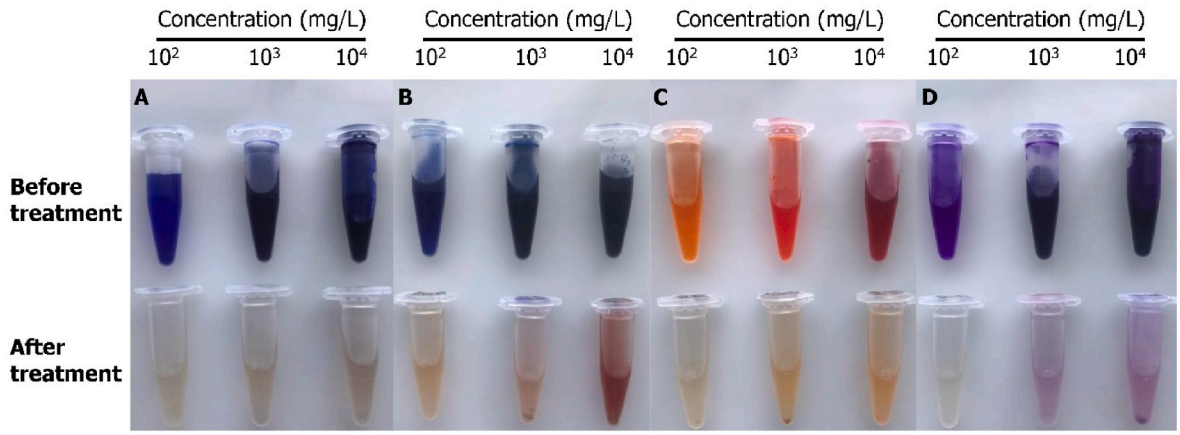

Fig. 1. Color reduction of synthetic dyes before and after treatment by *F. oxysporum* HUIB02 at different concentration ranging from 10^2 mg/L to 10^4 mg/L. (A) Aniline blue; (B) Reactive black 5; (C) Orange II; (D) Crystal violet.

degradation capacity (Almeida and Corso, 2019). Thus, increasing initial fungus biomass may not suitable in order to accelerate synthetic dyes degradation and should be combined with other modification methods such as addition of nutrients, inducers or mediators that improve cells activity as well as enhances tolerance against environmental stresses (Przystas et al., 2018).

Glucose is a nutrient factor that shows strong effect to laccase secretion by fungi. Numerous reports indicated that the presence of glucose improves laccase production and azo dyes degradation (Strong, 2011; Huy et al., 2020). On the contrary, other reports demonstrated glucose inhibiting lignin-degrading enzyme including laccase and reducing dye degradation (Janusz et al., 2015; An et al., 2016; Eskandari et al., 2019). In this study, glucose significantly helps the fungus in degradation of synthetic dyes up to 4 folds than that of control, and this value is higher than that by another research group (Strong, 2011). Meanwhile, glucose concentration higher than 1% leads to reduction in the synthetic dyes removal. Basically, synthetic dyes can be used as carbon source by microorganisms, but its low carbon content may limit the cells growth. Hence, the degradation process is more difficult and takes longer time (Eskandari et al., 2019). Therefore, addition of external carbon sources is necessary, and glucose is the most optimal carbon source to accelerate dyes degradation (Song et al., 2018; Jamee and Siddique, 2019). However, when the ratio of carbon source in culture medium exceeds a certain range, microorganisms declines to utilize dyes as carbon source, leading to the regulation of dyes metabolism system (Al-Tohamy et al., 2020).

Mediators are well-known substances that significantly increase the oxidation efficiency of synthetic dyes by the laccase (Viswanath et al., 2014; Bharathi et al., 2022). The results demonstrate that the presence of natural mediators (vanillin and SA) or synthetic mediator (1-HOBT) enhances the degradation percentage of synthetic dyes up to 95.3%, indicating a very effective factor for all dyes group degradation. RBBR was rapidly degraded by *F. oxysporum* HUIB02 after 3 day of culture along with an induced level of laccase production (Huy et al., 2020). In the present study, the fungus was inoculated with RBBR for 3 days. After RBBR being removed, the dyes were added into the cultures. It is observed that the synthetic dyes were removed faster in comparison to control. The degradation degree was higher than that of mediator's supplementation for aniline blue, reactive black 5, and orange II (refer to Table 3). A study by Eichlerova et al. (2006) showed that RBBR increases the laccase activity from *Ischnoderma resinosum* by 10 times after 20 days of co-culture period as dye degradation increases in relative with enzyme accumulation (Eichlerova et al., 2006). Similarly, RBBR has been found to improve laccase production by *M. cladophyllus* up to 70 folds (Sing et al., 2017). Furthermore, RBBR has been demonstrated to regulate the laccase expression system in fungus by inducing the expression of specific enzyme targeting the synthetic dyes (Huy et al., 2020). This finding suggests that addition of easier-degrading synthetic dyes may be a good strategy not only for laccase production but also to promote the efficiency for degradation of other synthetic dyes. Generally, the induction of laccase production in fungi by xenobiotics or phenolic compounds is linked with the cells response against toxicity of these compounds (Piscitelli et al., 2011). Up to date, the induction mechanism of laccase genes system in *F. oxysporum* by RBBR is unclear. However, laccase genes systems in fungi typically are regulated through different pathways including metal response elements, xenobiotic response elements, cAMP-mediated glucose repression sites, nitrogen repression response elements, heat shock response elements and antioxidant response elements (Wang et al., 2021). Thus, a xenobiotic such as RBBR may activate laccase genes though the xenobiotic response element pathway. For example, a recent study reported that emodin, an aromatic compound, induced laccase expression in *Trametes versicolor* by the same mechanism (Wang et al., 2021). However, the detail mechanism of induction by RBBR on laccase expression in *F. oxysporum* is worth for further study because laccase induction by specific substrate is host dependent and laccase genes are regulated by different pathways (Piscitelli et al., 2011; Wang et al., 2021). Furthermore, it is noticeable that an increased concentration of mediators or RBBR results in a decreasing performance of total synthetic dyes degradation. It may be explained that a highly excess accumulation of intermediate transformation substances prevent the binding of substrate onto active site of enzyme or inhibit enzyme activity. Table 7 compares the efficiency of biodegradation of recalcitrant synthetic dyes by *F. oxysporum* HUIB02 and other microorganisms.

The fungus efficiently removed synthetic dyes with concentration ranging from 10^2 to 10^4 mg/L, with degree nearly 80% to more than 95%. The degradation efficiency of aniline blue by *F. oxysporum* HUIB02 is higher than that of *Shewanella oneidensis* MR-1 (Wu et al., 2013). Meanwhile, *F. oxysporum* HUIB02 significantly removed reactive black 5 better than *P. chrysosporium* MUM 94.15 (Ottoni et al., 2013), *Sterigmatomyces halophilus* SSA1575 (Al-Tohamy et al., 2020) and the bacterial consortium (Seyedi et al., 2020). However, the reactive black 5 biodegradation degrees by the fungus are lower than that by using *T. versicolor* MUM 94.04 (Ottoni et al., 2013) and *Aspergillus* sp. (Gul et al., 2023). On the other hand, degradation ability of *F. oxysporum* HUIB02 for crystal violet is comparable to that of *Aeromonas hydrophila* SJ4 (Bharagava et al., 2018), *Aspergillus niger* A1 (Ali et al., 2016) and *P. chrysosporium* ATCC24725 (Ansari et al., 2017), with removal percentages of approximately 90%. Interestingly, *F. oxysporum* HUIB02 strongly exhibited the orange II degradability at concentration of 10^2 mg/L or above, whereas *T. versicolor* HEMIM-9, *Bacillus subtilis* ATCC6633, *Coriolus versicolor* ATTC200801, and *Funalia trogii*

Table 7
Comparison of the biodegradation efficiency of recalcitrant synthetic dyes between *F. oxysporum* HUIB02 and other microorganisms.

Microorganism	Dye type	Dye concentration (mg/L)	Efficiency (%)	References
F. oxysporum HUIB02	Aniline blue	100	97.06	This study
S. oneidensis MR-1		100	90	Wu et al. (2013)
F. oxysporum HUIB02	Reactive black 5	100	89.86	This study
Bacteria consortium		50	87	Seyedi et al. (2020)
T. versicolor MUM 94.04		100	100	Ottoni et al. (2013)
P. chrysosporium MUM 94.15		100	75	Ottoni et al. (2013)
S. halophilus SSA1575		100	~84	Al-Tohamy et al. (2020)
Aspergillus sp.		100	95.24	Gul et al. (2023)
F. oxysporum HUIB02	Orange II	100	91.38	This study
T. versicolor HEMIM-9		25	~84	Riegas-Villalobos et al. (2020)
B. subtilis ATCC6633		25	83.37	Ikram et al. (2022)
C. versicolor ATTC200801		–	73.1	Sam and Yesilada (2001)
F. trogii ATTC200800		–	64.5	Sam and Yesilada (2001)
F. oxysporum HUIB02	Crystal violet	100	95.3	This study
A. hydrophila SJ4		100	99	Bharagava et al. (2018)
A. niger A1		40	84.6	Ali et al. (2016)
P. chrysosporium ATCC24725		10	87	Ansari et al. (2017)

-: no data available.

ATTC200800 exhibited a lower degradation ability even at lower concentration of dyes (Sam and Yesilada, 2001; Riegas-Villalobos et al., 2020; Ikram et al., 2022). The high efficiency of biodegradation of various synthetic dyes classes by *F. oxysporum* HUIB02 demonstrates that this fungus can be applied to the treatment of a mixture of dyes which is commonly found in industry wastewater. Increasing dye concentration in medium culture dramatically reduced degradation efficiency. The low degradation may due to the toxic effects of dyes or its degraded products on the cells (Chen and Yien Ting, 2015). On the other hand, azo dyes including orange II and reactive black 5 structures consist of sulfonic acid group (HSO_3^-) on their aromatic ring which inhibits cells growth when the dye concentration is high (Ayed et al., 2011). Typically, industrial wastewater contains dye concentration in the range of 10–250 mg/L (Yaseen and Scholz, 2019). Thus, the results of this study indicate that the fungus is a promising candidate to efficiently remove synthetic dyes from dye effluent wastewater.

5. Conclusion

The microbial biodegradation of different classes of recalcitrant synthetic dyes by *F. oxysporum*was successfully demonstrated in this study, where the degradation levels of orange II, crystal violet aniline blue, and reactive black 5 were increased by 8.32, 6.17 2.73, and 2.59 folds, respectively. Glucose is an essential factor that improves the synthetic dyes degradation. Addition of mediators including SA, vanillin, and 1-HBT enhanced the biodegradation of dyes. Interestingly, pre-culture containing RBBR strongly accelerated the synthetic dyes degradation. However, increasing the concentrations of glucose, mediators or RBBR inhibited the biodegradation efficiency. The biodegradation process can be applied to treat different types of dye in the concentration range of 10^2–10^3 mg/L, at which the concentration used in this study is considered higher than that concentration in real industrial effluent wastewater. The biological treatment of dye waste water as demonstrated in the present study can also be integrated into other chemical or physical treatment processes to biodegrade a broad range of substances, i.e., not only synthetic dyes but also other organic pollutants.

Credit author statement

Le Thi Kim Thoa: Investigation, Writing-Original Draft. Trinh Thi Phuong Thao: Investigation My-Le Nguyen-Thi: Investigation, Resources Nguyen Duc Chung: Writing-Reviewing and Editing Ooi Chien Wei: Writing-Reviewing and Editing Seung-Moon Park: Writing-Reviewing and Editing, Supervision. Tran Thuy Lan: Investigation Hoang Tan Quang: Resources. Kuan Shiong Khoo: Literature, Reviewing and Editing Nguyen Duc Huy: Conceptualization, Reviewing and Editing, Funding acquisition Pau Loke Show: Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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