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Exploring *Aspergillus* biomass for fast and effective Direct Black 22-dye removal

Explorando a biomassa de *Aspergillus* para a remoção rápida e eficaz do corante Direct Black 22

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ABSTRACT

Azo dyes are widely used in the textile industry due to their stability and resistance. These properties also make them recalcitrant xenobiotics, toxic, mutagenic, and carcinogenic, even at low concentrations. Considered emerging pollutants, there is an urgency to address mechanisms capable of remediating these contaminants, with *Aspergillus* fungi standing out as an effective solution. Fifteen strains of *Aspergillus* were investigated for the decolorization of the tetra azo dye Direct Black 22. The influence of different culture media was evaluated on fungi biomass production, dye concentrations (50–300 mg/L), biomass concentrations (1–5 g), and the reuse of biomass in continuous batches. The strains that stood out the most were *Aspergillus japonicus* URM 5620, *Aspergillus niger* URM 5741, and *A. niger* URM 5838. Obtaining biomass in less nutrient-rich medium favored decolorization by forming more organized pellets. The live biomass of these fungi was 59% more efficient than the dead biomass. The decolorization efficiency was not affected at lower dye concentrations, showing a decrease in decolorization only when the concentration reached 300 mg/L. Increasing the amount of biomass resulted in proportionally greater decolorization. Even with just 1 g of biomass, the three fungi could remove more than 90% of the dye in less than 60 minutes, and with 5 g, the dye was completely removed in 10 minutes. The biomass was reused in three consecutive decolorization cycles, and the fungus that best withstood the cycles

RESUMO

Os corantes azo são amplamente utilizados na indústria têxtil devido à sua estabilidade e resistência. Essas propriedades também os tornam xenobióticos recalcitrantes, tóxicos, mutagênicos e carcinogênicos, mesmo em baixas concentrações. Considerados poluentes emergentes, há urgência em abordar mecanismos capazes de remediar esses contaminantes, com os fungos *Aspergillus* destacando-se como uma solução eficaz. Quinze linhagens de *Aspergillus* foram investigadas para a descoloração do corante tetra azo Direct Black 22. A influência de diferentes meios de cultura foi avaliada na obtenção da biomassa dos fungos, das concentrações de corante (50–300 mg/L), das concentrações de biomassa (1–5 g) e da reutilização da biomassa em bateladas contínuas. As linhagens que mais se destacaram foram *Aspergillus japonicus* URM 5620, *Aspergillus niger* URM 5741 e *A. niger* URM 5838. A obtenção da biomassa em meio menos nutritivo favoreceu a descoloração, pela formação de pellets mais organizados. A biomassa viva desses fungos foi 59% mais eficiente que a biomassa morta. A eficiência de descoloração não foi afetada em concentrações mais baixas do corante, mostrando uma diminuição na descoloração somente quando a concentração atingiu 300 mg/L. O aumento da quantidade de biomassa resultou em uma descoloração proporcionalmente maior. Mesmo com apenas 1 g de biomassa, os três fungos foram capazes de remover mais de 90% do corante em menos de 60 minutos, e com 5 g, o corante foi completamente removido em 10 minutos. A biomassa foi reutilizada em três ciclos consecutivos de descoloração e o fungo que suportou melhor os ciclos foi

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was *A. niger* URM 5741. These results demonstrate the potential of the genus *Aspergillus* fungi tested in this study as sustainable and efficient biosorbents for the remediation of azo dyes such as Direct Black 22, with potential for colored industrial effluent treatment.

Keywords: decolorization; azo dyes; mycoremediation; biosorption; green technologies.

o *A. niger* URM 5741. Esses resultados mostram o potencial dos fungos do gênero *Aspergillus* testados nesse estudo como biossorventes sustentáveis e eficientes para a remediação de corantes azo como o Direct Black 22, com potencial para o tratamento de efluentes industriais coloridos.

Palavras-chave: descoloração; corantes azo; micorremediação; biossorção; tecnologias verdes.

Introduction

The diversity of colors and intensities of azo dyes and their resistance to physical, chemical, and biological effects make them the most widely used dyes in the textile industry. This class consists of synthetic dyes characterized by at least one azo bond (-N=N-) and at least one aromatic group as a radical (Kataria et al., 2022). The annual production of azo dyes is estimated at 9×10^6 tons per year (Maganha de Almeida et al., 2018); however, 10–25% of the dyes used in textile processing are lost during garment fabrication, and up to 20% are discharged as effluents into water bodies without any treatment (Ali et al., 2023).

Some azo dyes are visible at concentrations lower than one part per million (ppm). The environmental damage caused by this contaminant is associated with the mixture of dyes and their chemical composition (Alzain et al., 2023). The presence of aromatic groups (benzene, xylene, toluene, naphthalene, and others) makes these molecules recalcitrant and reactive, resistant to light, humidity, and the action of oxidizing agents. They can be toxic, mutagenic, carcinogenic, and bioaccumulative at a concentration of just 3%, even after treatment (Przystaś et al., 2018).

The ecological and public health problems generated by the release of this colored wastewater highlight the need to develop various techniques to remediate these pollutants. Applications with microorganisms, such as bacteria, microalgae, unicellular, and filamentous fungi, stand out, especially as they enable contaminants' partial or complete bioconversion (Chowdhary et al., 2022).

Filamentous fungi have attracted particular attention as agents for bioremediation. This is due to their economic application, widespread presence, and rapid metabolic adaptation to various carbon and nitrogen sources, including waste (Harper and Moody, 2023). They also can treat insoluble and recalcitrant compounds via extracellular enzymes (Latif et al., 2023). Additionally, they have the potential for biodegradation, bioaccumulation, and/or adsorbing pollutants, depending on the fungi's metabolism, tolerance, and other characteristics (Przystaś et al., 2018). Especially those of the genus *Aspergillus* stand out for their self-pelletization capability, which enhances the surface-to-volume ratio and, thereby, contact with contaminants (Gao et al., 2020).

Fungi of the genus *Aspergillus* can treat dyes of different classes. Species such as *A. flavus* (Qin et al., 2024), *A. terreus* (Chandukishore et al., 2024), *A. niger* (Bilgi et al., 2023), *A. oryzae* (Kadam et al., 2024), *A. arcoverdensis* (Skanda et al., 2023), *A. fumigatus* (Pullapukuri and Reddy, 2024), *A. ustus* (Mohamed et al., 2023), *A. tamarii* (Rai and Vijayakumar, 2023), and *A. tubingensis* (Karatay et al., 2023) have been applied in the treatment of acid, azo, basic, triphenylmethane, anthraquinone, reactive, direct, and disperse dyes. The most common mechanisms applied in these studies for dye bioremediation were biosorption, using pelleted biomass or immobilized cells on surfaces and/or nanocomposites, and/or enzymatic degradation, mainly with laccases, manganese peroxidase, and lignin peroxidase.

This study investigated the ability of filamentous fungi of the genus *Aspergillus* to remove the Direct Black 22 (DB22) dye. This is a sulfonated anionic tetra azo dye, containing phenyl and naphthyl linkages, with the presence of amino, chlorine, hydroxyl, methyl, and nitro groups. This chemical composition makes DB22 extremely stable and resistant, which is why it is widely used in the textile industry, mainly for dyeing cotton, wool, and viscose (Hien et al., 2020). The release of this dye without treatment or with inefficient treatment can lead to the breakdown into aromatic amines, posing a high risk to environmental health (Menezes et al., 2019). It was also evaluated whether the type of culture medium used to obtain the fungal biomass, the amount of fungal biomass, the condition of the biomass (live/active and dead/inactive), and the concentration of DB22 dye influence the efficacy of DB22 dye decolorization. Additionally, it was assessed whether the reuse of biomass in continuous batches maintains or improves dye removal efficiency.

Methodology

Preparation of the dye

The commercial dye used in this study was tetra azo Direct Black 22 (DB22; CI 35435; CAS 6473-13-8, molar mass 1083,97 g/mol) (Menezes et al., 2019) (Figure 1), obtained from Exatacor©, Brazil. Dye preparation was based on Amorim et al. (2013) and Carvalho et al. (2020), being the dye solution prepared at 50, 150, or 300 mg/L and adjusted to pH 11.0 standard error (±) 0.05 using 20% sodium hydroxide (NaOH), to hydrolyze the dye, and then heated to 80°C for one hour. After cooling, it was neutralized to pH 7.0±0.05 with hydrochloric acid (HCL).

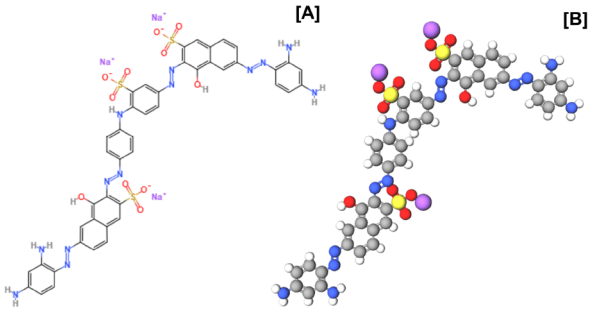


Figure 1 – Molecular structure of the dye Direct Black 22 (A) and its 3D model (B).

Microorganisms

Fifteen strains of *Aspergillus* were tested for their ability to decolorize the DB22 dye. They were: *A. janus* URM 4456, *A. aculeatus* URM 4953, *A. carbonarius* URM 5012, *A. terreus* URM 5093, *A. japonicus* URM 5620, *A. niger* URM 5654, *A. niger* URM 5741, *A. parasiticus* URM 5778, *A. sclerotiorum* URM 5792, *A. niger* URM 5837, *A. niger* URM 5838, *A. niger* URM 5863, *A. terreus* URM 5864, *A. terreus* URM 5896, and *A. niveus* URM 5930. These strains were obtained from the fungal collection URM of the Federal University of Pernambuco (UFPE) (https://www.ufpe.br/micoteca/catalogo), Brazil. The fungi were maintained on a Potato Dextrose Agar (PDA) medium and preserved in mineral oil at room temperature.

Selection of *Aspergillus* strains for the removal of Direct Black 22 dye

The fungi were inoculated on a PDA medium and incubated at 30°C for seven days. The spores were adjusted to a concentration of 10^4 CFU/mL and inoculated into 125 mL Erlenmeyer flasks with 50 mL of Czapek Dox Broth (Himedia®) or Sabouraud Dextrose Broth (Himedia®). The flasks were incubated at 30°C with agitation of 120 rpm for 96 hours to evaluate the morphological variation of the biomass in both media. After growth time, 1 mL of the corrected DB22 tetra azo dye at a concentration of 50 mg/L was added to the flasks and analyzed for 90 minutes, with aliquots taken every 30 minutes for spectrophotometric analysis. This monitored period allowed the best strains to be pre-selected. At the end of the decolorization process, the pre-selected strains were subjected to a further decolorization step by adding 1 mL of the dye at 1,000 mg/L. The flasks were agitated again at 120 rpm at 30°C for 24 hours. The experiments were performed in triplicate, and the supernatant was spectrophotometrically evaluated at each stage. The criteria used for selection were initially visual, based on observation of the supernatant and fungal biomass. They were as follows: 1. More discolored supernatants; 2. Biomass with higher dye capture; and 3. Biomass less degraded by agitation. The results were compared to spectrophotometric data, determining which fungi would proceed to the second selection phase.

Preparation of biomass for decolorization analysis

The selected strains were inoculated into Czapek Dox Agar test tubes (Himedia®) and incubated at 30°C for 120 hours. Once completely sporulated, they were inoculated at a concentration of 10^4 CFU/mL into 125 mL Erlenmeyer flasks containing 50 mL of Czapek Dox Broth (Himedia®). The strains were then incubated at 30°C with agitation (120 rpm) for 96 hours. An average of 145 mg was produced and used for decolorization.

Decolorization process with live and dead biomass

For the decolorization process with dead biomass, the flasks were autoclaved at 121°C for 30 minutes to inactivate the biomass (Khelifi et al., 2015). In the flasks containing live or dead biomass, 1 mL of DB22 dye at 50 mg/L was applied and placed under agitation (120 rpm). Samples were withdrawn every 10 minutes during the first hour after dye application and every 30 minutes during the second hour of monitoring.

Influence of dye concentration on decolorization

The assay to determine the effect of DB22 dye concentration on decolorization was conducted in 125 mL Erlenmeyer flasks containing 50 mL of DB22. The dye was applied at 50, 150, and 300 mg/L. All assays were performed in triplicate, under agitation (120 rpm) for 120 minutes.

Effect of biomass quantity on discoloration

The obtained biomass was washed with deionized water, vacuum filtered (45 µm filter paper), and weighed on an analytical balance. Biomass quantities of 1, 3, and 5 g were tested in the decolorization of DB22 (Almeida and Corso, 2019). This biomass was placed in 250 mL flasks, hydrated with 90 mL deionized water, and agitated (120 rpm) for 15 minutes. Subsequently, 10 mL of dye (50 mg/L) was added to the flasks, which were then mechanically agitated (120 rpm) at 30°C. The process was monitored for 120 minutes, with observations made every 10 minutes in triplicate.

Analysis of biomass reuse for decolorization

To assess biomass reuse, two separate experiments were conducted. In the first, three dye concentrations (50, 150, and 300 mg/L) were varied through two consecutive decolorization runs. The second experiment tested three biomass quantities (1, 3, and 5 g), performing three consecutive batches of decolorization. After each 2-hour cycle, the dye was replenished and monitored for additional two hours, following the described experimental conditions (Lu et al., 2017).

Analysis of the Direct Black 22 discoloration efficiency

To identify the wavelength with a maximum absorbance of DB22 dye, a scan was performed using ultraviolet-visible spectroscopy (UV-VIS) on the Ultrospec 53™ 7000 (Ge Healthcare), ranging λ=330–

1000 nm, with deionized water used as a reference, as described by Gao et al. (2020). Aliquots taken during the discoloration processes were centrifuged at 10,000 rpm for 10 minutes, the supernatant was analyzed at the wavelength of maximum dye absorbance (475 nm), and the results were presented as a percentage, according to Equation 1:

$$Decolorization\ (\%) = \frac{Initial\ absorbance - Observed\ absorbance}{Initial\ absorbance} * 100 \quad (1)$$

Statistical analysis

The experiments were conducted in triplicate, and the experimental data were presented as mean±standard error from the triplicates. Analysis of variance (ANOVA or Kruskal-Wallis) and analysis of least significant difference were performed, assuming a $p<0.05$ for statistically significant differences. The data were analyzed using the open-source software RStudio version 2023.12.1+402.

Results and Discussion

Selection of the strains with the greatest potential for decolorizing Direct Black 22

Fifteen strains of *Aspergillus* were tested for their ability to remove the textile dye DB22. These fungi were inoculated in two types of broths, Czapek Dox (Himedia®) and Sabouraud Dextrose Broth (Himedia®), called Czapek and Sabouraud, respectively, to assess whether biomass production and discoloration would be affected. The former is a less nutritious broth, rich in salts and ions, while the latter is a peptone-based nutrient medium. Although both cultures were maintained under the same conditions (30°C, 120 rpm, for 96 hours), a difference was noted in the biomass obtained. In Sabouraud broth, the fungi mycelia exhibited varied morphologies, with non-pelleted and extremely long clumps, pellets, and free hyphae. Meanwhile, in Czapek broth, a growth of well-defined pellets was generally observed. Almeida and Corso (2019) noted that the variation in mycelial structure affects decolorization efficiency since biosorption is proportional to the surface-to-volume ratio. Consequently, pellet formation favors dye adhesion to the fungal structure. However, in this study, the variation in decolorization efficiency was more related to the applied strain than to the biomass condition, so both media were efficient for biomass cultivation.

A pre-selection of the best strains was conducted for fungi that visually (Figure 2) and quantitatively exhibited a higher dye removal rate in both culture media (Figure 3A). The pre-selected fungi were *A. aculeatus* URM 4953, *A. carbonarius* URM 5012, *A. japonicus* URM 5620, *A. niger* URM 5741, and *A. niger* URM 5838. These fungi exhibited between 72.07 and 85.74% decolorization when cultured in Sabouraud medium and between 69.69 and 100% when grown in Czapek broth (Figure 3A).

The pre-selected fungi were subjected to a second decolorization of the dye at 1,000 mg/L for 24 hours. Figure 3B shows the decolorization achieved using the biomass cultivated in Czapek or Sabouraud. The variance analysis was performed by comparing the decolorization achieved with the biomass cultivated in Czapek or Sabouraud, and no significant difference was found. However, when comparing the decolorization efficiency among the fungi (Figure 3C) after 24 hours of dye removal at 1,000 mg/L, *A. japonicus* URM 5620, *A. niger* URM 5741, and *A. niger* URM 5838 were the fungi that decolorized DB22 with the biomass cultivated in both Czapek and Sabouraud (Figure 3B). The variance analysis of these data also shows that these fungi do not present a significant difference among themselves and have the best data distribution in the boxplot, with a mean and median decolorization of approximately 90%. Therefore, they were selected for further analyses.

Aspergillus niger has been efficiently applied in the decolorization of textile dyes such as Remazol Black B (Iscen et al., 2022), Congo Red (Hamad and Saied, 2021), Indigo Carmine (Rodrigues et al., 2024), and Vat Blue (Lira Pérez et al., 2024). However, our searches revealed that *A. japonicus* was found only in two studies. El-Kassas (2008) applied pelleted *A. japonicus* HK to decolorize Acid Fast Red 1, Congo Red 4, and Direct Fast Red 8B dyes, within a range of 25–200 mg/L, being more efficient in static conditions than under agitation, and removing up to 98.2% of the dye after two days. Meanwhile, Sharma et al. (2008) observed that *A. japonicus* could biosorb and biodegrade Congo Red, Methyl Orange, and Methyl Red dyes, decolorizing 96 to 99% of the dyes within 2–4 days. These studies demonstrate the adsorptive capacity of the genus. The results found in the present study prove this efficiency by being able to perform discoloration in just a few minutes, differentiating itself from the others.

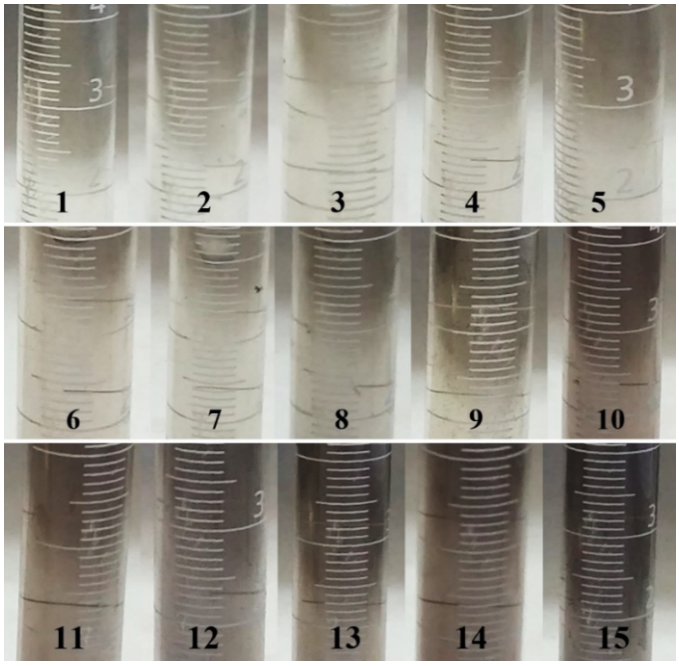
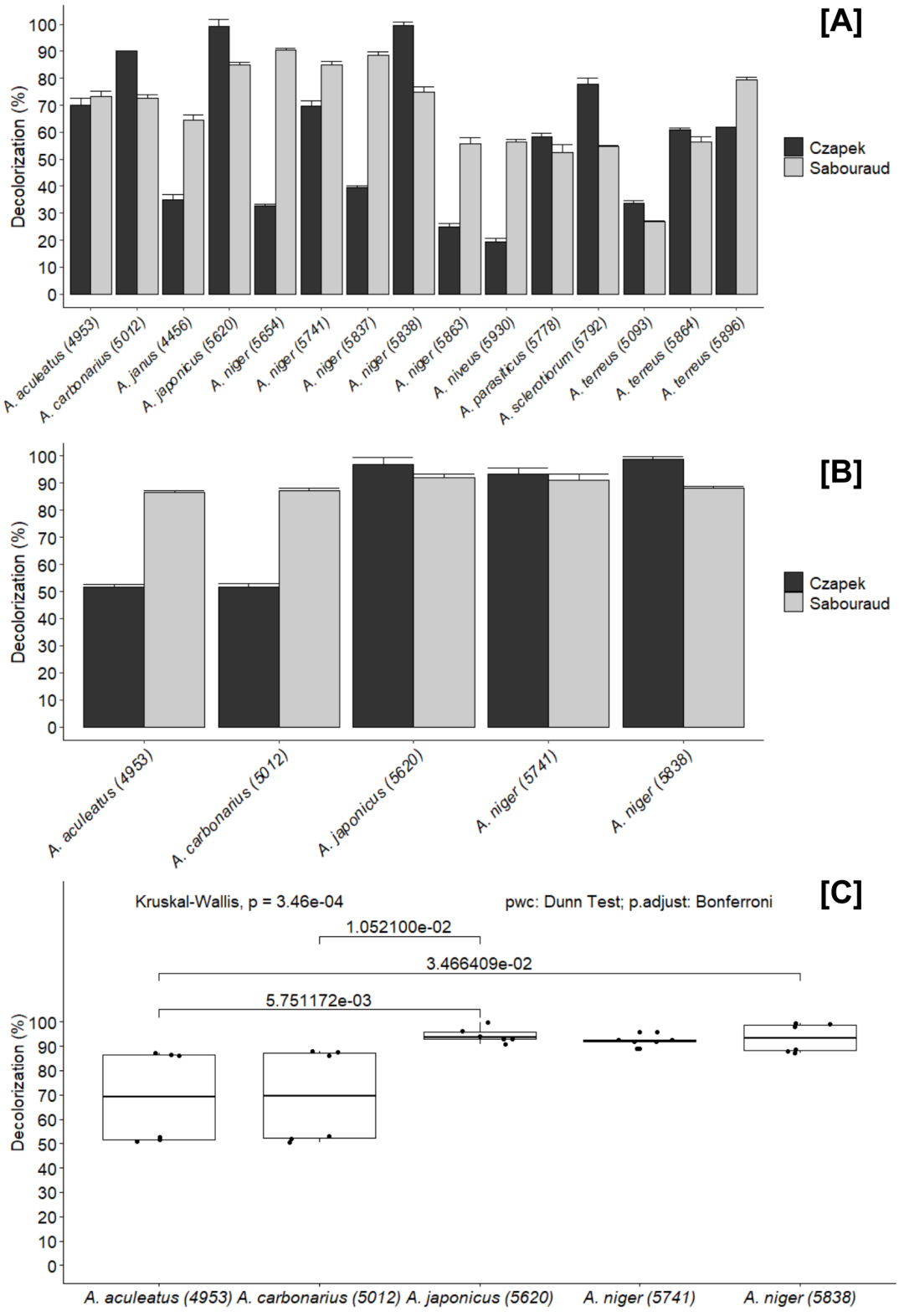


Figure 2 – Supernatant from the discoloration of the textile dye Direct Black 22 (50 mg/L) by filamentous fungi after 90 minutes of monitoring. Images used as visual pre-selection criteria. (1=*Aspergillus niger* (5838); 2=*Aspergillus japonicus* (5620); 3=*Aspergillus carbonarius* (5012); 4=*Aspergillus sclerotiorum* (5792); 5=*Aspergillus aculeatus* (4953); 6=*Aspergillus parasiticus* (5778); 7=*Aspergillus niger* (5741); 8=*Aspergillus terreus* (5896); 9=*Aspergillus terreus* (5864); 10=*Aspergillus terreus* (5093); 11=*Aspergillus niger* (5837); 12=*Aspergillus janus* (4456); 13=*Aspergillus niger* (5654); 14=*Aspergillus niveus* (5930); and 15=*Aspergillus niger* (5863)).



pwc: pairwise comparisons; p: p.adjust: adjust p-value.

Figure 3 – Decolorization of textile dye Direct Black 22 using filamentous fungi biomass of the genus *Aspergillus*. A. Screening of DB22 decolorization efficiency (50 mg/L) in 90 minutes with biomass fermented in commercial Sabouraud and Czapek broths; B. Screening of pre-selected strains for DB22 decolorization (1,000 mg/L) after 24 hours of monitoring; C. Boxplot of the variance analysis of decolorization in the pre-selection of fungi λ_{max}DB22 = 475 nm.

Efficiency of Direct Black 22 discoloration with live and dead biomass

The efficiency of dye adsorption by fungi of the genera *Aspergillus* has long been evaluated concerning the application of dead biomass, mainly because the biomass is not affected by the toxicity of the contaminant and does not require nutrient supplementation during its use as a biosorbent. Within this investigation, the dead biomass derived from *Aspergillus* exhibited a removal efficiency ranging from 32 to 46% (Figure 4), surpassing the efficacy observed in recent researches (Castro et al., 2021; Chau et al., 2023). Other studies suggested that live and dead biomass of different fungi species evidenced promising decolorization abilities, with *Fusarium*, *Trichoderma*, *Humicola*, *Aspergillus*, *Penicillium*, *Paecilomyces*, *Alternaria*, *and Beauveria* being evaluated for their effectiveness (Seyis and Subasioglu, 2008; Abdel-Ghany et al., 2019). However, research trends show greater interest in live biomass due to its higher efficiency (Ekanayake and Manage, 2022; Singh and Dwivedi, 2022). The present study revealed that live biomass showed up to 59% higher efficiency than dead biomass (Figure 4). In addition to enhanced adsorption, live biomass offers other removal mechanisms that contribute to its effectiveness. These mechanisms include bioaccumulation and biodegradation. The presence of active metabolic processes in live biomass also allows for the continuous uptake and transformation of contaminants, further improving the removal efficiency and sustainability of the process (Filote et al., 2022). This article focused on the adsorption potential of the studied microorganisms; thus, since the adsorptive capacity of the live biomass proved superior to that of the dead biomass, the remaining tests were conducted only on the live biomass of *Aspergillus*.

Effect of initial concentrations of Direct Black 22 on discoloration

The adsorptive efficiency of *Aspergillus* for DB22 at different initial concentrations is shown in Figure 5A. The three fungi were able to efficiently remove the dye at DB22 concentrations of 50 and 150 mg/L, decolorizing between 87.11–90.97 and 86.65–93.90%, respectively. When the concentration was increased to 300 mg/L, the removal efficiency of *A. japonicus* URM 5620 and *A. niger* URM 5838 decreased. It is likely that the binding sites in the biomass of these fungi saturated more quickly at the highest concentration (Nouri et al., 2021).

This effect is confirmed by variance analysis, where the increase in concentration interferes with the interquartile data distribution and the median decolorization with fungal biomass (Figure 5B). Hamad and Saied (2021) and Azeez and Al-Zuhairi (2022) also observed that removal efficiency is higher at lower initial concentrations and decreases with increasing concentrations due to the saturation of adsorption sites. In the studies mentioned, the maximum concentrations removed were 200 and 150 mg/L, with 69.4 and 69.6% removals, respectively. In contrast, in the present study, *A. niger* URM 5741 was able to remove over 80% of the dye even at a concentration of 300 mg/L.

Effect of biomass quantity on Direct Black 22 decolorization

The influence of the biosorbent quantity on the process was measured using 1, 3, and 5 g of biomass (Figure 6A). Decolorization was directly proportional to the amount of biosorbent applied; thus, the more biomass, the faster the decolorization. This result is consistent with the concept that an increased biosorbent surface area provides more binding sites for dye molecules, enhancing the overall adsorption capacity (Nouri et al., 2021). It corroborates researches that indicate that varying the biomass amount significantly influences the decolorization process, with higher biomass quantities leading to more effective dye removal (Azeez and Al-Zuhairi, 2022). For example, experiments with *A. oryzae* show that the removal of Rhodamine B increases exponentially until reaching the optimal biomass concentration of the fungus, removing up to 60% of the dye with biomass at a concentration of 10 g/L. However, surpassing the optimal biosorbent concentration reduces the fungal adsorptive capacity. This is because adsorption depends not only on the availability of active sites but also on the concentration gradient and mass flow in the adsorbent-adsorbate complex, that is, biomass-dye (Souza et al., 2020).

There is a statistical difference between the masses of biosorbent applied and the discoloration efficiency (Figure 6B). As a result, in the first 10 minutes of decolorization with 5 g of biomass, 97–99% was removed by all fungi. However, even with only 1 g of biomass, it was possible to remove the dye altogether after 120 minutes. This indicates that while a higher biomass quantity accelerates the process, lower amounts can still be effective over a longer period. Almeida and Corso (2019) also evaluated the effect of biomass quantity on the decolorization of Acid Blue 161, Procion Red MX-5B, and a solution containing both dyes, using *A. niger* and *A. terreus*. Between 15–25% of the dyes were decolorized with 1 g and up to 55% with 5 g of biomass. These results corroborate the idea that using larger quantities of biomass could be advantageous in industrial settings where rapid decolorization is required, whereas smaller quantities could be more suitable for batch processes where extended contact times are feasible (Muthukumaran et al., 2023).

Reuse of biomass

The use of batches for dye reapplication allows understanding the biosorbent's reuse capacity, making it suitable for large-scale applications, and assessing its overall efficiency. The consecutive use of the biosorbent makes the treatment less costly and more sustainable, reducing waste production (Karagöz et al., 2018; Horciu et al., 2020; Celik et al., 2021). In this study, the reuse of biomass was evaluated after the assays on the influence of initial dye concentrations and in the trials with higher concentrations of fungal biomass.

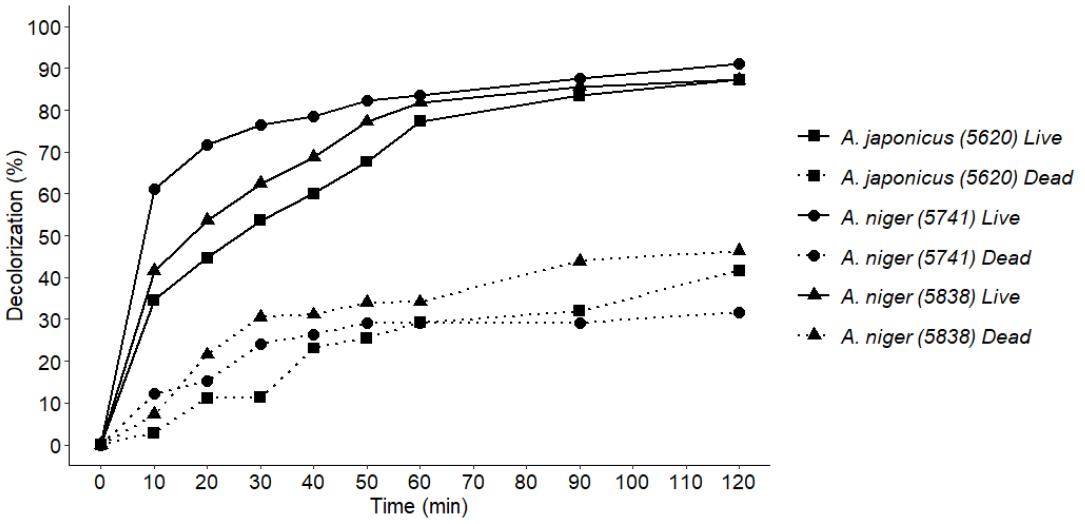


Figure 4 – Decolorization of the textile dye Direct Black 22 (50 mg/L) with live and dead biomass of *Aspergillus*. λ_{max}DB22 = 475 nm.

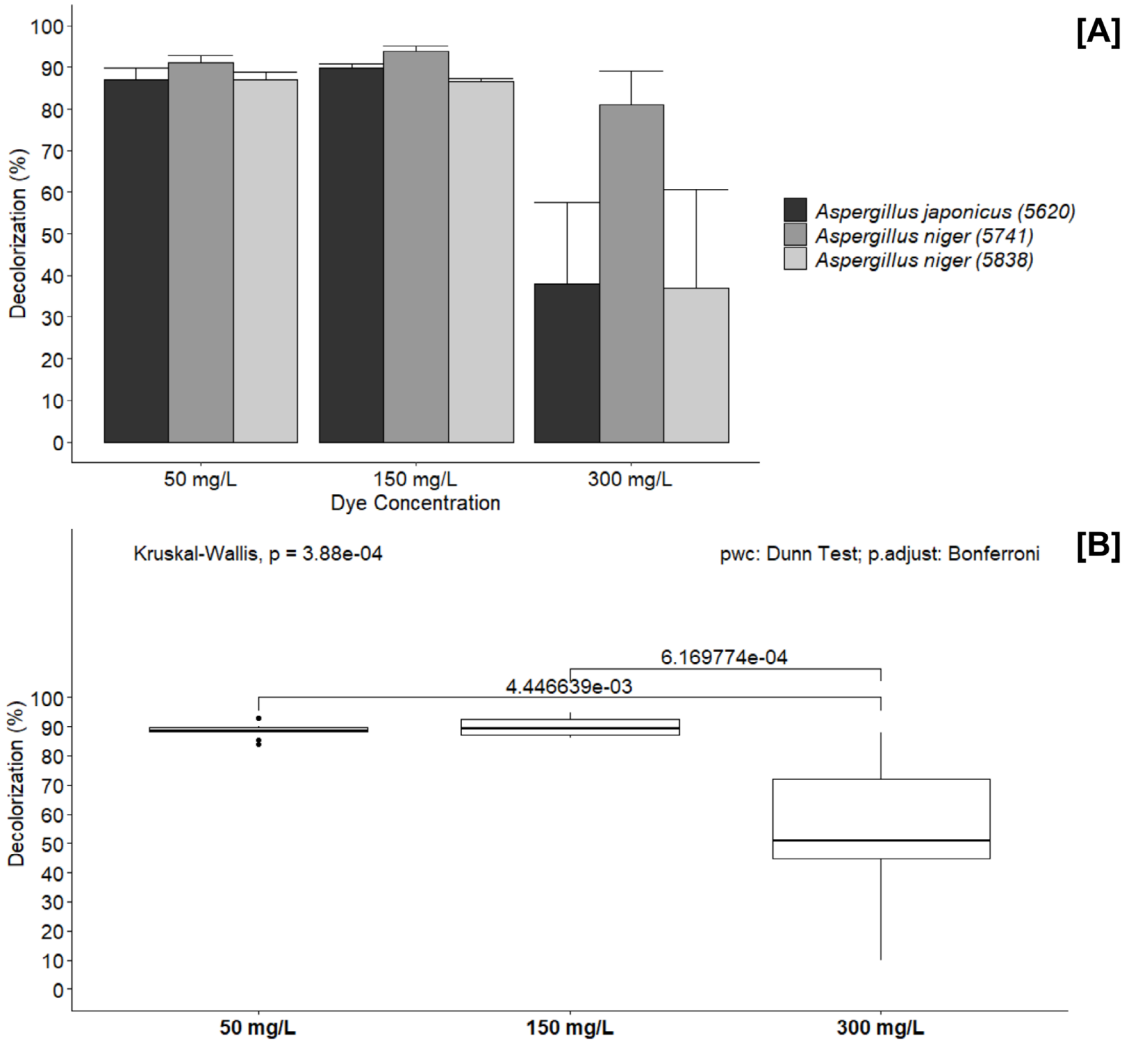


Figure 5 – Influence of initial dye concentration on Direct Black 22 decolorization. (A) Decolorization by *Aspergillus* fungi after 120 minutes; (B) Statistical analysis of the concentration effect on discoloration. λ_{max}DB22 = 475 nm.

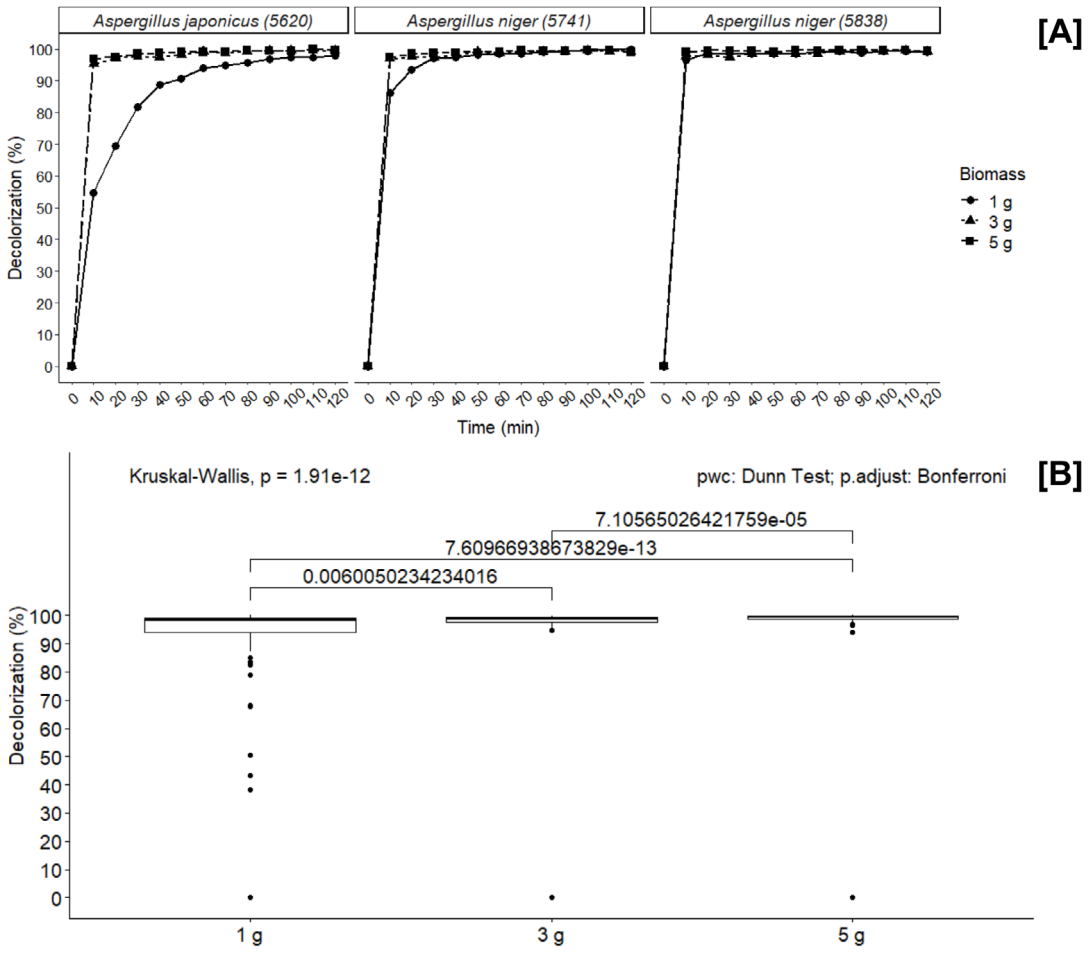


Figure 6 – The effect of increasing biomass concentration on removing textile dye Direct Black 22 after 120 minutes (A) and the statistical analysis (B). λ_{max}DB22 = 475 nm.

When examining the impact of different dye concentrations in the experiment, it was observed that the most effective agent for dye removal was *A. niger* (5741). Even when exposed to the highest initial dye concentration (300 mg/L), this particular fungus exhibited an adsorption rate exceeding 50% (Figure 7A). This performance was achieved even with a relatively small amount of biosorbent (145 mg). The boxplot of the statistical tests (Figure 7B) shows that there is no significant difference between the fungi *A. japonicus* (5620) and *A. niger* (5838) but both are significantly different from *A. niger* (5741). Additionally, the interquartile height of *A. niger* (5741) is lower than that of the others, with a higher median, indicating greater efficiency of this fungus.

The analysis of biomass reuse with 1, 3, and 5 g of fungal biomass was conducted with DB22 at an initial concentration of 50 mg/L (Figure 7C). In the reuse with larger volumes of fungal biomass, especially with 3 and 5 g of biosorbent, there was no significant difference in the decolorization efficiency between *A. japonicus* (5620), *A. niger* (5741), and *A. niger* (5838) ($p<0.05$). However, in decolorizations with 1 g of biomass, *A. niger* (5741) showed better removal after three cycles, being significantly different from *A. japonicus* (5620) ($p=0.002$) and *A. niger* (5838) ($p=0.011$). The results presented are consistent with those observed by Lu et al. (2017) when conducting batch decolorization experiments with the azo dye Congo Red, using 4 g of *A. niger* ZJUBE-1 biomass. The authors indicated a gradual reduction in decolorization efficiency with each cycle, but it was subtle. However, in this study, with the application of 3 and 5 g of biomass from *Aspergillus*, there was no reduction in efficiency in the decolorization of DB22, even after three consecutive cycles. This indicates that fungi of the genus *Aspergillus* have high potential for application as biosorbents for industrial dyes with high efficiency and the possibility of biomass recycling.

Conclusion

This study highlights the efficiency of pelletized biomass as a biosorbent for textile dyes, especially the decolorization of the tetra azo dye DB22 by *Aspergillus* sp. The evaluated *Aspergillus* strains fully eliminated the dye without requiring variations in environmental conditions such as pH and temperature. Three strains were singled out as the most effective among the fifteen tested: *A. japonicus* URM 5620, *A. niger* URM 5741, and *A. niger* URM 5838. The selected fungi were able to remove the dye at concentrations of up to 300 mg/L, with an average of 145 mg of biomass applied.

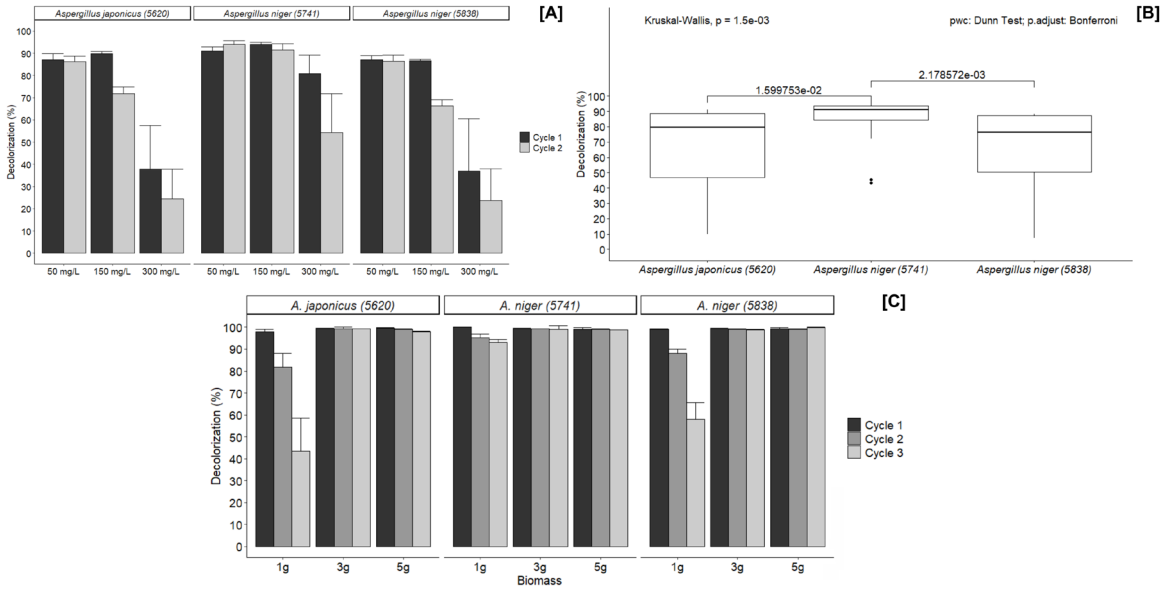


Figure 7 – Reuse of *Aspergillus* biomass for new decolorization of textile dye Direct Black 22. (A) Reuse at different initial dye concentrations; (B) Boxplot of the statistical analysis of the best fungus in biomass reuse; (C) Reuse to varying concentrations of fungal biomass. λ_{max}DB22 = 475 nm.

With 1 g, they could completely remove DB22 between 10 and 90 minutes. The strains maintained decolorization efficiency after multiple cycles of biomass reuse, with the fungus *A. niger* URM 5741 showing the highest decolorization efficiency. This underscores the feasibility of applying these fungi as a sustainable approach to industrial pollutant remediation and enables applications through filtering columns, tanks, and bioreactors. Therefore, bioremediation technologies using *Aspergillus* fungi show potential for mitigating the environmental impacts associated with the textile industry, and consequently for the remediation and preservation of water resources.

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Authors' Contributions

Neves, A.G.D.: conceptualization, experimentation, data curation, formal analysis, writing. **Silva**, R.L.A.: experimentation; data curation, formal analysis, writing. **Cardoso**, K.B.B.: data curation, formal analysis; writing. **Brito-Júnior**, J.J.R.T.: data curation, formal analysis, visualization. **Ferreira**, K.R.C.: data curation, formal analysis; writing. **Nascimento**, T.P.: contributed significantly to the research and analysis, adding depth to the study's conclusions. **Costa**, R.M.P.B.: data curation; visualization; investigation. **Silva**, M.V.: Data curation; visualization; investigation. **Porto**, A.L.F.: data curation; visualization; investigation.

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