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Treatment of the Textile Wastewater using Malaysian *Ganoderma lucidum* Mycelial Pellets

(Rawatan Air Sisa Tekstil menggunakan Pelet Miselium *Ganoderma lucidum* Malaysia)

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ABSTRACT

Purification of textile wastewater using biomass and in particular different fungi is gaining exponential interest to minimize the impacts of current physical-chemical and biological wastewater treatment by-products. This study investigates the potential of Malaysian *Ganoderma lucidum* mycelium pellets (GLMP) for the decontamination of wastewater samples received from a commercial textile manufacturer. All studies were performed under ambient temperature (26-35 °C) and unsterilized conditions using a simple bioreactor design (stirred batch bioreactor) for a more practical assimilation of the current available wastewater treatment process system. The optimal conditions of adsorption by GLMP were determined by variation effects of adsorbent concentration (0, 8.75, 12.5 and 25 g/L), pH (unadjusted 7.10 – 8.22, 4 and 6), and wastewater dilution factor (1:0, 1:4, and 2:3, v/v). This method was proved to be effective in both decolorization and chemical oxygen demand (COD) reduction, simultaneously. The most significant percentage of decolorization observed was 77.24% in a 72 h treatment, whereas COD reductions were 78.32% in a 36 h treatment. The present study fits both Langmuir and Freundlich adsorption isotherms as the values of R^2 both model were close to 1, indicating the favorable adsorption of dyes towards Malaysian GLMP.

Keywords: Bio-adsorption; COD; decontamination; dyes; *Ganoderma lucidum*; textile wastewater

ABSTRAK

Pembersihan air sisa tekstil menggunakan biojisim dan khususnya kulat yang berbeza semakin mendapat minat untuk meminimumkan impak produk sampingan yang terhasil daripada rawatan air sisa semasa secara fizikal-kimia dan biologi. Penyelidikan ini mengkaji potensi pelet miselium *Ganoderma lucidum* dari Malaysia (GLMP) untuk mengenyah lumuskan sampel air sisa yang diterima daripada pengeluar tekstil komersial. Semua kajian dilakukan di bawah suhu ambien (26-35 °C) dan keadaan tidak disterilkan menggunakan reka bentuk bioreaktor ringkas (bioreaktor kelompok berpengaduk) untuk simulasi yang lebih praktikal bagi sistem proses rawatan air sisa yang sedia ada. Keadaan optimum penjerapan oleh GLMP ditentukan oleh kesan variasi kepekatan penjerap (0, 8.75, 12.5 dan 25 g/L), pH (tidak diselaras 7.10 - 8.22, 4 dan 6), dan faktor pencairan air sisa (1:0, 1:4, dan 2:3, v/v). Kaedah ini terbukti berkesan dalam kedua-dua penyahwarnaan dan pengurangan permintaan oksigen kimia (COD), secara serentak. Peratusan penyahwarnaan yang paling ketara diperhatikan ialah 77.24% dalam rawatan 72 jam, manakala pengurangan COD adalah 78.32% dalam rawatan 36 jam. Kajian ini sesuai dengan kedua-dua model isoterma penjerapan Langmuir dan Freundlich kerana nilai R^2 kedua-duanya adalah hampir 1, menunjukkan penjerapan yang terbaik bagi pewarna terhadap GLMP Malaysia.

Kata kunci: Air sisa tekstil; bio-penjerapan; COD; mengenyah lumuskan; *Ganoderma lucidum;* pewarna

Introduction

Purification of industrial textile effluent is extensively being researched to create an environmentally friendly treatment method for such wastewater (Idris et al. 2007; Rafaqat et al. 2022). Conventional wastewater treatment systems face challenges in meeting discharge limits for all pollutants due to factors like the inefficiency of coagulants, high costs associated with adsorbent materials, and the substantial consumption of chemicals and electrical power. Current industrial wastewater treatment methods involve chemicals that produce additional contaminants as by-products, as well as waste (sludge) that requires further treatment before disposal in landfills. By using fungi as a single treatment medium, instead of commercially available chemicals, the waste generated, including sludge, can be safely reused as soil fertilizer.

Fungi can safely break down waste materials such organic biomass, thermoset polymers, toxic colors, and obsolete electronics into mature compost and non-toxic biodegradable chemicals (Bibbins-Martínez et al. 2023; Pereira, Serbent & Skoronski 2021). Additionally, the effluent discharge may serve as a valuable plant fertilizer, potentially containing beneficial enzymes (laccase, manganese peroxidase, and lignin peroxidase) for soils and plants (Ikehata 2015). *Ganoderma lucidum* contains a variety of biologically active compounds that have antioxidant, antitumor, anti-inflammatory, antifungal, and antimicrobial properties (Cör Andrejč, Knez & Knez Marevci 2022; Wan-Mohtar et al. 2016). Fungi may significantly affect wastewater treatment economics and surpass bacteria in waste-activated sludge treatment; the biomass generated by fungal wastewater treatment has a higher value than the bacterial-activated sludge treatment.

Fungi are used to produce valuable biochemicals and they can also serve as a protein source (Zhu et al. 2022). The biomass of Malaysian GLMP is non-toxic to Zebrafish embryos (Taufek et al. 2020) and has the potential to be a functional feed item for aquaculture-farmed red hybrid Tilapia (*Oreochromis* sp.) (Wan-Mohtar et al. 2021). It was proven that mycelial extract was non-toxic and safe for living organisms. One of the industries that contribute to a high volume of wastewater discharge is the textile industry. The Malaysian apparel market is expected to reach US$5.2 billion in revenues in 2023, with the industry expected to grow at a rate of more than four percent over the next few years to 2027 with 19 new projects approved (Wan-Mohtar et al. 2021). Textile and textile products manufacturing will result in a high volume of wastewater discharge and contribute to a series of dye-related water pollution issues (Pang & Abdullah 2013). These discharges contain a high load of contaminants during the runoff processes of textile dyeing and the rinsing of natural fabrics.

Dyes represent one of the major contaminants which, due to their chemical structure, resist degradation and fading when exposed to light, water, and many chemicals (Renu, Agarwal & Singh 2023; Robinson et al. 2001). They also contribute to an increase in biological oxygen demand (BOD) and chemical oxygen demand (COD) in industrial wastewater, leading to an increase in the cost of treatment (Lellis et al. 2019). It has been estimated that 200 L of water is consumed in the production of 1 kg of textile material during the chemical application process on the fabrics and during the rinse process of the final products (R Ananthashankar 2013). About 10,000 chemicals, including pigments, dyes, and dyeing auxiliaries, are produced along with the textile processing and the wastewater produced about 5–35% of these chemicals in it when it is discharged (Oyetade, Machunda & Hilonga 2023). This colored wastewater needs to be treated using environmentally friendly wastewater treatment methods, where the cost to set up such a system is much lower than the conventional wastewater treatment system available in the markets.

Scientific literature has demonstrated that microorganisms, including bacteria, yeast, algae, actinomycetes, and fungi, perform well in dye decolorization using mostly synthetically prepared dye-containing wastewater (Shah 2018; Usman et al. 2022). Investigation into how microbes can decolorize and mineralize organic molecules by rupturing intra-molecular bonds has been conducted (Olivito, Jagdale & Oza 2023). The use of microorganisms can also significantly contribute to the advancement of various bioremediation methods (Tiwari, Tripathi & Gaur 2017). Both bacteria and fungi can break down and discolor polycyclic aromatic hydrocarbons and azo dyes (Ahsan et al. 2021). Fungi have been reported to promote the transfer of hydrophobic pollutants from the aqueous phase (Espinosa-Ortiz, Rene & Gerlach 2022). Bacteria have also been discovered to efficiently degrade azo dyes into amino groups (Gupta & Raviya 2022). Fungi have several benefits, and some of their features make them more effective degraders than bacteria (Mostafa et al. 2019). They can break down any organic substance by using enzymes, making them excellent bio degraders (Singh & Vyas 2022). Fungi are also metabolically versatile, with the ability to degrade chemically stable substances such as recalcitrant xenobiotics (Asadollahzadeh, Mohammadi & Lennartsson 2023). They can efficiently degrade herbicides like glyphosate, with certain strains of Penicillium able to use glyphosate as a phosphorus source and degrade it into sarcosine and aminomethylphosphonic acid (Correa et al. 2021). Fungi are also useful in environmental cleanup, agriculture, food technology, textile industry, and pharmaceutical industry (Koul & Farooq 2020; Usmani et al. 2021).

In applications of color removal from water, numerous studies have been reported using fungi in the literature. For instance, Hanafiah et al. (2022) used *Ganoderma lucidum* (GL) from Malaysia in treating color from a lake water reservoir. The result showed that GL was able to remove about 90% of the color in 48 h of the batch treatment and 10% of the GL volume. Selvakumar, Manivasagan and Chinnappan (2013) treating textile dye wastewater using *Ganoderma lucidum* from India with optimal conditions of wastewater dilution (1:2), 100 rpm agitation, and pH 6.6, found that 81.4% and 90% of color and COD were removed, respectively. In other work done by Yesilada et al. (2003) which studied a few types of fungi (*Coriolus versicolor, Funalia trogii, Phanerochaete chrysosporium, Pleurotus florida, Pleurotus ostreatus*, and *Pleurotus sajorcaju*) in treating textile colors (Astrazone Black FDL, Astrazone Blue FGRL, and Astrazone Red FBL), more than 75% of the colors were removed by the culture in 24 h. Previous work has shown the potential of using fungi to treat different types of color from different types of water sources. It was anticipated that various cultural groups would respond differently to specific contaminants and have varying ideal environments. The applications of Malaysian *Ganoderma lucidum* in treating real industrial textiles as biosorbents are still unclear.

Thus, the aim of this study was to determine the potential of using Malaysian GLMP as a bio-adsorption media for decolorizing dyes from real textile wastewater, as well as for the chemical oxygen demand (COD) removal. Current studies mostly employ artificial wastewater, which involves diluting commercial textile azo dyes mixed with distilled or deionized water (Rainert et al. 2021). The decolorization capabilities of the real textile wastewater by Malaysian GLMP were observed during the experiment using a batch bioreactor under ambient temperature and unsterilized conditions. The COD removal capabilities were also monitored under the same experimental conditions. Subsequently, microscopic images of the mycelium cell structures were generated to obtain hints on the biosorption process in real textile wastewater. The findings from this study are useful for the implementation and assimilation of fungal-based bio-adsorption media into existing industrial wastewater treatment methods. Overall, these biological approaches provide safer, cleaner, and more cost-effective options, by the introduction of more sustainable and environmentally friendly processes.

Materials and Methods

MALAYSIAN *Ganoderma lucidum* MYCELIUM PELLETS

Malaysian *Ganoderma lucidum* mycelium pellets (GLMP) were cultured and obtained from Malaysia, as identified by Supramani et al. (2019a, 2019b). For plate subculture and seed culture, the medium contained 39 g/L of potato dextrose agar (PDA; Sigma-Aldrich, Dorset, UK), whereas the fermentation medium consisted of 30.0 g/L glucose, 1.0 g/L yeast, 0.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4$, and 4.0 g/L NH_4Cl. The ideal procedure recommended was followed for all these operations, which were performed at 30 °C and shaken at 100 rpm for 11 days (Wan Mohtar et al. 2016). The first seed culture mycelium was homogenized in a sterile waring blender for 20 s after 10 days to produce extra growing hyphae tips. It was then transferred to the bioreactor using the same media as the first seed culture and used as the inoculum for the second seed culture in a 500 mL Erlenmeyer flask containing 200 mL medium. The mycelium pellets were removed from the liquid media and prepared for experimental use as a biosorption media.

TEXTILE DYE WASTEWATER SAMPLE

The purplish red real textile wastewater (RTWW) was received from the textile industry located in Batu Pahat, a district of Johor, Malaysia. The wastewater's initial pH, Color, BOD, TSS and COD were measured and referred for this experiment as shown in Table 1.

EXPERIMENTAL SET UP

The treatment was performed in duplicate, and the weighted dosages of Malaysian GLMP added to the 400 mL RTWW, which set at a stirring speed of 150 rpm and consistent aeration at 4 L/min. The initial pH was adjusted to the required level upon the initiation of treatment. Samples were collected at 24 h intervals, allowed to settle for 30 min sedimentation, filtered and then analyzed.

This process continued for a total treatment duration of 72 h. The variables examined included the GLMP adsorbent amount (0, 3.5, 5 and 10 g, corresponding to initial concentration 0, 8.75, 12.5 and 25 g/L), the initial treatment pH (4, 6 and unadjusted) and dilution ratio of real textile wastewater (RTWW) (RTWW: distilled water) for the treatment (1:0, 1:4, and 2:3, v/v %). All treatments were performed at room temperature (26-35 °C) and unsterilized conditions. An overview of the methods used is shown in Figure 1.

MICROSCOPIC ANALYSIS

The structures of the Malaysian GLMP were observed microscopically and was performed under a dissecting microscope (Leica EZ4 microscope). The observation was conducted on pre- and post- treatment of the pellets.

TABLE 1. Characteristic of the real textile wastewater effluent

Parameters	Value
pH @ 25 °C	7.10 - 8.22
Color Appearances	Purplish red
Color (PtCo)	1116.0
Total Suspended Solid, TSS (mg/L)	39.0
Biological Oxygen Demand, BOD (mg/L)	50.0
Chemical Oxygen Demand, COD (mg/L)	326.0 – 541.0

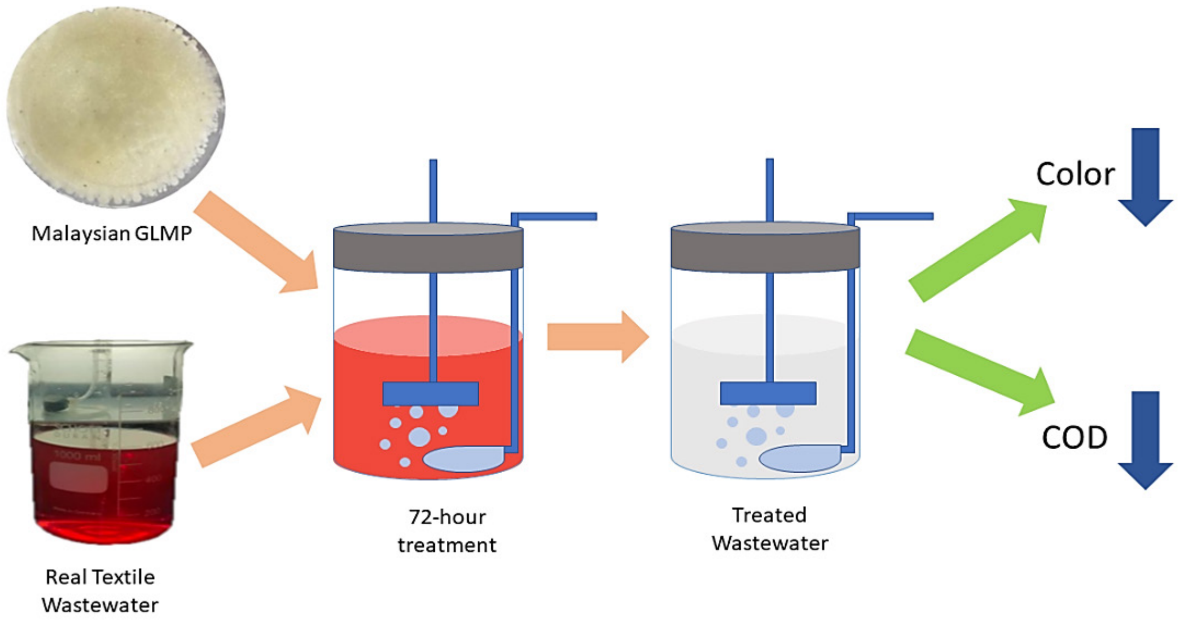

FIGURE 1. Graphical representation of the wastewater treatment methodology

COLOR AND COD REMOVAL

Each sample was left sedimented for 30 min, and then 5 mL of supernatant was withdrawn using a syringe and was subsequently diluted five times in a measuring cylinder with a stopper. The sample was filtered using a Whatman 0.45 µm syringe filter, transferred into a cuvette, and subjected to color analysis using a HACH DR900 colorimeter, according to the standard protocol provided by the manufacturer (Hach Method 8025, Malaysia at wavelength for 420 nm). Meanwhile, the COD values were determined by the colorimetric method using the HACH vial high-range reactor digestion method, 20-1500 mg/L (HACH method 8000, Oxygen Demand, Chemical). The color reduction and COD removal was calculated using Equation (1)

$$Percentage\ removal\ (\%) = \frac{(C_0 - C_t)}{C_o}\ x\ 100 \qquad (1)$$

where C_o is initial PtCo color or initial COD; and C_f is final PtCo color or final COD.

ADSORPTION ISOTHERM

Isotherms, or functions connecting the quantity of adsorbate on the adsorbent, are typically used to characterize adsorption. Numerous isotherm models, including Langmuir and Freundlich, can be used to describe the distribution of real textile wastewater color dyes between the liquid and solid phases. By calculating the correlation coefficients, or R^2, the isotherm equation's applicability was assessed (Syauqiah et al. 2022). The sorption capacity was estimated according to the following equation using Formula 2.

$$Q_e = \frac{C_i - C_e}{W} \qquad (2)$$

where Q_e is the quantity of dye color uptake from solution by mass of adsorbent (mg/g); C_i and C_e are the initial and equilibrium color concentrations (mg/L), and W is the weight of the adsorbent added (g).

The Langmuir isotherm presumes monolayer adsorption onto a surface with a finite number of uniformly distributed adsorption sites and no adsorbate transmigration in the mycelium pellet surface. No more sorption may occur at a spot once it has been filled, suggesting that the surface achieves a point of saturation at which the surface's maximal adsorption is realized. The representation for the Langmuir isotherm refers to Formula 3. The linear representation of specific adsorption (C_e/Q_e) vs equilibrium concentration (C_e) demonstrates that adsorption follows the Langmuir model. The values of the constants and Q_m, which refer to the adsorption energy and maximum adsorption capacity, are derived from the slope and interception of the plot.

$$\frac{C_e}{Q_e} = \frac{1}{K_L Q_m} + \frac{Ce}{Q_m} \qquad (3)$$

As an empirical model, the Freundlich isotherm is presented, where Q_e is the quantity adsorbed per unit of adsorbent at equilibrium (mg/g), C_e denotes the equilibrium concentration (mg/L), and K_f and n denote parameters that rely on the adsorbate and adsorbent, respectively. The representation for the Freundlich isotherm refers to Formula 4. The dependent constants K_f and $1/n$ can be obtained by linear regression, Formula 5, where K_f and n, which represent adsorption capacity and intensity, respectively, are the Freundlich constants. The plot of ln Q_e vs. ln C_e was used to calculate the Freundlich equilibrium constants.

$$Q_e = K_f C_e^{1/n} \qquad (4)$$

$$ln\ Q_e = ln\ K_f + \left(\frac{1}{n}\right) ln\ C_e \qquad (5)$$

RESULTS

PHYSICAL AND MICROSCOPIC OBSERVATION

The decolorization process involves the initial biosorption of the dye onto the fungal mycelium, followed by subsequent enzymatic degradation to colorless end products. Biosorption occurs because of the interaction between the reactive groups in the dyes and the active sites on the surface of the fungi (Karim et al. 2020). In this study, observation of Malaysian GLMP was done using a dissecting microscope (DM) to investigate physical characteristics of the adsorbent surface. This was advantageous for supervising the adsorption process, whether it occurred naturally, assessing its adsorption capabilities, and observing cell changes upon the adsorption process. Figure 2(a) is a view of the initial shape of Malaysian GLMP, showing the pellets has a round shape, and initially is yellowish white color. After 72 h of the treatment, there was presence color on the GLMP pellets indicates that adsorption has occurred as shown in Figure 2(b), 2(c) and 2(d). As longer in contact time (24 to 72 h) it can be observed that the color become darker showing increasing capacity of the adsorption process. The physical adsorption of dyes to certain membrane receptors on the surface of the strain aids the decolorization process (Chen & Yien Ting 2015). The dye molecules can bind to the active groups on the cell walls of the fungal mycelium via van der Waals forces, electrostatic interactions or chemical bonds (Thampraphaphon et al. 2022). The mycelium of fungi has notable adsorbing-like functional properties, since its cells can use organic and inorganic materials as a food source, making it a potential biosorbent for pollution control (Li et al. 2022).

EFFECT OF THE ADSORBENT CONCENTRATION

The amount of Malaysian GLMP use as adsorbent significantly influenced the effectiveness of the decolorization the RTWW in the bioreactor. Figure 3 indicates that the different concentrations of Malaysian GLMP (0, 8,75, 12,5 and 25 g/L) had a substantial impact on dye adsorption in terms of percentage removal of undiluted RTWW. Result shows that the decolorization percentage consequently rise with the adsorbent concentration up to 10 g (25 g/L) in 400 mL RTWW. Thus, an adsorbent concentration of 25 g/L was chosen for the subsequent adsorption experiments. These results are in agreement with a different study on the removal of two different classes of dyes using immobilized fungal biomass (Przystaś, Zabłocka-Godlewska & Grabińska-Sota 2018), with results showed that the fungal biomass was effective in decolorizing the dyes, and its quantity influenced the efficiency of the process. Larger amounts of adsorbent increased the availability of adsorption sites and pore surface areas, making it easier for the dye molecules to penetrate and be adsorbed (Abdulsalam et al. 2020). A higher quantity of fungal adsorbent can enhance the decolorization process by providing more adsorption sites for the dye molecules.

EFFECT OF INITIAL pH

Figure 4 shows the results of the decolorization percentage of undiluted RTWW at different set of initial pH. The highest color removal of 61.55% was obtained at pH 4, compared to 46.28% at pH 6, while the unadjusted pH of RTWW (in the range 7.10 - 8.22) corresponds to maximum a removal of 12.57%. It was demonstrated that the highest removal percentage can be achieved through acidification of the wastewater sample at pH 4, hence further experiments were conducted at this pH.

This finding aligns with the research conducted by Mohd Hanafiah et al. (2019), which indicated that fungi are most efficient in reducing COD at lower pH levels. It was found that COD values of wastewaters reduce as pH decreases from its starting value and further reductions occur with extended treatment times (Yang et al. 2021). However, it was demonstrated that very low pH values are detrimental for the cleaning activity of fungi (Birgani et al. 2016). In fact, as the pH rise from 1 to 3, the percentages of COD that were removed increased. A substantial COD decrease was observed at pH 3. Fungi thrive and produce enzymes in the pH range of 3 to 5, which is ideal for wastewater treatment using fungi (Hadibarata et al. 2013; Wang et al. 2019). An increase in pH tends to reduce the decolorization capabilities of

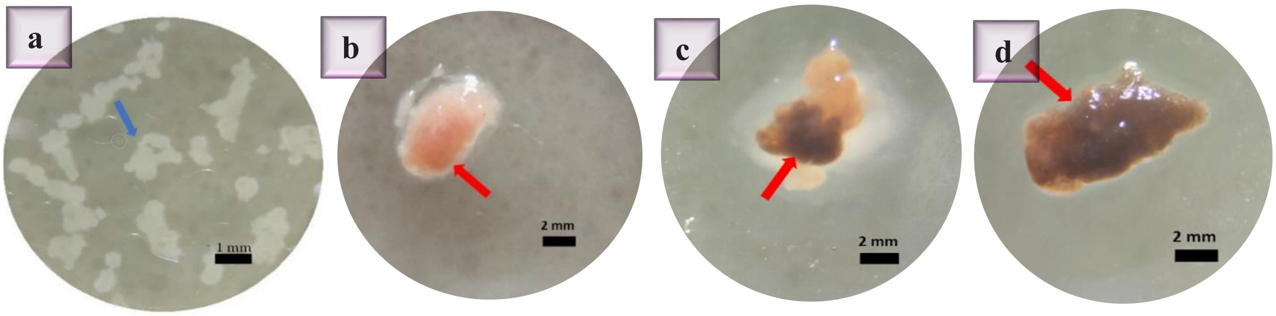

FIGURE 2. Images of Malaysian GLMP treated with RTWW (1:0) obtained with an optical microscope, after different treatment times: (a) 0 h, (b) 24 h, (c) 48 h and (d) 72 h. The blue arrow indicates no dye adsorption while red arrows indicate dye adsorption on the pellet (dense colored on mycelium cells)

fungi. However, the optimal pH for dye decolorization depends on factors such as the type of fungus, the dyes, the medium, and environmental conditions (Mostafa et al. 2019). Mycelium has been shown to have a wide range of application conditions, including temperature and pH ranges, which allow it to maintain high processing capacity for various types of wastewaters, including dye wastewater (Guo et al. 2020).

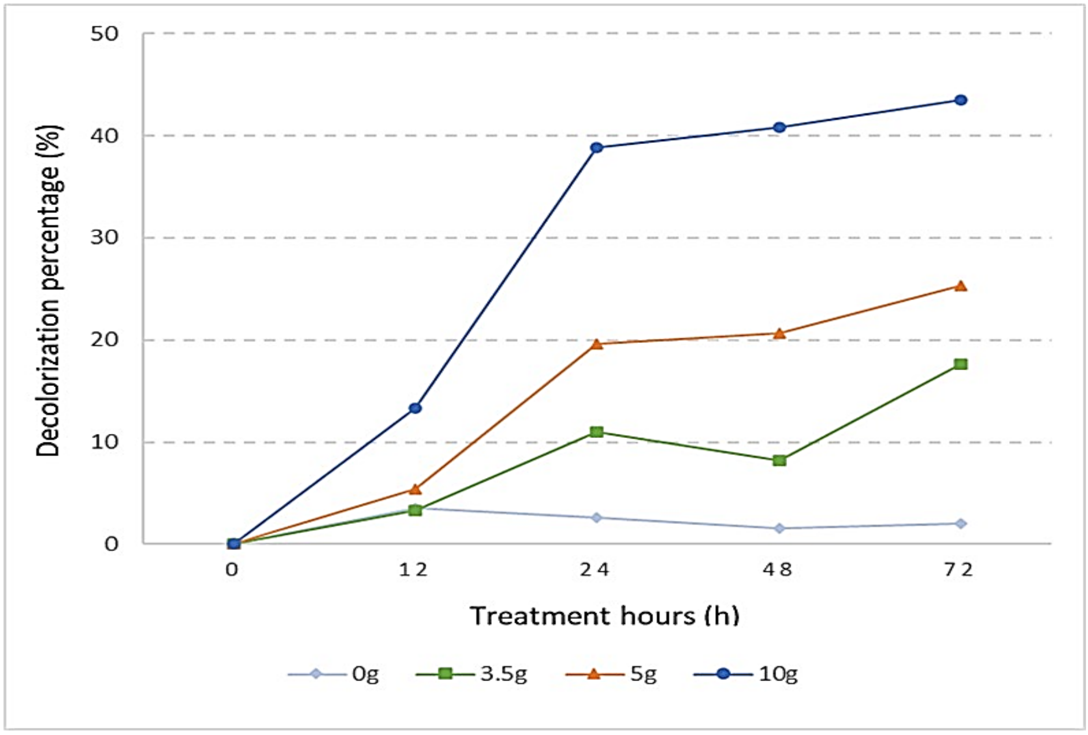

FIGURE 3. The decolorization percentage of undiluted (1:0) real textile wastewater (RTWW) treated with different GLMP amounts, corresponding to 0, 8,75, 12,5 and 25 g/L (0, 3.5, 5 and 10 g)

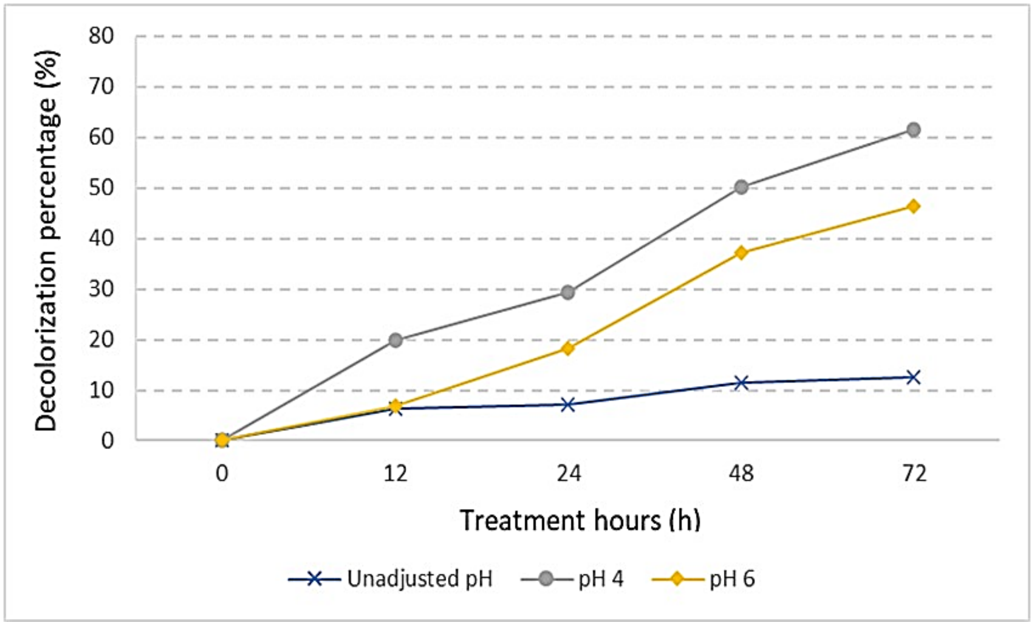

FIGURE 4. Extent of decolorization of RTWW (1:0) at different initial pH values with 25 g/L of Malaysian GLMP

EFFECT OF INITIAL TEXTILE WASTEWATER CONCENTRATION

The effect of the color concentration in the wastewater has been investigated to the 25 g/L of GLMP. This is important in view of industrial application whether it has significant effect to the adsorbent performance. Therefore, three different of concentrations (v/v, %) which were 1:0 (raw), 1:4 (lowest concentration) and 2:3 (intermediate concentration) has been investigated and result of decolorization percentage are shown in Figure 5. It can be observed that the highest percentage of color removal achieved at 72 h of treatment was in highest diluted samples (1:4) of 77.24%, followed by water samples 2:3 with 42.15% and as expected undiluted samples achieved lowest at maximum percentage of 38.75%.

The method of dilution of the initial wastewater can be one of the effective methods for treating high concentrated dye-containing wastewater. Dilution involves mixing the wastewater with a larger volume of clean water, which can help reduce the concentration of dyes and other pollutants in the wastewater. This makes it easier to remove the dyes through various treatment processes efficiently. For the treatment of textile dye wastewater, the impact of dye wastewater concentration is more important than agitation speed (Pratiwi et al. 2017).

Dilution can enhance the effectiveness of adsorption by increasing the contact between the dye molecules and the adsorbent, thus improving the efficiency of the adsorption process, besides enhance the degradation of the organic compound (read as COD) in the wastewater (de Farias Silva, da Silva Gonçalves & de Souza Abud 2016). Adsorption involves the attachment of dye molecules to a solid surface, such as activated carbon, nanomaterials, and bio-adsorbent materials. Lower pollutant concentrations are generally easier to treat, and dilution can enhance the performance of treatment processes, such as biological or chemical treatment, by reducing the inhibitory effects of the pollutants on microorganisms. By diluting the wastewater, the concentration of toxic dyes can be reduced, making the effluent less harmful to the Malaysian GLMP, and resulting in shorter treatment times for specific pollutants (Selvakumar, Manivasagan & Chinnappan 2013). The amount of decolorization increases as the concentration of the initial textile dye wastewater decreases, however, dilution can facilitate easier and shorter treatment

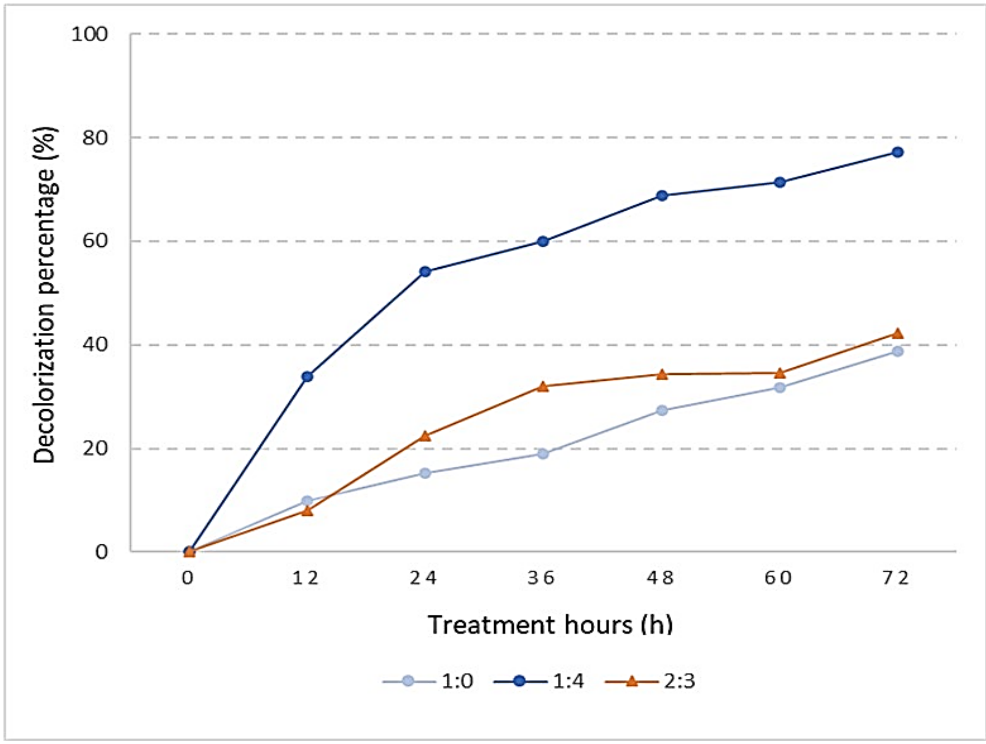

FIGURE 5. Extent of decolorization of real textile wastewater (RTWW) at initial dilutions of 1:0, 1:4 and 2:3 at experimental condition of initial pH = 4 and 25 g/L GLMP concentration

time of textile wastewater, it is important to strike a balance. Excessive dilution may lead to excessive water consumption, increased treatment volumes, and higher operational costs. Therefore, careful consideration should be given to find an optimal dilution ratio that maximizes the treatment efficiency while minimizing associated drawbacks. Competitive adsorption among various colors and other chemicals may occur.

CHEMICAL OXYGEN DEMAND (COD) REDUCTION

Figure 6 shows percentage reduction of chemical oxygen demand (COD) in different effect of initial wastewater concentration in order to assess the bio-adsorbent's suitability for varying wastewater dilution levels. The highest percentage was determined at 78.32% of treatment with a higher wastewater dilution of 1:4 v/v, 10 g of Malaysian GLMP at 36 h of similar treatment. A study found that Malaysian *Ganoderma lucidum* inoculum exhibited excellent COD and ammonia degradation, achieving elimination percentages ranging from 95% to 100% within 30 h of treatment of synthetic domestic wastewater due to presence of carbon source (Mooralitharan et al. 2023).

Beyond 36 h, for the 1:4 v/v wastewater dilution ratio, the COD percentage drops again (resulting increasing in organic content), most likely due to the release of the metabolites generated by the degradation process induced by the fungi pellets (Khouni, Marrot & Ben Amar 2012). On the other hand, the reduction in nutrient content in the solution due to degradation and adsorption GLMP in the previous period, leads to the limited nutrients required by the subsequent GLMP, thus, some of the pellets died and produced certain toxic substances that increase the COD concentration (Wu et al. 2022). More concentrated RTWW contains higher pollutant and dye concentrations, which can inhibit fungi growth, disrupt their metabolic processes, and even lead to fungi cell death, consequently increasing the COD of the RTWW. The COD reduction percentage for the 1:0 v/v wastewater dilution was low. More concentrated wastewater often requires a higher adsorbent concentration for effective treatment, as wastewater treatment with fungi typically starts with physical rather than chemical processes. Competitive adsorption between chemicals may occur, resulting in the declining of COD removal capabilities. In the presence of higher toxic compounds, fungi may struggle to thrive and effectively reduce COD.

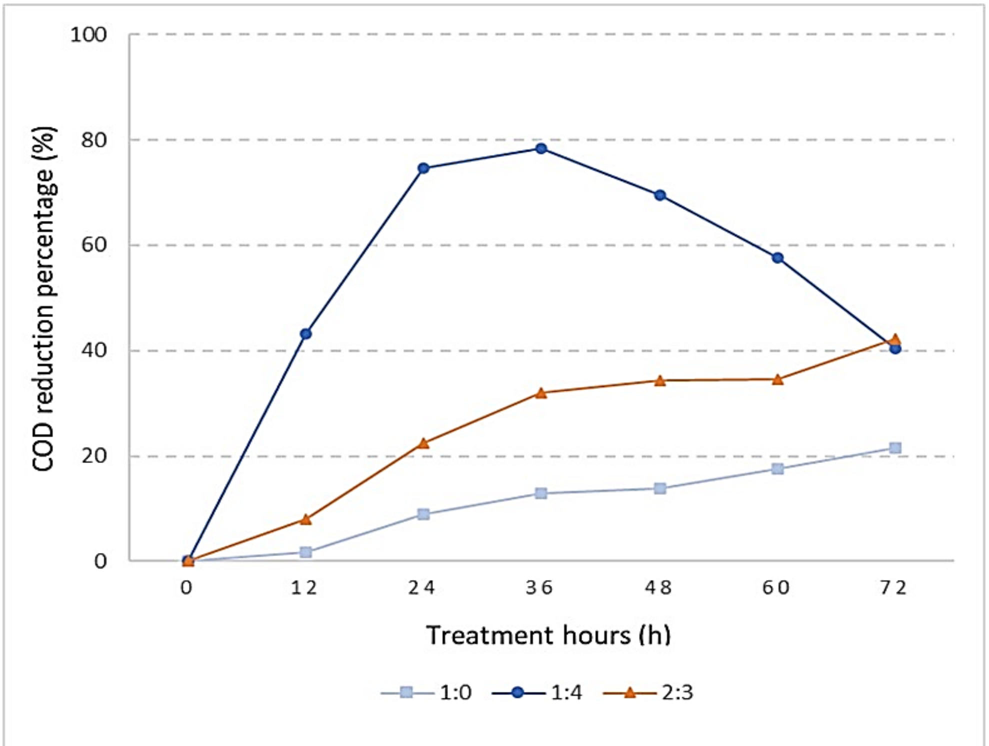

FIGURE 6. The COD reduction percentage of RTWW based on RTWW dilution ratios

ADSORPTION ISOTHERMS

Langmuir and Freundlich models are generally employed to rationalize the equilibrium isotherms of various biomass-derived sorbents used to remove dyes from wastewater (Olivito et al. 2021). Table 2 shows the adsorption constants and correlation coefficients (R^2) of the two models calculated from the data obtained in current study.

The decontamination by Malaysian GLMP fits both Langmuir and Freundlich adsorption isotherms for wastewater dilution of 1:4 v/v (Figure 7(a) & 7(b)). The R^2 correlation coefficient values are 0.9479 and 0.9438, respectively. For 2:3 v/v wastewater dilution (Figure 7(c) & 7(d)) the correlation coefficient for the linearity pattern derived from the Langmuir isotherm equation is R^2 = 0.9263, while for the Freundlich isotherm, it is R^2 = 0.8646. This shows that because the R^2 value is closer to linearity, the adsorption process is consistent with the Langmuir isotherm model. The number of groups that the adsorbent can exchange is known as its adsorption capacity. The term 'adsorption exchange capacity' refers to the quantity of ions that can be exchanged for each

TABLE 2. Langmuir and Freundlich adsorption isotherms parameters

Initial concentration (v/v, dilution)	Langmuir isotherm			Freundlich isotherm		
	Q_m (mg/g)	K_L (L/mg)	R^2	K_f	n	R^2
1:4	0.0866	2.6820	0.9479	0.9463	0.9463	0.9438
2:3	0.0257	0.3165	0.8646	0.0091	0.2561	0.9263

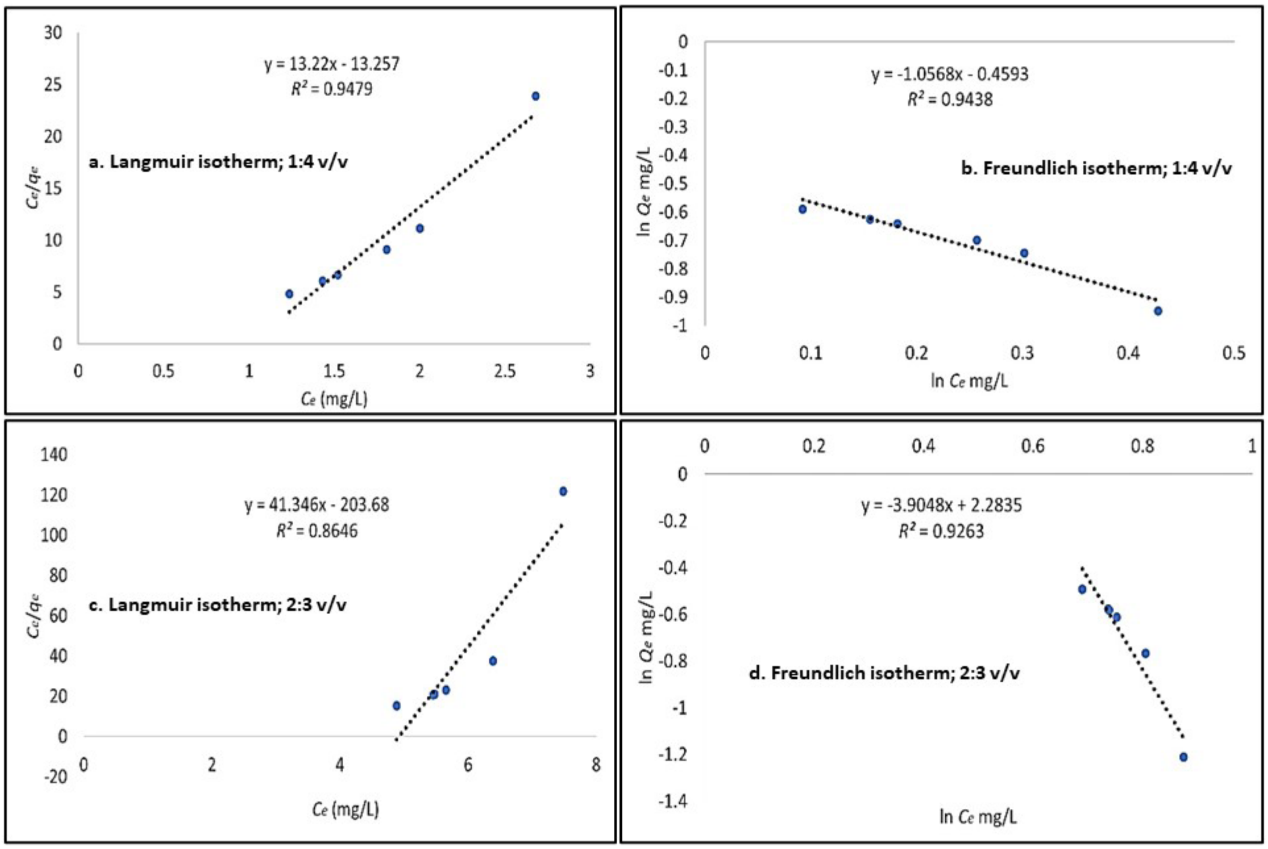

FIGURE 7. (a) Langmuir isotherm, (b) Freundlich isotherm showing the adsorption of RTWW color onto Malaysian GLMP for 1:4 v/v wastewater dilution; and (c) Langmuir isotherm, (d) Freundlich isotherm showing the adsorption of RTWW color onto Malaysian GLMP for 2:3 v/v wastewater dilution

gram of dry adsorbent or for each milliliter of wet adsorbent (Syauqiah et al. 2022). An empirical model known as the Langmuir isotherm makes the assumption that the adsorbed layer is one molecule thick (monolayer adsorption), with the adsorption process taking place at identical and equivalent specific localized sites (Al-Ghouti & Da'ana 2020). Based on the linear form of the Freundlich equation, if value $n = 1$, adsorption is linear; if $n < 1$, suggests chemical adsorption; and if $n > 1$, implies physical adsorption (Sah et al. 2022). The n number indicates the degree of nonlinearity between solution concentration and adsorption. From the result, $n = 0.9463$ for 1:4 wastewater dilution and $n = 0.25610$ for 2:3 wastewater dilution. All chemical interactions that take place in the single absorption layer on the surface of the adsorbent cell wall are due to the interaction between the adsorbent's active site and the adsorbate, which involves chemical bonds (Syauqiah et al. 2022). Due to chemical interaction ($n < 1$), commonly irreversible and monolayer adsorption process is described by the Freundlich adsorption at constant temperature model. This mechanism employed by Freundlich adsorption-chemisorption process also imitate Langmuir homogeneity on monolayer adsorption thus support GLMP as adsorbent fits with both models (Al-Ghouti & Da'ana 2020).

DISCUSSION

The adsorption of dye and COD using Malaysian GLMP was compared with studies using other dyes and wastewater, as shown in Table 3. This study showed the decolorization rate of 77.24% within 72 h of treatment using Malaysian GLMP as the primary adsorbent. When compared to the current works on adsorption of dyes using GLMP, this study recorded a shorter treatment time than those using real dye containing wastewater, maintaining a good removal efficiency (Pratiwi et al. 2017; Rainert et al. 2021; Selvakumar, Manivasagan & Chinnappan 2013).

TABLE 3. Comparison of the adsorption of dye and COD using GLMP

Type	Compound removed	Treatment time	COD reduction	Color reduction	References
Malaysian GLMP	Real textile wastewater	72 h	-	77.24%	This study
		36 h	78.32%	-	
Wild-Serbian *Ganoderma lucidum* mycelium	Synthetic domestic sewage	120 h	96.00%	Na	Mohd Hanafiah et al. (2019)
G. lucidum mycelium	Paper mill effluent	15 to 18 days	98.00%	94.00%	Perumal, Murugesan & Kalaichelvan (2000)
G. lucidum mycelium	Textile dye wastewater	120 h	81.40%	91.30%	Selvakumar, Manivasagan & Chinnappan (2013)
G. lucidum mycelium	Naphthol black	30 days	81.03%	60.53%	Pratiwi et al. (2017)
Wild-Serbian *Ganoderma lucidum* mycelium	Synthetic domestic wastewater	15 h	92.9%	na	Mohd Hanafiah et al. (2022)
Malaysian *Ganoderma lucidum*	Inoculum synthetic domestic wastewater	30 h	>95.00%	na	Mooralitharan et al. (2023)

na: not available

Similar achievement was obtained for the COD level, with a good compromise betweentime and removal percentage. The COD reduction in RTWW reached 78.32% within 36 h oftreatment. The variability of the data (Mohd Hanafiah et al. 2019; Mooralitharan et al. 2023;Perumal, Murugesan & Kalaichelvan 2000; Pratiwi et al. 2017; Selvakumar, Manivasagan &Chinnappan 2013) is influenced by the individual characteristics of RTWW, where textileproduction factories typically use a wide variety of dyes and toxic chemicals. A study usingGanoderma lucidum treated with Naphthol Black dye in batik effluent showed thatdecolorization increased as COD degradation increased. This might be due to the presence ofnutrient elements contained in batik effluent for the fungi to growth. With increasingincubation time and dye concentration, the percentage of decolorization and COD degradationincreased gradually, in contrast with the conclusions of a general study stating that theeffluent's capacity to remove COD is reduced by a higher dye concentration (Pratiwi et al.2017).

It was found that the decolorization of dye wastewater is positively impacted by agitation speed and wastewater concentration, and negatively impacted by pH and temperature. Temperature has a negative impact on COD reduction, while pH, agitation speed, and dye wastewater concentration demonstrate positive contributions. It was discovered that the maximum decolorization and COD reduction at the optimal conditions were 81.4 and 90.3%, respectively (Selvakumar, Manivasagan & Chinnappan 2013). Studies using domestic wastewater have been conducted to determine the COD reduction using *Ganoderma lucidum*. In an experiment, the optimal performance of the GLMPs within 15 h of retention time with a percentage removal of 92.9% for COD was demonstrated at an agitation speed of 100 rpm and an ambient temperature of 25 °C (Hanafiah et al. 2022). The robust performance demonstrated in this research highlights GLMP's capability to address the complex challenges of wastewater remediation.

While this eco-friendly approach shows great potential, it is not without its limitations and challenges. This brief introduction will touch upon the existing constraints, the challenges faced in the process, and outline potential future strategies to enhance and optimize fungi decolorization methods. Addressing these aspects is crucial for advancing the application of fungi in wastewater treatment and ensuring sustainable and effective decolorization processes (Figure 8):

Composition and characteristics of industrial wastewater

GLMP may not be equally effective for the decolorization and COD reduction of all types of industrial wastewater. The efficiency of decolorization may vary depending on the composition and characteristics of the effluent. The presence of toxicity in some industrial effluents and wastewater may inhibit the growth of GLMP, thereby limiting their ability to decolorize and reduce the COD of the effluent. The accumulation of metabolic waste during treatment induced cell death, potentially reducing the effectiveness of GLMP removal performance (Mohd Hanafiah et al. 2019).

Treatment efficiency enhancement

In terms of application, immobilized fungal mycelium has proven to be effective in treating dye wastewater. Immobilized mycelium exhibits a high processing capacity for various types of wastewaters, including dye wastewater. It can maintain high activity even after multiple batches of wastewater treatment, allowing for continuous treatment (Guo et al. 2020). Advancements in genetic engineering could be used to enhance the efficiency of GLMP for decolorization and COD reduction by modifying their metabolic pathways or enhancing their ability to degrade specific compounds. Combining GLMP with other microorganisms, enzymes, or other types of natural adsorbents like zeolite or activated carbon could improve decolorization efficiency, particularly for challenging effluents. Immobilizing mycelium on suitable supports, such as nylon sponges, can enhance the decolorization efficiency (Thampraphaphon et al. 2022). Combining GLMP with activated Zeolite may reduce a great amount of color and COD which recorded high color removal percentage of 98.7% and COD reduction percentage of 66.7% using real textile wastewater. Continual optimization of the process parameters, such as temperature, pH, and agitation, can lead to increased efficiency and reduced costs for GLMP decolorization and COD reduction.

Lack of research using the same fungi

Without sufficient research, there may be a significant knowledge gap regarding the effectiveness, optimal conditions, and limitations of using mycelium pellets for textile wastewater treatment. This lack of understanding can hinder the development of appropriate policies and guidelines for implementation. The absence of

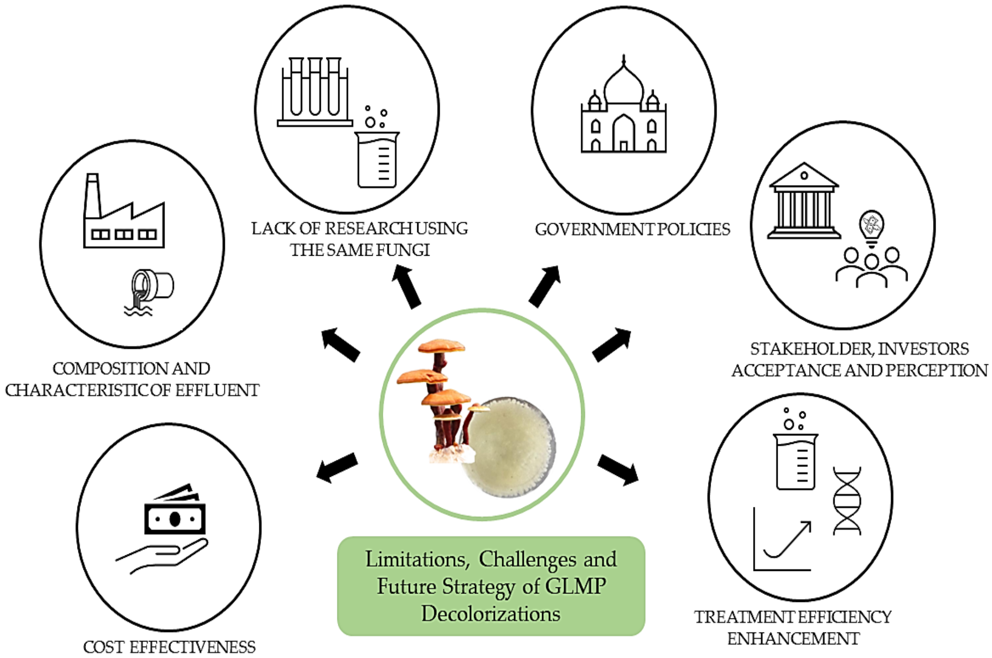

FIGURE 8. Limitations, challenges and future strategy of GLMP decolorizations

comprehensive research can lead to uncertainty about the performance and reliability of mycelium-based bioremediation techniques for textile wastewater. Without robust data on removal efficiency, treatment capacity, and the potential for byproduct formation, decision-makers may hesitate to promote or invest in such methods.

Government policies

Government policies may be limited by a lack of awareness and understanding of the potential benefits and effectiveness of using mycelium pellets for bioremediation. This could result in inadequate support, funding, or incentives for the implementation of such technologies. The lack of standardized protocols and certification processes for mycelium-based bioremediation methods could hinder their widespread adoption. Governments may be hesitant to endorse or incentivize these techniques without clear guidelines or proven efficacy.

Conclusions

In conclusion, the use of GLMP for the decolorization and COD reduction of industrial wastewater furnished promising results. Adsorbent dose of 10 g, initial pH of 4 and wastewater dilution of 1:4 v/v resulting in the maximum removal of color and COD. Assimilating this natural bio-adsorbent within the existing wastewater treatment system will be able to improve the treatment mechanism of the toxic dye containing textile wastewater. Further research and development in this area, along with advancements in genetic engineering and process optimization, could lead to more efficient and cost-effective methods for decolorization and COD reduction of general industrial wastewater. Harnessing the unique properties of GLMP will offer a sustainable and efficient pathway for advancing wastewater treatment technologies, marking a significant stride towards a cleaner and healthier environment.

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