



Fungal interactions of co-cultures in the degradation of industrial dyes

Interacciones fúngicas de co-cultivos durante la degradación de colorantes industriales

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ABSTRACT

This study explores the possible microbial interactions between two species that have the potential to be exploited in the degradation of industrial dyes. The antagonism index (AI) of four distinct fungal strains was evaluated within two co-cultures (*Phanerochaete chrysosporium* and *Aspergillus niger*, *Trametes versicolor* and *Trichoderma* sp.) in culture media enriched with industrial dyes. The main interaction in the co-cultures was type A, deadlock at contact, except for *Trichoderma* sp. which exhibited antagonistic behavior and partial replacement interactions. The fungal co-cultures demonstrated a higher affinity for the degradation of blue and black dyes, azo dyes, phthalocyanine, and anthraquinone. The co-culture of *P. chrysosporium* and *A. niger* exhibited no antagonistic interactions, suggesting a mutual inhibition pattern that maintained strain compatibility. In the given context, it is proposed that the strains in fungal co-cultures exhibit a range of responses that are contingent upon their metabolic capabilities. These responses may include the production of reactive oxygen species (ROS), hydrogen peroxide, and the presence of intracellular and extracellular enzymes. These enzymes have the potential to be utilized in

the degradation of industrial dyes. Fungal co-cultures have been demonstrated to be both suitable and viable systems for the treatment of water colored with industrial dyes.

Key words: Antagonism index, co-cultures, degradation, industrial dyes, interactions.

RESUMEN

En este estudio se exploran las posibles interacciones microbianas entre dos especies fúngicas para ser aprovechadas en la degradación de colorantes industriales. Se evaluaron los índices de antagonismo (IA) de cuatro cepas en dos co-cultivos (*Phanerochaete chrysosporium* y *Aspergillus niger*, *Trametes versicolor* y *Trichoderma* sp.) en medios enriquecidos con colorantes industriales. La principal interacción en los co-cultivos fue tipo A, bloqueo al contacto, excepto para *Trichoderma* sp. que presentó un comportamiento antagónico e interacciones tipo reemplazo parcial. Los co-cultivos fúngicos presentaron mayor afinidad a degradar colorantes azules y negros, tipo azo, ftalocianina y antraquinona. El co-cultivo de *P. chrysosporium* y *A. niger* no presentó interacciones antagónicas manteniendo una inhibición mutua y logrando una compatibilidad entre cepas. Se propone que las cepas fúngicas en los co-cultivos emplean distintas respuestas metabólicas que cada especie dispone, que incluyen la producción de especies reactivas de oxígeno (EROs), enzimas intracelulares y extracelulares que pueden ser aprovechadas en la degradación de colorantes industriales. Los co-cultivos fúngicos son los sistemas adecuados y viables para el tratamiento de aguas coloreadas con colorantes industriales.

Palabras clave: Co-cultivos, colorantes industriales, degradación, índice de antagonismo, interacciones.

1. Introduction

The presence of industrial dyes in the environment poses a considerable threat to ecological sustainability. Industries frequently discharge untreated or incompletely treated wastewater into the environment, thereby causing water and soil pollution. The composition of this wastewater is determined by the specific nature of industrial activity. However, in effluents from the pharmaceutical, textile, food, paper, and cosmetics industries, industrial dyes used during manufacturing processes constitute one of the predominant pollutants (Shindhal *et al.*, 2020). The release of industrial dyes into the environment has been demonstrated to cause ecological problems. Due to their recalcitrant nature and toxicity, they have the potential to persist and accumulate in the environment, exerting detrimental effects on water quality and ecosystem health. Numerous studies have documented the deleterious effects of azo dyes (Chung, 2016; Martínez *et al.*, 2018), halogenated and anthraquinone industrial dyes (Lira *et al.*, 2024) on both humans and plants (Singh and Arora, 2011; Bhatia *et al.*, 2017).

In this context, the use of fungi for the treatment of colored wastewater has emerged as a promising strategy for industrial dye removal. The most relevant and effective examples of species in dye removal are those of the genera *Phanerochaete*, *Pleurotus*, *Trametes*,

Aspergillus and *Trichoderma*, especially the white rot fungi with ligninolytic capacity (Martínez *et al.*, 2018; Barbelli *et al.*, 2024 ; Abd El, 2025). In the last decade, research has focused on identifying strategies to enhance the production of degradative enzymes and metabolites involved in dye removal. One promising approach involves fungal co-cultures, which entail the cultivation of two or three distinct strains in a single culture. Co-cultures promote fungus-fungus interactions, which produce several metabolic and physiological changes (Ujor *et al.*, 2018; Sperandio and Filho, 2019). Interspecific interactions in co-cultures have been shown to be advantageous for dye removal (Lira *et al.*, 2020; Abd El, 2025), because they are able to generate increases in degradative enzymes and/or other metabolites related to contaminant removal (Simpal and Ram, 2016; Ujor *et al.*, 2018). Nevertheless, the primary limitation of fungal co-cultures relates to limited knowledge concerning their functional dynamics.

The use of fungal co-cultures in research has seen a marked increase in recent years, as their application has been shown to enhance the efficiency of pollutant removal. It is imperative to ascertain compatibility between strains during the development of co-cultures. The antagonistic behavior exhibited by these strains can be measured using the antagonism index (AI) (Sperandio and Filho, 2019), which describes the dynamics and type of interaction between the strains. The term AI denotes the antagonistic capacity of strains. This capacity can be classified into three categories: inhibition, deterioration, or killing of one microorganism by the action of another. It can also be classified as competition for space and nutrients (Sperandio and Filho, 2019; Iglesias *et al.*, 2022). Antagonistic interactions between fungi allow for increased metabolic production of compounds that can eliminate contaminants such as reactive oxygen species (ROS), H₂O₂ and enzymes, both intracellularly and extracellularly. Therefore, interactions in fungal co-cultures have been exploited for the removal of environmental pollutants such as industrial dyes (Kasonga *et al.*, 2020; Lira *et al.*, 2020; Abd El, 2025). Fungi of the genera *Phanerochaete*, *Trametes*, *Aspergillus* and *Trichoderma* were used to evaluate the different fungal interactions due to their diverse biotechnological applications, especially in bioremediation and the removal of organic contaminants. The present study has three objectives: (i) to evaluate the antagonism index in fungal co-cultures during the degradation of industrial dyes, (ii) to establish the most effective co-culture for dye degradation, and (iii) to propose a mechanism of interaction between two fungal species in a solid medium co-culture during the degradation of industrial dyes.

2. Materials and methods

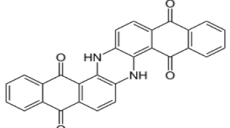
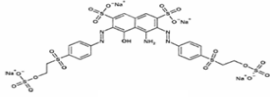
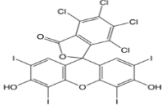
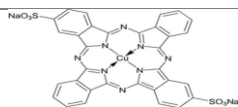
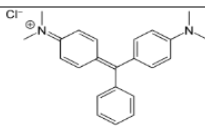
2.1. Microorganisms

The strains *A. niger* and *Trichoderma* sp. were obtained from the National Collection of Microbial Strains and Cell Cultures of the Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional. *T. versicolor* and *P. chrysosporium* were obtained from the Enzymatic Catalysis Laboratory of the Tecnológico de Estudios Superiores de Ecatepec. This strain was grown on potato dextrose agar (PDA) at 28°C for eight days.

2.2. Co-cultures on agar plates

Fungal interspecific interactions were evaluated in dual culture experiments using Petri dishes (90 mm Ø) with PDA added with 100 mg/L of industrial dyes: vat blue, reactive black 5, rose bengal, turquoise blue 86, malachite green. Table 1 presents the chemical structure and other specific characteristics of the dyes. As controls, some plates were inoculated with medium without dyes. Two separate co-cultures were performed: *P. chrysosporium* and *A. niger*, *T. versicolor* and *Trichoderma* sp. On the right side of the petri dish a 6 mm mycelial agar disc of *P. chrysosporium* or *T. versicolor* was placed and on the opposite side of the petri dish a mycelial agar disc of *A. niger* or *Trichoderma* sp. was placed as appropriate. Co-cultures were incubated at 28°C for at least 10 d. Petri dishes inoculated with individual fungal species were used as controls. For each co-culture, five replicates and their respective controls (without dye) were prepared. The radial growth of the strains in the petri dishes was meticulously monitored using a vernier caliper, with measurements taken at 24-hour intervals for a period of six days. The radial growth rate (RGR) (mm/h^{-1}) was calculated by using the linear equation. RGR is equivalent to the gradient of the exponential growth phase of an organism's growth curve.

Table 1. Characteristics of the industrial dyes.

Name	Characteristics	Chemical structure
Vat blue	Chemical formula: $\text{C}_{28}\text{H}_{14}\text{N}_2\text{O}_4$ Molecular weight: 442.4 g/mol Type: Azo	
Reactive black 5	Chemical formula: $\text{C}_{26}\text{H}_{21}\text{N}_5\text{Na}_4\text{O}_{19}\text{S}_6$ Molecular weight: 991.8 g/mol Type: Azo	
Rose bengal	Chemical formula: $\text{C}_{20}\text{H}_{14}\text{Cl}_4\text{O}_5$ Molecular weight: 973.6 g/mol Type: Halogenated	
Turquoise blue 86	Chemical formula: $\text{C}_{32}\text{H}_{14}\text{CuN}_8\text{Na}_2\text{O}_6\text{S}_2$ Molecular weight: 780.1 g/mol Type: Phthalocyanine	
Malachite green	Chemical formula: $\text{C}_{23}\text{H}_{25}\text{ClN}_2$ Molecular weight: 364.9 g/mol Type: Triarylmethane	

2.3. Antagonism index

The antagonism index (AI) was determined using the rating scale proposed by Badalyan *et al.* (2004). The scale employed in the study was a visual rating scale, which encompassed

three types of interspecific interactions (A, B, and C) and four subtypes (CA1, CB1, CA2, and CB2) for each species, where: A= deadlock at contact where there is mutual inhibition, where no species is able to outgrow another; B= deadlock at distance without mycelial contact where both species do not make contact and are kept at a distance; C= replacement, overgrowth without initial deadlock, where one species is able to displace another by mycelial growth; CA1= partial replacement after deadlock at contact, after mutual inhibition, one species is able to replace the other; CB1= partial replacement after deadlock at distance; CA2= complete replacement after deadlock at contact; CB2= complete replacement after deadlock at distance. The following score was assigned to each type or sub-type of reaction: A=1; B=2; C=3; CA1=3.5; CB1=4; CA2=4.5; CB2=5 (Badalyan *et al.*, 2004). The antagonism index (AI) was then calculated for each fungal species using the following formula 1:

$$AI = A(n \times 1) + B(n \times 2) + C(n \times 3) + CA1(n \times 3.5) + CB1(n \times 4) + CA2(n \times 4.5) + CB2(n \times 5) \quad (1)$$

where n = frequency of each type or sub-type of reaction.

2.4. Statistical analysis

Each experiment was carried out in quintupled. A comparison of the means of evaluating fungal radial growth rates was executed using the method of least significant difference (LSD), with an α of 0.05, in which different letters have different levels of statistical significance, and the same letters have the same statistical level of significance.

3. Results

Figure 1 shows the interactions of the co-cultures *P. chrysosporium* and *A. niger*, *T. versicolor* and *Trichoderma* sp. in solid medium enriched with industrial dyes. In each trial, the AI of each species was determined according to equation 1. In the co-culture, *P. chrysosporium* was observed to interact with *A. niger* (Fig. 1a,1b,1c,1d,1e) in the presence of dyes, resulting in a deadlock at contact. Mutual inhibition was observed, resulting in an AI equal to 4 for each species. In these experiments, it was observed that the presence of different dyes in the medium did not induce any alterations in the interactions between the strains. A decline in growth was observed for both strains in the medium with rose bengal, thus precluding an assessment of their AI.

In the co-culture of *T. versicolor* and *Trichoderma* sp. (Fig. 1f,1g,1h, 1i, 1j), the *T. versicolor* strain exhibited type A interactions, resulting in a deadlock at contact across all media. This observation led to the attainment of an AI value of 4. The *Trichoderma* sp. strain in the control medium exhibited interactions of type A in the medium with vat blue, interactions of type C and CA2 with turquoise blue, and interactions of type A and CA1 with reactive black 5. These interactions resulted in an AI of 17.5. In this essay, it was observed that the presence of the different dyes caused changes in the co-culture interactions. On rose bengal media, only mycelial growth of mycomycetes fungi was observed (*A. niger* and *Trichoderma* sp.) (Fig

1e and 1j). Consequently, no interaction occurred at the fungal level, and no results were obtained on their Als.

Mycelial growth was not observed for any of the strains employed in media enriched with malachite green; consequently, no results are presented.

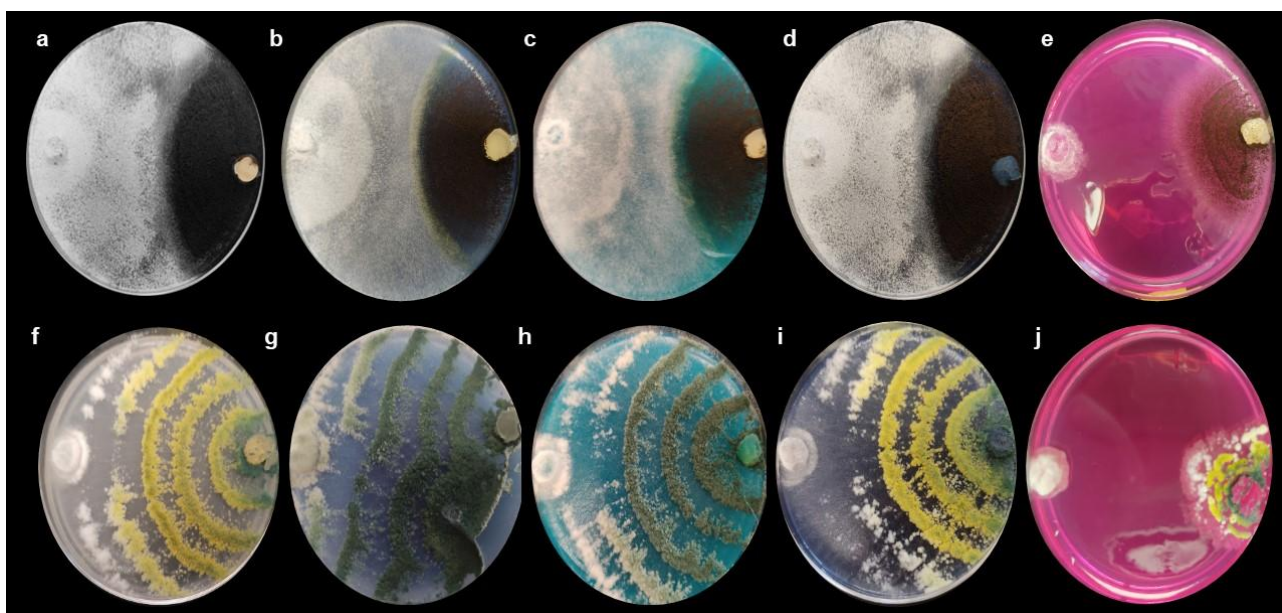


Fig. 1. Co-cultures of *P. chrysosporium* and *A. niger* in control medium (a), media enriched with: vat blue (b), turquoise blue 86 (c), reactive black 5 (d), and rose bengal (e); *T. versicolor* and *Trichoderma* sp. in control medium (f), media enriched with: vat blue (g), turquoise blue 86 (h), reactive black 5 (i), and rose bengal (j) in solid medium with industrial dyes.

Figure 2 shows a change in the morphology and mycelial coloration of *P. chrysosporium* in the co-culture with *A. niger*, due to a possible increase in pigments throughout the zone of confrontation (CZ) with *A. niger*. It has been documented that these alterations are initiated to deter an aggressive competitor and that fungal strains can augment the synthesis of intra- and extracellular pigments in CZ (Lynne, 2000; Hiscox and Boddy, 2017).

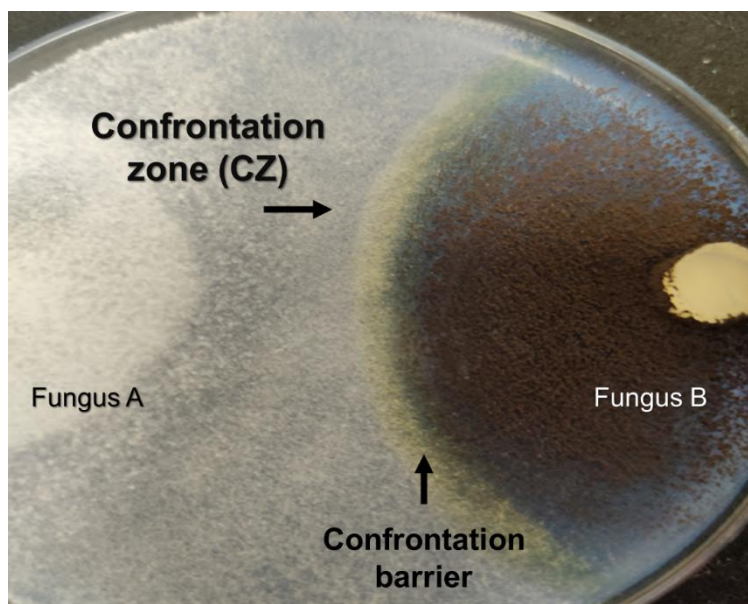


Fig. 2. Confrontation zone in a fungal co-culture: *P. chrysosporium* (fungus A) and *A. niger* (fungus B) on solid medium.

Figure 3 illustrates the proposed mechanism of possible responses in fungal interactions in co-cultures, which can be exploited for industrial dye degradation.

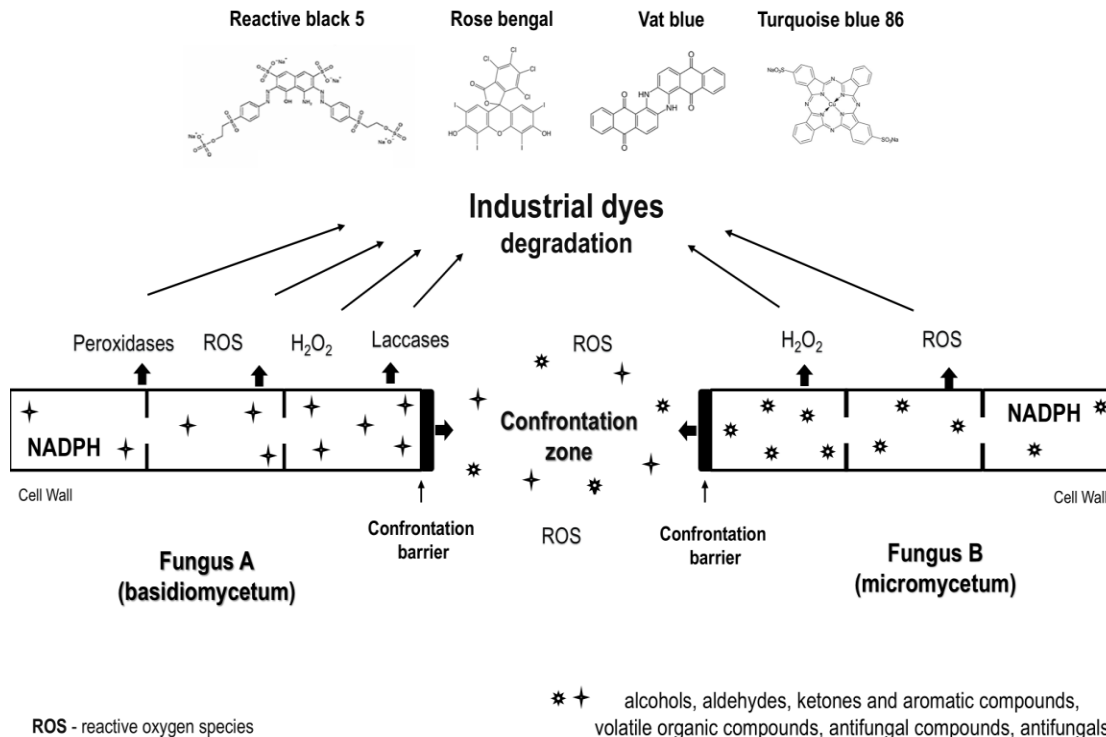


Fig. 3. Proposed mechanism of fungal interactions between two strains in a co-culture on solid medium during industrial dyes degradation.

Figure 4 shows the radial growth of each strain in the different media enriched with industrial dyes. A significant decrease in radial growth of *P. chrysosporium*, *T. versicolor* and *Trichoderma* sp. strains was observed in the media with dyes compared to the control without dyes. This confirms that there are negative effects and metabolic modifications during mycelial propagation caused by dyes. However, the *A. niger* strain demonstrated enhanced growth compared to the control medium in the presence of turquoise blue (phthalocyanine type), vat blue (anthraquinone type) and reactive black 5 (azo type) dyes. All co-cultures showed a decrease in radial growth in media with the pink bengal dye (halogenated type).

The basidiomycete fungi (Fig. 4a and 4c), *P. chrysosporium* and *T. versicolor*, showed greater tolerance to media with anthraquinone, phthalocyanin and azo dyes. *Trichoderma* sp. showed no significant difference in radial growth in media with turquoise blue and vat blue dyes compared to the control.

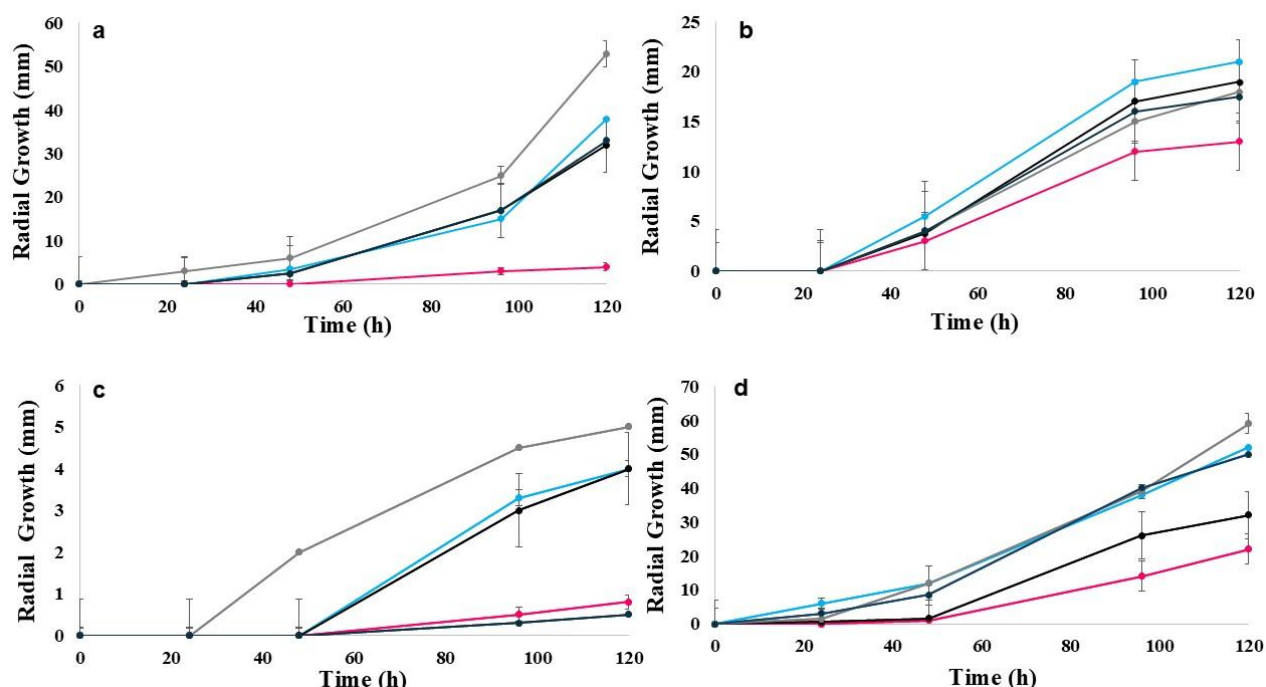


Fig. 4. Fungal radial growth of *P. chrysosporium* (a), *A. niger* (b), *T. versicolor* (c), *Trichoderma* sp (d). A control solid medium without dye (gray line), solid medium with 100 mg/L industrial dyes: turquoise blue 86 (turquoise line), rose bengal (pink line), reactive black 5 (black line) and vat blue (blue line).

Table 2 shows the RGR at 120 h of growth of the strains in the co-cultures with industrial dyes. *A. niger* shows a 21.8% reduction in the medium with rose bengal compared to the control (without dye) and a 21.2% increase in the medium with turquoise blue. *P. chrysosporium* demonstrated a significant reduction in the presence of rose bengal, with a recorded decrease of 92.6%. Furthermore, a 12-13% reduction was observed in the presence of vat blue and reactive black 5, while turquoise blue resulted in a 1.4% reduction.

Trichoderma sp. exhibited a 63% reduction in vat blue and rose bengal media, and *T. versicolor* showed a 24.4% decrease in vat blue and a substantial 91% reduction in rose bengal media. The reduction of RGRs in fungal co-cultures could be due to the influence of industrial dyes, as they contain chromophore groups and auxochromes that can be detrimental to strain propagation.

Table 2. Fungal radial growth rates (mm/h⁻¹) in co-cultures with industrial dyes.

Co-Culture	Control	Industrial Dye			
		Vat blue	Rose bengal	Turquoise blue	Reactive black 5
<i>A. niger</i>	0.16 ^b	0.169 ^b	0.125 ^c	0.194 ^a	0.174 ^b
<i>P. chrysosporium</i>	0.392 ^a	0.341 ^b	0.029 ^c	0.347 ^b	0.344 ^b
<i>Trichoderma</i> sp.	0.406 ^{ab}	0.148 ^c	0.149 ^c	0.419 ^a	0.378 ^b
<i>T. versicolor</i>	0.045 ^a	0.034 ^b	0.004 ^c	0.036 ^{ab}	0.036 ^{ab}

Means with the same letter in row are not significantly different (LSD, *p*= 0.05).

4. Discussion

During the development of fungal co-cultures on industrial dye media, antagonistic interactions characterized by mutual inhibition occurred, in which neither species was able to grow on the other. Research has demonstrated that various fungi have the capacity to proliferate on a single substrate, and that they can interact with one another both remotely and through direct mycelial contact. Such interactions are facilitated by the exchange of chemical signals, which can result in either mutual or selective inhibition (Muñiz and Loera, 2016; Hiscox *et al.*, 2017; Ujor *et al.*, 2018).

In the present study, it was hypothesized that interactions in co-cultures are driven by competition for nutrients from the culture medium. Consequently, the fungi were able to develop various accelerated colonization strategies that may reflect the attempt of both species to consume the substrates. Exploitative nutrient competition may occur when one species exhibits enhanced efficiency in the consumption of resources required by the other. Nutrient competition may also occur through interference through the secretion of chemicals such as volatile organic compounds, ROS or antibiotics, which affect the growth of a second species (Lynne, 2000; El ariebi *et al.*, 2016; Hiscox and Boddy, 2017). These interactions are driven by the need to ensure the provision of nutrients for carbon and energy consumption, thereby facilitating the maintenance of competitive ability and the preservation of already propagated territory (Castro *et al.*, 2022). However, the presence of industrial dyes has been demonstrated to cause alterations in these interactions, resulting in dye consumption being prioritized in the confrontation zone (CZ) (Simpal and Ram, 2016; Barbelli *et al.*, 2024; Abd El., 2025).

In the context of deadlock at contact interactions, the generation of combative scenarios involved the modification of the mycelium and hyphae of the strains within the zone of direct

mycelial contact (CZ) (Fig. 2). These modifications were observed to resist the invading opponent (Lynne, 2000; Eyre *et al.*, 2010; Ujor *et al.*, 2018). Badalyan *et al.* (2002) reported that aggregated structures may be present, including mycelial cords, pigmented hyphae, exudate droplets, dark pseudosclerotial lines, and fruiting body primordia, within the interaction zones.

In addition to this capacity, fungi possess the ability to form a dense barrier of aerial mycelia (referred to as a confrontation barrier) for the protection of a mycelial invasion by an antagonistic strain (Badalyan *et al.* 2002; Abd El, 2025). Ujor *et al.* (2018) reported that fungi can form confrontational barriers and generate modifications in their cell wall plasticity as transient resistance when confronted with another fungal aggressor that compromises their cell wall.

In the interactions of *T. versicolor* and *Trichoderma* sp. there were more antagonistic behaviors by *Trichoderma* sp. which obtained AI values of 17.5. Partial and total replacement interactions were generated where the confrontational barrier formed by *T. versicolor* did not resist the invasion (Badalyan *et al.*, 2002). It has been reported that interactions with high AI values have the capacity to modify fungal biomass functioning. The alterations encompass mycelial growth patterns, the distribution and allocation of nutrients within mycelia, and respiration. Consequently, there is an elevated probability of mycelia being invaded by more antagonistic strains (El ariebe *et al.*, 2016; Castro *et al.*, 2022).

Figure 3 shows the potential responses of fungal interactions in co-cultures through several key mediators: i) Volatile organic compounds (VOCs), including alcohols, aldehydes, ketones and aromatic compounds, which often exhibit antifungal properties (Muñiz and Loera, 2016; Barbelli *et al.*, 2024; Rodríguez *et al.* 2025); ii) Non-volatile antifungal compounds (antibiosis) secreted by one or both interacting species (Lynne *et al.*, 2000; Castro *et al.*, 2022); iii) NADPH acting as a regulator of oxidative stress (Ujor *et al.*, 2018); iv) Elevated ROS production in the CZ (Lynne *et al.*, 2000); v) Enhanced laccase and peroxidase enzyme generation by basidiomycete fungi; and vi) H₂O₂ production by micromycetes (Muñiz and Loera, 2016; Lira *et al.*, 2024). The interconnected processes of increased ROS, H₂O₂ and enzyme production (laccases and peroxidases) have been linked to enhanced industrial dye degradation (Lira *et al.*, 2020). Consequently, the biochemical responses generated through fungal interactions can be strategically harnessed for the reduction or elimination of pollutants (Simpal and Ram, 2016; Barbelli *et al.*, 2024; Abd El, 2025).

In the figure 3 showed numerous biochemical and physiological changes that have been reported in CZ, including mycelial pigmentation, up-regulation of mycelial temperature, increased ROS production, mycelial barrier formation to repel opposing fungal mycelia, and sealing of the mycelial front (Eyre *et al.*, 2010; Castro *et al.*, 2022; Rodríguez *et al.* 2025). These defensive reactions lead to increased oxidative stress aimed at strengthening resistance to mycelial invasion or replacement (Lynne, 2000; Muñiz and Loera, 2016; Hiscox *et al.*, 2017; Ujor *et al.*, 2018). When CZ formation occurs on industrial dye media, a competitive dynamic emerges for simple substrates, potentially leading to nutrient depletion and the subsequent consumption of more complex substrates, such as dyes (Sperandio and

Ferrería, 2019; Lira *et al.*, 2020; Abd El., 2025). This is an attempt to consume nutrients from the interaction zone more rapidly by one or both fungi.

The increased ROS production in CZ has been reported as a signaling related and it is considered as an attack or defense mechanism. This may produce an augmentation of oxidative stress regulatory mechanisms, thereby increasing NADPH generation via the pentose phosphate pathway (Ujor *et al.*, 2018). In fungal co-cultures, NADPH provides the critical reductive power necessary for physiological defense against oxidative stress generated by ROS (Lynne, 2000). Multiple studies have documented that mycorrhizal fungi in both co-culture and axenic cultures have been shown to produce hydrogen peroxide (H_2O_2) in situ. This compound serves as an oxidizing agent and a precursor of free radicals, playing an important role in advanced oxidation processes (Iglesias *et al.*, 2022; Lira *et al.*, 2024). Furthermore, hydrogen peroxide generation in fungal co-cultures grown on industrial dyes has been shown to enhance dye degradation efficiency.

In basidiomycete co-cultures, the production of oxidoreductase enzymes undergoes significant changes (either increases or decreases) as part of defensive or aggressive strategies against fungal competitors (Lynne, 2000; Barbelli *et al.*, 2024). Production of laccase and peroxidase enzymes is increased in CZ (Lira *et al.*, 2020; Iglesias *et al.*, 2022; Abd El., 2025). The production of these enzymes is a natural detoxification response to phenolic compounds, which tend to accumulate in the medium and consequently increase oxidative stress in the CZ (Badalyan *et al.*, 2004; Iglesias *et al.*, 2022; Barbelli *et al.*, 2024). The enhanced enzyme production in these zones can be exploited for the transformation and removal of dyes (Simpal and Ram, 2016; Abd Abd El., 2025). Ujor *et al.* (2018) suggest that laccases and peroxidases can be synthesized and stored latently prior to contact with the opposing mycelia and rapidly released from intracellular stores upon contact. Castor *et al.* (2022) reported that in fungal interactions, laccases are involved in multiple physiological processes such as oxidative stress response, mycelial invasion, detoxification mechanisms, mycelial morphogenesis and melanin pigment production.

In general, antagonistic interactions between fungal co-cultures remain minimal during dye degradation processes, and achieving strain compatibility has been shown to yield benefits absent in monocultures. These advantages include an increase in production of laccase enzymes, peroxidases, H_2O_2 y ROS (Simpal and Ram, 2016; Sperandio y Filho, 2019; Castro *et al.*, 2022). However, despite the clear advantages of co-cultures over axenic cultures, continuous monitoring of interactions during incubation is essential to ensure the stability of fungal interactions.

The primary limitation of co-cultures for industrial dye degradation lies in the scarcity of information regarding their operational dynamics and the absence of data on instabilities arising from initial micro-environmental disturbances or species competitiveness. Despite this acknowledged scarcity of data relating to fungal interactions, emerging research has begun to elucidate the molecular mechanisms underlying specific interaction responses, as well as identifying the genes and proteins governing these responses (Eyre *et al.*, 2010). This knowledge is essential to understanding the importance of fungal community dynamics and how these interactions can enhance the removal of environmental pollutants, such as industrial dyes.

5. Conclusion

The compatibility between *P. chrysosporium* strains and *A. niger* was evidenced by the absence of highly antagonistic interactions. Additionally, balanced mutual inhibition was maintained throughout the co-culture period. The co-cultivation of *P. chrysosporium* with *A. niger* has proven to be an effective approach for industrial dye degradation applications. Given their capacity to degrade complex compounds, fungal co-cultures represent a promising and viable alternative for treating colored wastewater, particularly that contaminated with industrial dyes. In fungal co-cultures, continuous competition for space and nutrients drives diverse interactions between participating species. The mechanisms employed to combat threats of mycelial invasion from strains with higher antagonistic indices generate a wide spectrum of metabolic responses. These responses can be exploited strategically for the industrial degradation of dyes.

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Contribution of the authors

Mayola Garcia: Conceptualization and Proofreading, Editing. **Juan Carlos Figueroa:** Writing, Proofreading and Editing. **Refugio Rodriguez:** Research and Resources. **Juana Lira:** Conceptualization, Writing, Proofreading and Editing.

Conflict of interest

No potential conflict of interest was reported by the authors

References

- Abd El Aty, A., 2025. Enhancing laccase enzyme production by co-culture of endophytic fungi with potential application in bioremediation of dyes. JMBFS. 14(4). <https://doi.org/10.55251/jmbfs.5542>
- Badalyan, S., Innocenti, G., Garibyan, N., 2004. Interactions between xylotrophic mushrooms and mycoparasitic fungi in dual-culture experiments. Phytopathol. Mediterr. 43, 44-48. https://doi.org/10.14601/Phytopathol_Mediterr-1733
- Badalyan, S., Innocenti, G., Grigory, N., 2002. Antagonistic activity of xylotrophic mushrooms against pathogenic fungi of cereals in dual culture. Phytopathol. Mediterr. 41, 200-225.

- Barbelli, M., Peralta, M., Levin, L., and Lechner B., 2024. Effect of co-cultivation of white and brown rot species on basidiome production, lignocellulolytic enzyme activity and dye decolourisation. *Bioresour. Technol.* 395, 130397. <https://doi.org/10.1016/j.biortech.2024.130397>
- Bhatia, D., Sharma, N., Singh, J., and Kanwar, R., 2017. Biological methods for textile dye removal from wastewater: A review. *Crit. Rev. Env. Sci. Tec.* 47(19), 1836–1876. <https://doi.org/10.1080/10643389.2017.1393263>
- Castro, J., Valle, V. and Afonso, M., 2022. Lignin degradation by co-cultured fungi: status and future perspectives. *Lilloa*. 59 (suplemento), 39–62. <https://doi.org/10.30550/j.lil/2022.59.S/2022.08.10>
- Chung, K. T., 2016. Azo dyes and human health: A review. *J. Environ. Sci. Health. Part C*, 34(4), 233–261. <https://doi.org/10.1080/10590501.2016.1236602>
- El Ariebe N., Hiscox, J., Scriven, S., Müller, C. and Boddy L., 2016. Production and effects of volatile organic compounds during interspecific interactions. *Fungal Ecol.* 20,144-154. <https://doi.org/10.1016/j.funeco.2015.12.013>
- Eyre, C., Muftah, W., Hiscox, J., Hunt, J., Kille, P., Boddy, L. and Rogers, H., 2010. Microarray analysis of differential gene expression elicited in *Trametes versicolor* during interspecific mycelial interactions. *Fungal Biol.* 114 (8), 646-660. <https://doi.org/10.1016/j.funbio.2010.05.006>
- Hiscox, J. and Boddy, L. (2017). Armed and dangerous - Chemical warfare in wood decay communities. *Fungal Biol. Rev.* 31 (4): 169-184. <https://doi.org/10.1016/j.fbr.2017.07.001>
- Iglesias, S., Chan, W., Osuna, J., Hernández, H. and Centeno, S., 2022. Antagonismo in vitro, actividad ligninolítica y de hidrolasas de pared celular en la interacción de especies de *Trichoderma* spp. con *Curvularia eragrostidis* aislado de pña. *Sci. fungorum*. 53, e1430. <https://doi.org/10.33885/sf.2022.53.1430>
- Kasonga, T., Coetzee, M., Kamika, I. and Momba, M., 2020. Assessing a co-culture fungal granule ability to remove pharmaceuticals in a sequencing batch reactor. *Environ. Technol.* 43(11), 1684–1699. <https://doi.org/10.1080/09593330.2020.1847204>
- Lira, J., Figueroa, J., García, M. and Rodríguez, R., 2024. Evaluation of vat blue removal by *Aspergillus niger* using scanning electron microscopy and glucose oxidase enzyme activity. *Vietnam J. Chem.* v. 1. <https://doi.org/10.1002/vjch.202300374>
- Lira, J., Rodríguez, R., Chan, W., 2020. Effect of fungal co-cultures on ligninolytic enzyme activities, H₂O₂ production, and orange G discoloration. *Prep. Biochem. Biotechnol.* 50(6), 607-618. <https://doi.org/10.1080/10826068.2020.1721534>

Lynne, B., 2000. Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiol. Ecol. 31(3), 185–194. <https://doi.org/10.1111/j.1574-6941.2000.tb00683.x>

Martínez, C., Díaz. R., Sánchez. L., Díaz. G., 2018. Biodegradación de Colorantes Azo por *pleurotus ostreatus*. Mex. J. Biotechnol. 3(1), 43–59. <https://doi.org/10.29267/mxjb.2018.3.1.43>

Muñiz. F. and Loera. O., 2016. The importance of strong inocula in fungal cultures. Mex. J. Biotechnol. 1(1), 120-134. <https://doi.org/10.29267/mxjb.2016.1.1.120-134>

Rodríguez, L., Martínez. G., Torres. C., Chavez. M., Lafuente. D., Rodriguez. J., Flores. E., Aguilar. C., Ramírez. N., 2025. Agroindustrial residues exploration to maximize metabolites production in fungal biocontrol co-culture. Mex. J. Biotechnol. 10 (2), 64-88. <https://doi.org/10.29267/mxjb.2025.10.2.64-88>

Shindhal, T., Rakholiya, P., Varjani, S., Pandey, A., Ngo, H., Guo, W., and Taherzadeh, M., 2020. A critical review on advances in the practices and perspectives for the treatment of dye industry wastewater. Bioengineered. 12(1), 70–87. <https://doi.org/10.1080/21655979.2020.1863034>

Simpal, K. and Ram, N., 2016. Decolorization of synthetic brilliant green carpet industry dye through fungal co-culture technology. J. Environ. Manage. 180, 172-179. <https://doi.org/10.1016/j.jenvman.2016.04.060>

Singh, K., and Arora, S., 2011. Removal of Synthetic Textile Dyes from Wastewaters: A Critical Review on Present Treatment Technologies. Cri. Rev. Env. Sci. Tec. 41(9), 807–878. <https://doi.org/10.1080/10643380903218376>

Sperandio, G. and Ferreira, E., 2019. Fungal co-cultures in the lignocellulosic biorefinery context: A review. Int. Biodeterior. Biodegrad. 142, 109-123. <https://doi.org/10.1016/j.ibiod.2019.05.014>

Ujor, V., Adukwu, E. and Okonkwo, C., 2018. Fungal wars: The underlying molecular repertoire of combating mycelia. Fungal Biol. 122 (4), 191-202. <https://doi.org/10.1016/j.funbio.2018.01.001>