PERFORMANCE OF MALAYSIAN *Ganoderma lucidum* MYCELIUM IN TREATING TEXTILE WASTEWATER DYES USING BATCH BIOREACTOR

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FACULTY OF SCIENCE
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PERFORMANCE OF MALAYSIAN *Ganoderma lucidum* MYCELIUM IN TREATING TEXTILE WASTEWATER DYES USING BATCH BIOREACTOR

ABSTRACT

The treatment of textile wastewater dyes that contain many types of toxic and harmful pollutants is often challenging for the industries themselves to meet the effluent discharge quality requirements, which mostly use physical-chemical and adsorption as their primary treatment methods. The level of toxic and hazardous organic substances in a wastewater effluent was easily quantify by chemical oxygen demand (COD). The higher the COD value, the more serious the organic contamination of the water. Over the years, there is an exponential interest in using the antioxidant, antitumor, anti-inflammatory, antifungal, and antimicrobial *Ganoderma lucidum* fungi as bioadsorption material to minimize the impacts of harmful by-products derived from current wastewater treatment on the ecosystem. Less studies have been conducted using real wastewater, makes this study is important to determine its effectiveness. This research determines the bioadsorption capacity of the native Malaysian *Ganoderma lucidum* in its mycelium pellets (Malaysian GLMP) in treating real textile wastewater dyes (RTWW) by measuring the decolourization and COD reduction, under unsterilized and ambient temperature (26-34 °C) condition. This new treatment method uses a stirred batch bioreactor, as it is more practical and easier to be applied towards current available wastewater treatment process system. The treatment studies vary in the Malaysian GLMP volume in grams (g), initial treatment pH, and wastewater dilution ratios, with a fixed aeration rate of 4 L/min and agitation speed of 150 rpm for complete treatment cycle of 72 hours (h). The treatment proved to be effective in both dye decolorization and COD reduction which the highest percentage of decolorization observed was 77.24% in 72-h, whereas COD reductions were 78.32% in 36-h, with initial treatment pH of 4 and wastewater dilution ratio of 1:4.

Langmuir and Freundlich adsorption isotherm models gave a superior fit ($R^2<1$), indicating the existence of both homogenous and heterogeneous binding sites on the cells. Using Malaysian GLMP as bioadsortion material in a stirred batch bioreactor offers a sustainable and environmentally friendly wastewater treatment solution which shows great potential in reducing the colour intensity and organic pollutants of real textile wastewater dyes.

Findings from this study are useful for implementing and assimilating bioremediation using Malaysian GLMP as bioadsorption material to the existing wastewater treatment in the industry. Further studies could be made by replicating this treatment method using Malaysian GLMP together with the other commercially available adsorption agents.

Keywords: Malaysian *Ganoderma lucidum;* Lingzhi mushroom; real textile wastewater; Azodyes; bioreactor wastewater treatment; bioadsorbent; decolorization; chemical oxygen demand (COD).

PRESTASI *Ganoderma lucidum* MISELIUM DALAM MERAWAT AIR SISA BUANGAN TEKSTIL MENGGUNAKAN BIOREAKTOR KUMPULAN

ABSTRAK

Rawatan air sisa kumbahan tekstil yang mengandungi pewarna serta mengandungi banyak jenis bahan pencemar beracun dan berbahaya seringkali memberi cabaran kepada bioreact itu sendiri untuk memenuhi keperluan kualiti pelepasan air sisa kumbahan bioreact, yang kebanyakan menggunakan kaedah rawatan fizikal-kimia dan adsorpsi sebagai kaedah rawatan utama mereka. Tahap kandungan bahan toksik dan organik merbahaya dapat ditentukan oleh permintaan oksigen kimia (COD). Semakin tinggi bacaan nilai COD, semakin serius pencemaran organik tersebut di dalam air. Tahun demi tahun, terdapat minat eksponensial dalam penggunaan anti-oksidan, antitumor, anti-radang, antifungal dan antimikrob cendawan *Ganoderma lucidum* sebagai bahan bioadsorpsi untuk meminimakan kesan sampingan berbahaya yang berasal daripada rawatan air sisa kumbahan kepada ekosistem. Kurang kajian yang dijalankan menggunakan air sisa sebenar, menjadikan kajian ini penting untuk menentukan tahap keberkesanannya. Penyelidikan ini menentukan keupayaan bioadsorpsi *Ganoderma lucidum* asli Malaysia dalam bentuk pallet miselium (GLMP Malaysia) dalam rawatan pewarna air sisa kumbahan tekstil sebenar dengan mengukur tahap penyahwarnaan dan pengurangan permintaan oksigen kimia (COD), di dalam keadaan tidak steril dan suhu persekitaran (26-34 °C). Kaedah rawatan baru ini menggunakan bioreactor batch bercampur (Stirred Batch Bioreactor), kerana ia lebih praktikal dan lebih mudah digunakan terhadap sistem proses rawatan air sisa kumbahan sedia ada. Kajian rawatan dijalankan dengan perbezaan dalam jumlah palet miselium yang digunakan didalam gram (g), pH rawatan permulaan, dan nisbah pencairan air sisa kumbahan, dengan kadar pengudaraan tetap 4 L/min dan kelajuan agitasi 150 rpm untuk kitaran rawatan lengkap selama 72 jam (h). Rawatan ini terbukti berkesan dalam kedua-dua penyahwarnaan dan pengurangan COD yang peratusan tertinggi penyahwarnaan yang direkod ialah 77.24% pada 72-h, manakala pengurangan COD ialah 78.32% pada 36-h, dengan pH rawatan awal 4 dan nisbah pencairan air sisa kumbahan 1:4. Model isoterma penjerapan Langmuir dan Freundlich memberikan padanan yang baik ($R^2<1$), menunjukkan kewujudan tapak pengikatan bioreactor. Menggunakan GLMP Malaysia sebagai bahan biopenjerap dalam bioreactor batch campuran menawarkan penyelesaian rawatan air sisa kumbahan yang mampan dan mesra alam sekitar yang menunjukkan potensi yang besar dalam mengurangkan intensiti warna dan pencemar organik dari pewarna air sisa kumbahan tekstil sebenar.

Hasil daripada kajian ini berguna untuk digunapakai dan assimilasi rawatan secara bioremediasi menggunakan cendawan GLMP Malaysia sebagai bahan bioadsorpsi kepada rawatan air sisa yang sedia ada di dalam industri. Kajian lanjut boleh dilakukan dengan mengulangi kaedah rawatan ini menggunakan GLMP Malaysia bersama dengan agen adsorpsi lain yang boleh didapati secara komersial.

Kata kunci: *Ganoderma lucidum* Malaysia; cendawan Lingzhi; air sisa kumbahan tekstil; Azodyes; rawatan bioreaktor air sisa kumbahan; biopenjerap; penyahwarnaan; permintaan oksigen kimia (COD).

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Allah blesses all of you.

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LIST OF SYMBOLS AND ABBREVIATIONS

C_0	:	Initial colour
C_t	:	End of treatment colour
COD_0	:	Initial chemical oxygen demand
$CODt$	:	End of treatment chemical oxygen demand
C_i	:	Initial dye colour concentration
C_e	:	Equilibrium dye colour concentration
K_f		Adsorption capacity
K_L		Adsorption energy
n		Adsorption intensity
Q_e	:	Quantity of dye colour uptake from solution by mass of adsorbent
Q_m	:	Maximum adsorption capacity
W	:	Weight of the adsorbent
%	:	Percentage
°C	:	Degree Celsius
g	:	Gram
h	:	Hour
L	:	Liters
L/min	:	Liter per minute
mL	:	Milliliters
mm	:	Millimeters
mg/L	:	Milligrams per Liter
mg/g	:	Milligram per gram
µg/mL	:	Microgram per mililiters
AC	:	Activated Carbon

AD	:	Aerobic Digesters
ADMI	:	American Dye Manufacturers Institute
BF	:	Biofilter
BOD	:	Biological Oxygen Demand
COD	:	Chemical Oxygen Demand
CSTR	:	Continuous Stirred Tanks Reactors
DM	:	Dissecting Microscope
GLMP	:	*Ganoderma lucidum* Mycelium Pallet
KH_2PO_4	:	Potassium dihydrogen phosphate
LC50	:	Lethal Concentration 50%
MBR	:	Membrane Bioreactors
MBBR	:	Moving Bed Biofilm Reactor
MFC	:	Microbial Fuel Cell
$MgSO_4$	:	Magnesium sulfate
MIDA	:	Malaysian Investment Development Authority
MLSS	:	Mixed Liquor Suspended Solid
NA	:	Not Available
NH_4Cl	:	Ammonium chloride
NH_3–N	:	Ammoniacal Nitrogen
PAH	:	Polycyclic aromatic hydrocarbons
PBB	:	Packed Bed Bioreactor
PtCo	:	Platinum-Cobalt
rpm	:	Revolutions per minute
RTWW	:	Real Textile Wastewater
SBR	:	Sequencing Batch Reactor
SDG	:	Sustainable Development Goal

STR	:	Stirred Tank Reactors
TSS	:	Total Suspended Solids
UASB	:	Upflow Anaerobic Sludge Blanket

CHAPTER 1: INTRODUCTION

1.1 Research background

Bioremediation of textile wastewater is extensively being researched to create an environmentally friendly treatment method for such wastewater (Al-Tohamy et al., 2022); Idris et al. (2007). There are some challenges in meeting the discharge limits for the conventional wastewater treatment system to treat all the pollutants in the wastewater, such as the inefficiency of coagulants that lead to high consumption of chemicals used, high pricing of absorbance materials (e.g.: activated carbon), and needs of high electrical power of the wastewater treatment system (Idris et al., 2007; Legorreta-Castañeda et al., 2020). This wastewater contains various pollutants, including a high content of organic matter and colour traces. Thus, the industries are responsible for treating the highly polluted wastewater to meet the local country's regulation to minimize the pollution effects on the respective water bodies. In Malaysia, the related regulation is the Environmental Quality (Industrial Effluent) Regulations, 2009 which legislated by the Department of Environment, Malaysia.

Manufacturing of textile and textile products in Malaysia is one of the largest exporting industries, which contributing approximately RM 15.82 billion in total manufacturing goods export (Malaysia Investment Performance Report 2019, Malaysian Investment Development Authority, MIDA). It was expected to reach approximately RM 24 billion in revenue in 2023, with the industry expected to grow at a rate of more than four per cent over the next few years to 2027, with 19 new projects approved (Malaysia Investment Performance Report 2022, Malaysian Investment Development Authority, MIDA).

The manufacturing of textile and textile products is expected to result in a significant amount of wastewater discharge, characterized by a substantial concentration of pollutants during the runoff stages of textile dyeing and the rinsing of natural fabrics. It has been estimated that 200 L of water is applied in the production of 1 kg of textile material. Water is consumed during the chemical application process on the fabrics and during the rinse process of the final products (Ghaly et al., 2014). One of the significant contaminants is dyes, which because of their chemical structure, are resistant to fading on exposure to light, water, and many chemicals (Al-Tohamy et al., 2022; Przystaś et al., 2018; Robinson et al., 2001). They also contribute to the increase of biological oxygen demand (BOD) and chemical oxygen demand (COD) in industrial wastewater, which will lead to an increase in the cost of treatment (Al-Tohamy et al., 2022; Lellis et al., 2019).

Current wastewater treatment methods in industries use chemicals that produce other contaminants as their by-products, and waste (sludge) that needs to be further treated before being disposed to landfill. This coloured 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, as dyes may remain longer in the environment (20 to 50 years or more) because of their high stability in the effluents (Ardila-Leal et al., 2021; Pereira & Alves, 2012; Przystaś et al., 2018; Tkaczyk et al., 2020).

Fungi may employ an extracellular enzymatic system to convert aromatic compounds including lignin, polycyclic aromatic hydrocarbons (PAH) and pesticides (Przystaś et al., 2018). Using mycelium pallets of a fungi as bioadsorption material shown high potential in treating dye contain wastewater as it is eco-friendly, un-toxic, have an excellent self-immobilization capability, and are simple to filter (Wang et al., 2019).

By using fungi as treatment media instead of chemicals, the waste generated, such as sludge, can be safely reused as soil fertilizer and effluent discharge can be used as plant fertilizer as the presence of certain enzymes within the composition of the substance under consideration potentially contributes advantageous effects to both the soil and the plants (Bergsten-Torralba et al., 2009; Hossain et al., 2016; Legorreta-Castañeda et al., 2020; Wang et al., 2019).

Treatment utilizing this strategy has been shown to be less effective and consume longer time. Further research is required to create an improved, zero-waste technique for wastewater treatment, as well as to reduce environmental and hazard towards public health within the transition from laboratory to a pilot scale. (Al-Tohamy et al., 2022). Several wastewater treatment research using Malaysian *Ganoderma lucidum* mycelium pallets (Malaysian GLMP) in bioreactors has revealed a high potential of employing fungi in its mycelium pallet forms in the wastewater treatment process, resulting higher treatment capabilities and shorter time needed (Mohd Hanafiah et al., 2019; Mooralitharan et al., 2023).

A research using wild-Serbian *Ganoderma lucidum* mycelium in treating synthetic domestic wastewater recorded removal rate of 96.0% of COD and 93.2 % of NH_3-N under its optimal conditions using batch bioreactor (Mohd Hanafiah et al., 2019). Another research using Malaysian GLMP in treatment of synthetically prepared wastewater obtained highest COD and ammonia degradation with removal of 95% to 100% rate within 30 hours (Mooralitharan et al., 2023). This shows that by using the mycelium pallets in bioreactor treatment process, have potential to further enhance the treatment capabilities and improve the treatment time of using the real sample obtained from wastewater of the specified industries (ElMekawy et al., 2015; Wan-Mohtar et al., 2023).

These environmentally friendly treatment method are aligned with the United Nation's SDG 6: Clean Water and Sanitation as its ability to treat high polluted textile wastewater effluent will contribute to the more cleaner water sources, SDG 3: Good Health and Well Being as it will improving human health by reducing exposure to harmful substances or pollutants and also SDG 11: Sustainable Cities and Communities where it can help reducing the pollution level, creating healthier environment for the communities around this particular industrial area.

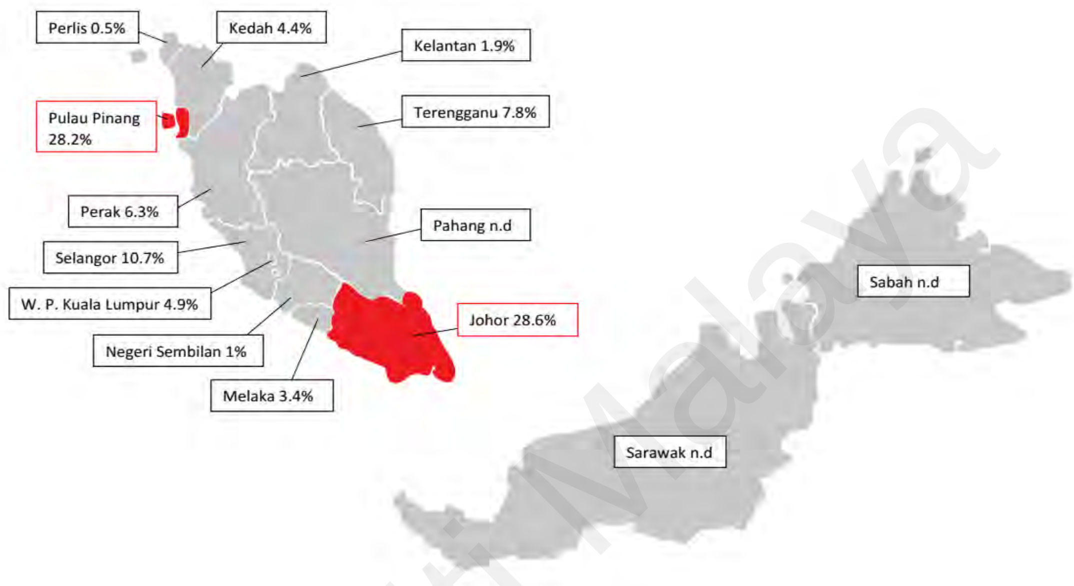
The usage of Malaysian GLMP as bioadsorption media will promote in using natural, sustainable alternatives for wastewater treatment, aligning with principles of the SDG 12: Responsible Consumptiona and Production. Reducing pollutants in water and soil will contributes to the preservation effort towards marine and terrestrial ecosystems, supporting biodiversity conservations, which aligned with the SDG 14: Life Below Water and SDG 15: Life on Land.

1.2 Problem Statement

Untreated wastewater that is being discharged directly through waterbodies such as rivers, drainage, or dams poses a threat to water treatment plants that using it as their source of raw water. There are several cases from 2020 to 2023 whereby pollution in river water led to disruption and stoppage of major water treatment plants in Malaysia. The pollution has been detected by referring to the abnormality of the river water colours and smells. Colour is typically the first contaminant identified because very small amounts of synthetic dyes (less than 1 mg/L) in the water are very visible, impacting the aesthetics, transparency and solubility of the water bodies (Pereira & Alves, 2012). This has impacted hundreds of thousands of consumers where clean tap water cannot be supplied to them resulting in interference in their daily life activities. Suppose this polluted water source being processed by a conventional water treatment plant. In that case, some of the toxicant and harmful substances may still contains in the treated water supplied, will poisoning the consumers.

Wastewater originating from the textile industry poses a significant environmental hazard in numerous regions across the globe due to the potential carcinogenic properties of the degradation byproducts of textile dyes. Furthermore, the presence of textile dyes obstructs light absorption, thereby causing detrimental effects on photosynthetic aquatic flora and algae. Consequently, this leads to anaerobic conditions in water bodies, resulting in the depletion of oxygen levels. It also contributes to an increase in biological oxygen demand (BOD) and chemical oxygen demand (COD) in industrial wastewater (Al-Tohamy et al., 2022; Ardila-Leal et al., 2021; Singh et al., 2022; Thampraphaphon et al., 2022).

Figure 1.1 shows the available data provided by the Department of Environment, Malaysia for the year 2013 which the highest textile water pollution occurred in the states of Johor (28.6%) and Penang (28.2%). These two states have the biggest textile industrial area compared to the other states in Malaysia (Pang & Abdullah, 2013).



*The n.d means not detected or not reported

Figure 1.1: Contribution of textile water pollution in Malaysia (Pang & Abdullah 2013).

During the processing stage, a certain percentage (ranging from 5-20%) of the utilized dyestuffs are discharged into the process water (Ghaly et al., 2014; Sahu & Singh, 2019). Among all the constituents, the dye component poses the greatest challenge when it comes to conventional biological wastewater treatment (Bhatia et al., 2017). Synthetic dyes, apart from their visual impact and negative influence on chemical oxygen demand, often exhibit toxic, mutagenic, and carcinogenic properties (Ardila-Leal et al., 2021; Pereira & Alves, 2012). The presently available methods for treating wastewater containing dyes are associated with high expenses, the generation of hazardous byproducts, and substantial energy consumption (Al-Tohamy et al., 2022; Idris et al., 2007; Lellis et al., 2019; Robinson et al., 2001). There is an imperative to foster the advancement of ecologically sustainable techniques as chemically physicochemical treatment methods possess the potential to inflict harm upon the environment, while conventional biological treatment methods encounter constraints in effectively eliminating colouration (Dhruv Patel & Bhatt, 2022; Holkar et al., 2016).

Bioremediation treatment methods have garnered significant attention in the treatment of diverse wastewaters due to their cost-effectiveness, operational feasibility, and notable efficiencies. Further to the high potential shown by recent synthetically prepared wastewater treatment using fungi mycelium pallets in batch bioreactor, using the real wastewater will further enhance the determination of optimal conditions in achieving the highest pollutants reduction rate (Mohd Hanafiah et al., 2019; Mooralitharan et al., 2023; Narayanan & Narayan, 2019). Consequently, the utilization of Malaysian *Ganoderma lucidum* mycelium pellets for dye treatment offers a viable and environmentally friendly approach, resulting in the generation of non-hazardous byproducts and waste while maintaining cost-effectiveness (Bhatia et al., 2017; Holkar et al., 2016; Narayanan & Narayan, 2019).

1.3 Research objectives

1. To investigate the decolorization capacity through bioremediation of mycelium pellets derived from Malaysian *Ganoderma lucidum* towards real textile's dye wastewater using a batch bioreactor.
2. To assess the efficacy of Malaysian *Ganoderma lucidum* mycelium pellets in the removal of chemical oxygen demand (COD) of the real textile's dye wastewater.
3. To determine the optimum conditions of the batch bioreactor treatment system under various conditions which the pH of initial treatment, adsorbent volumes with parallel conditions of aeration rate, agitation rate, and treatment time.

1.4 Scope of research

Evaluating the effectiveness of Malaysian *Ganoderma lucidum* mycelium pallets (Malaysian GLMP) in eliminating textile wastewater dyes using batch bioreactor processes. Through an examination of the efficiency of this natural bioabsorption medium, this research aims to contribute significant knowledge regarding sustainable and environmentally friendly strategies for addressing the issue of pollution caused by the textile industry.

Very small amounts of synthetic dyes (less than 1 mg/L) in the water are very visible, impacting the aesthetics, transparency, and solubility of the water bodies. This makes colour as the first typical contaminant to be identified in waterbodies and wastewater (Pereira & Alves, 2012).

Biological oxygen demand (BOD) and chemical oxygen demand (COD) in a wastewater effluent shows the presence of organic pollutants that are toxic and harmful to the environment. The higher degree of BOD and COD contains, the higher pollutants need to be treated before the effluent can be discharge into the waterbodies. COD are regularly monitored in wastewater treatment plant because it is much easier and shorter time to test which require about 4 hours compared to BOD which require 5 days in obtaining the test results.

Several research shows great potential in using *Ganoderma lucidum* mycelium pallets (GLMP) in treating the synthetically prepared wastewater. Research had found that treatment using *Ganoderma lucidum* shows great potential for synthetic domestic sewage which recorded 96% Ammonia (NH_3-N) reduction within 48 hours (Mohd Hanafiah et al., 2019). Another research found that the Malaysian *Ganoderma lucidum* reducing 95% of the chemical oxygen demand (COD) in treating synthetic domestic sewage (Mooralitharan et al., 2023). Lack of research conducted using Malaysian GLMP in treating the real wastewater obtained from the industries shows the critical necessity of assessing its efficacy towards real wastewater that has higher concentration of toxic and hazardous substances.

CHAPTER 2: LITERATURE REVIEW

2.1 Textile dye wastewater

The textile and textile products industry significantly contributed to economic growth in Malaysia as it was one of the nation's largest exporting industries in 2019 as per reported by Malaysian Investment Development Authority (MIDA), landing approximately RM 15.82 billion of Malaysia's total manufactured goods exports (Malaysia Investment Performance Report 2019, MIDA). The top market for Malaysia's exported textile products is the United States, which purchased RM1.6 billion, 13% of the industry's total exports (Malaysia Investment Performance Report 2018, MIDA). The Malaysian apparel market is expected to reach approximately RM 24 billion in revenue in 2023, with the industry expected to grow at a rate of more than four per cent over the next few years to 2027, with 19 new projects approved (Malaysia Investment Performance Report 2022, MIDA)

There are hundreds of thousands of dyes produced throughout the world, and the actual amount remains unknown. Production processes of textiles contribute to the high - coloured wastewater, which contains dyes from the runoff process of textile dyeing and the rinsing of natural fabrics (Dhruv Patel et al., 2022; Holkar et al., 2016). Figure 2.1 shows the main pollutants discharged from each processing step of textile production in textile manufacturing factories.

This category of wastewater poses challenges in terms of decolorization due to its intricate aromatic composition and synthetic source. The predominant utilization of synthetic dyes in these industries is primarily attributed to their lower cost compared to organic dyes (Al-Tohamy et al., 2022).

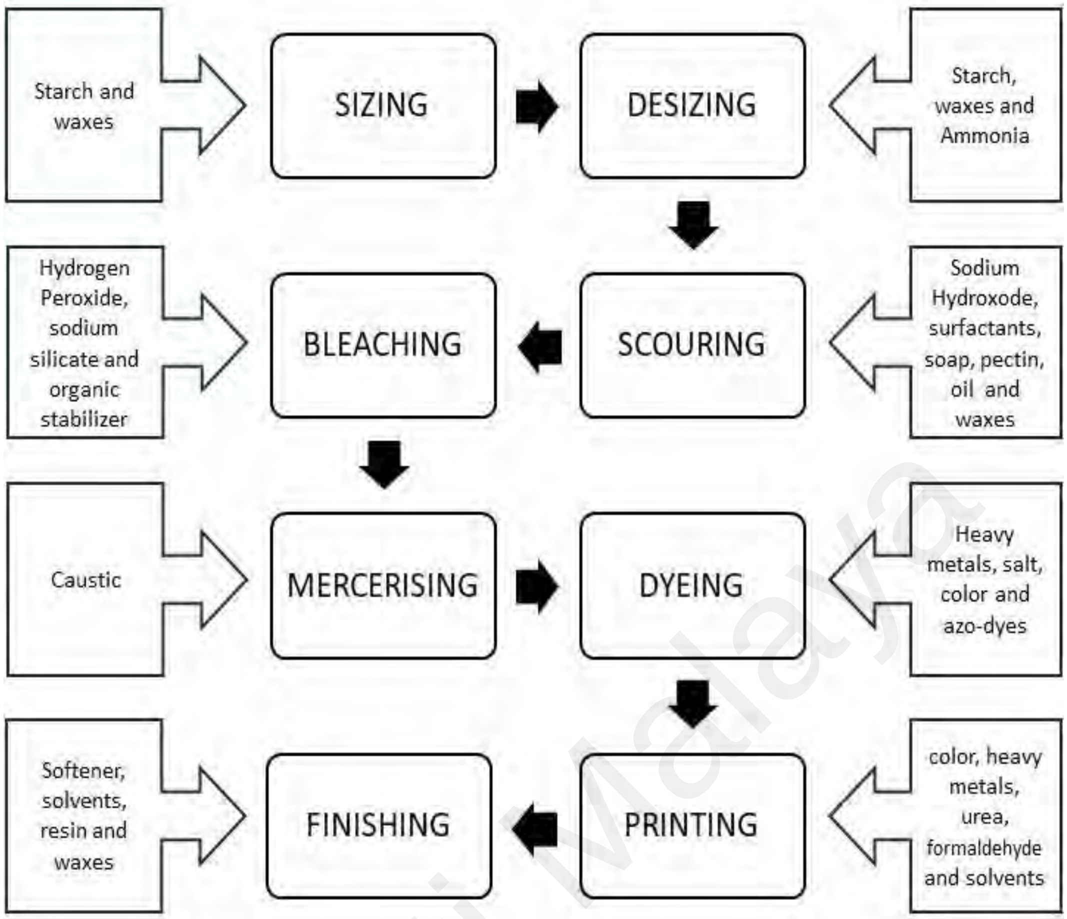


Figure 2.1: Main pollutants discharged from each processing step of textile production (Al-Tohamy et al., 2022; Holkar et al., 2016).

Dyes are often classified with reference to their chemical structure, method and domain of usage and/or chromogen (Bhatia et al., 2017). Toxic azo dyes, possessing a wide range of colours, have been extensively employed by various industries. However, it has been discovered that certain azo dyes exhibit carcinogenic and mutagenic properties subsequent to undergoing natural biotransformation. Dyes also contribute to the increase in biological oxygen demand (BOD) and chemical oxygen demand (COD) in industrial wastewater, which will lead to an increase in the cost of treatment (Lellis et al., 2019).

2.2 Conventional textile wastewater treatment methodology

Conventional methods used to treat dye-containing textile wastewater include chemical oxidation, coagulation and adsorption processes, and biological processes, but they cannot be individually sufficient to remove toxic dye from wastewater (Gosavi & Sharma, 2014). Current biological treatments need to use specific bacteria for dye decolorization because it may not remove any other parameters such as BOD, COD, and total suspended solids (TSS). The biological treatment process requires a longer retention time, and the higher value of heavy metals may hamper bacterial growth (Arslan et al., 2016).

Other than that, conventional treatment of physical-chemical coagulation and adsorption by using absorbance materials such as activated carbon (AC) is generally not feasible due to economic considerations and is not efficient as dye molecules are very complex in nature and are stable to heat and light. The treatment is apparently high cost of materials, costly operation, may not work with certain dyes and metals, and performance depends upon the material types (Wei et al., 2020). Table 1 shows the advantages and disadvantages of conventional textile wastewater treatment technologies that are widely used which comprising of physical, chemical and biological treatment.

There are also concerns about the sludge derived by this treatment method as disposing it into the environment will result in pollution by the release of pathogens derived from the bacteria itself (from biological treatment methods) and sludge containing heavy metals (from physical-chemical and adsorption method).

Sludges derived from the treatment process need to be further treated by thickening (removal of water to reduce volume of sludge), stabilization (removal of mass-organic matter and volatile solids), conditioning (preparation before dewatering process), dewatering (removal of water via mechanical process), disinfection (removal of pathogenic organisms), and final disposal (destination of sludge either in sanitary landfill or incinerator). This will further increase the total operational cost of such wastewater treatment facilities.

Table 1: Conventional textile wastewater treatment technologies (Table derived from Sahu & Singh, 2019).

Treatment Methods	Advantages	Disadvantages
1. Physical treatment **Adsorption**	Effective in the removal of dissolving organics. Media may be derived from sustainable and eco-friendly source. Specific applications that are not effective for all dyes. Larger contact times and enormous quantities required.	Expensive. Regeneration will decrease the adsorption capability, which impacts the cost.
2. Chemical treatment **Physicochemical**	Short detention time and low capital cost.	Selected operating conditions for a specific type of dyes. High chemical content sludge generated. Problem with sludge disposal
3. Biological treatment **Aerobic process (mixed culture)**	Partial or complete decolourization of all dyes.	Expensive treatment because of high energy usage.
Anaerobic process (mixed culture)	Treat a wide variety of complex compounds.	Longer acclimatization phase for the treatment.

Table 1, continued.

Treatment Methods	Advantages	Disadvantages
Single cell (fungal, algal and bacteria)	Good colour removal efficiency at low volumes and concentrations. Highly effective for specific colour removal.	Culture maintenance is still not available in large scale production, which is currently cost-intensive.

2.3 Bioremediation wastewater treatment

Bioremediation involving fungi commonly employs extracellular enzymes, particularly laccase, to degrade contaminants and mitigate the presence of hazardous substances within the ecosystem (Kulshreshtha et al., 2010). It uses its cells with biosorption, bioaccumulation, or biodegradation of the pollutants, and thus it will degrade or sequester contaminants into less harmful byproducts into the environment. This depends on the metabolism-independent process of the living or dead biomass itself.

The biosorption process arises when pollutants are bound to the fungal biomass. The bioaccumulation process occurs when actively grown fungi accumulate pollutants inside their cytoplasm. Biodegradation processes use certain enzymes particularly laccase and peroxidases derived from the fungi to degrade complex molecules into simpler and less toxic molecules (Ardila-Leal et al., 2021; Ikehata et al., 2015).

Fungi mycelium also has the potential to be used as a filter network to filter from farms, highways, and suburban zones in buffer zones around streams (Chiu et al., 2000). By inducing microbial and enzyme activity, the mycelium additionally diminishes toxins in their immediate environment. Certain fungi possess the ability to hyperaccumulate heavy metals in mushroom fruit bodies by consuming specific pollutants as their primary nutrient sources (Özcan et al., 2013).

2.3.1 Decolorization of dyes using fungal species

There is an increasing interest in the bioremediation studies using fungi for the decolorization of dye containing wqstewater from textile industries using synthetic or generic wastewater. Viable *F. trogii* pellets were found to be the most effective pellets to decolorize Astrazon black dye, and it was reported that viable pellets of all fungi during the first use tested were able to decolorize Astrazon black dye by approximately 90% effectively (Yesilada et al., 2010).

Penicillium simplicissimum was efficient in the decolorization of various dyes (Reactive Red 198, Reactive Blue 214, Reactive Blue 21, and a mixture of the three dyes). Additionally, it diminishes the level of toxicity associated with said dyes, transitioning them from a state of moderate acute toxicity to a state of minor acute toxicity (Bergsten-Torralba et al., 2009). Table 2 shows the type of dyes use in treatment, decolorization percentage and its optimal treatment conditions of research that used fungi to decolorize wastewater.

Table 2: Decolorization efficiencies of dyes and their optimum treatment conditions using fungi as bioadsorption agent.

Fungi species	Type of dye	Decolorization percentage	Optimal conditions	Reference
Penicillium simplicissimum	Reactive Red 198 Reactive Blue 214 Reactive Blue 21 Dyes Mixtures	100% 100% 100% 100%	7 days incubation 3 days incubation 2 days incubation 5 days incubation *140 rpm agitation	(Bergsten-Torralba et al., 2009)

Table 2, continued.

Fungi species	Type of dye	Decolorization percentage	Optimal conditions	Reference
Funalia trogii ***Phanerochaete chrysosporium*** ***Pleurotus ostreatus*** ***Pleurotus sajor-caju*** ***Trametes versicolor***	Astrazon Black	90%	24 hours incubation, 150 rpm agitation, 30^0C	(Yesilada et al., 2010)
Pleurotus eryngii F032	Reactive Black 5	93.57%	72 hours of incubation in dark condition, pH 3, 40^0C, 120 rpm agitation	(Hadibarata et al., 2013)
Myrothecium roridum	Malachite Green	97%	24 hours incubation, 150 rpm agitation, pH 4, 28^0C	(Jasińska et al., 2015)
Bjerkandera sp	Jean manufacturing wastewater	69%	180 hours treatment, 1 L/min aeration rate, 33^0C, pH 5.5	(Gaviria-Arroyave et al., 2018)
Cylindrocephalum aurelium	Mordant Orange-1	86%	30 days incubation	(Mostafa et al., 2019)

2.3.2 Chemical Oxygen Demand (COD) reduction using fungi

The use of fungi as bioadsorbent for COD reduction in wastewater has drawn a lot of interest in recent years. Fungi, a diverse group of microorganisms, have demonstrated remarkable proficiency in adhering to and decomposing various chemical compounds. The chemical oxygen demand, which refers to the quantity of oxygen required to oxidize organic matter in a water sample, can be used to measure the extent of this process (Azanaw et al., 2022). COD is a crucial parameter that measures the concentration of organic compounds capable of consuming oxygen during microbial degradation. High COD levels in wastewater bodies can lead to oxygen depletion, posing a severe threat to aquatic ecosystems. The high COD value indicates the presence of a high amount of chemical organic materials contained in the wastewater. These chemically organic materials are hard to break down physically or chemically and are hazardous to the environment. Table 3 shows a compilation of various research endeavors employing fungi to mitigate the COD in wastewater.

Table 3: The utilization of fungi as a bioadsorption agent for reduction of wastewater's COD with its the optimal treatment conditions.

Fungi species	Type of Wastewater	Percentage COD reduction	Optimal Conditions	Reference
Coriolus versicolor **Fungi strain - 30388** **Fungi strain- 9791**	Synthetic dye wastewater	67.47%	25 days 29±1°C pH 4.5±2 Hydraulic retention time: 15 hours Air flow: 2.5L/min Aeration rate: 5 L/min MLSS: 2000 mg/L	(Hossain et al., 2016)
Wild-Serbian *Ganoderma lucidum* mycelium	Synthetic domestic sewage	96.00%	48 hours pH 4 COD/N ratio of C17.8N1	(Mohd Hanafiah et al., 2019)
Chaetomium globosum **IMA1 KJ472923**	Industrial textile effluent	88.4%	10 days pH 5.5 28^0C Agitation: 150 rpm	(Manai et al., 2016)

Table 3, continued.

Fungi species	Type of Wastewater	Percentage COD reduction	Optimal Conditions	Reference
***Trametes villosa* SCS-10**	Tannery industry wastewater	40–60% without nutrient supply 80% with nutrient supply	11 days pH 4.0–6.0 30°C Agitation:200 rpm	(Ortiz-Monsalve et al., 2019)

2.4 Malaysian *Ganoderma lucidum*

Ganoderma lucidum, commonly referred to as "Lingzhi" in China and known as "Reishi" or "Mannentake" in Japan within the *Ganodermataceae* family, has a rich historical background in promoting health and longevity not only in these nations but also in various other Asian countries. It is a dark mushroom with a varnished glossy exterior (as per Figure 2.2). The term lucidus, derived from Latin, denotes "shiny" or "brilliant" specifically pertaining to the glossy exterior of the mushroom.

Figure 2.2: Malaysian *Ganoderma lucidum* (Figure is by Author).

The conventional approach to cultivating mushrooms poses challenges in terms of quality control and extended growth periods. However, the utilization of uncomplicated fermentation methods and cost-effective growth media can facilitate the economic production of fungal biomass of Malaysian *Ganoderma lucidum* within a span of three to seven days. Two primary aerobic technologies, submerged cultivation in a liquid medium and solid-state substrate bioprocessing, are widely employed to achieve higher yields of fungal mycelia within a significantly reduced timeframe (Berovic & Podgornik, 2016).

In studies being conducted by Wan-Mohtar (Wan et al., 2016) found that different method of production of exopolysaccharide of *Ganoderma lucidum* produce different results where shake flask produces 1.3-fold more with only 6 days of total fermentation time for repeated batch fermentation compared to 32 days for batch culture in a bioreactor. This research revealed a shorter production time of producing mycelium pallets.

The mycelium pallets of Malaysian *Ganoderma lucidum* have been discovered to possess a significant amount of beta-glucan and exhibit considerable antimicrobial properties against four prevalent pathogenic bacteria. These bacteria include two gram-positive strains, namely *S. epidermidis* and *S. aureus*, as well as two gram-negative strains, namely *E. coli* and *S. marcescens* (Wan-Mohtar et al., 2016; Supramani et al., 2019; Wan et al., 2021).

A study using wild-Serbian *Ganoderma lucidum* mycelium on the treatment of synthetic COD and NH_3–N (Ammoniacal Nitrogen) wastewater has found that the highest percentage for removal achieved was 96.0% (Mohd Hanafiah et al., 2019). This proved that *Ganoderma lucidum* has high potential in treating domestic wastewater, particularly at high organic content. More recent research recorded removal of 95.1% COD and 96.3% Ammonia Nitrogen, accordingly at the optimum temperature 25°C at the treatment time of 24 hours in treating synthetic domestic wastewater with *Ganoderma lucidum* in batch system (Mooralitharan et al., 2020). Studies using *Ganoderma lucidum* are listed as per Table 4.

Table 4: Current available studies on the adsorption of dye using *Ganoderma lucidum*.

Treatment Media	Wastewater Type	Treatment Time	COD Reduction	Colour Reduction	References
Wild-Serbian *Ganoderma lucidum* mycelium	Synthetic domestic sewage	120 hours	96.0%	NA	(Mohd Hanafiah et al., 2019)
***Ganoderma lucidum* mycelium**	Paper Mill Effluent	15 to 18 days	98%	94%	(Perumal et al., 2000)
***Ganoderma lucidum* mycelium**	Batik Dye Effluent	30 days	81.03%	60.53%	(Diah Pratiwi et al., 2017)
***Ganoderma lucidum* supported on PET-coated SBS paper**	Remazol Brilliant Blue R	30 days	NA	96%	(Karine Thaise_Rainert et al., 2020)
Malaysian *Ganoderma Lucidum* Inoculum	Synthetic Wastewater	30 hours	>95%	NA	(Mooralitharan, S et al., 2023)

A study conducted to assess the potential toxicity of Malaysian GLMP as a fish-feed supplement for fungivore red hybrid Tilapia (*Oreochromis sp.*) in Zebrafish embryo. The findings revealed that the Zebrafish embryo did not exhibit any toxic effects, with LC50 values of 1650 µg/mL and 2648.38 µg/mL, respectively. These results indicate that these natural compounds are safe for use as a substance in the aquaculture industry and other relevant applications (Taufek et al., 2020). A recent research investigation has revealed that the incorporation of *Ganoderma lucidum* mycelium as functional feed for aquaculture-farmed red hybrid Tilapia (*Oreochromis sp.*) has resulted in advantageous outcomes in terms of fish growth and health status. (Wan et al., 2021).

Following to this safe natural compound towards living organisms, it produces an interest in determining the performance capabilities in using Malaysian origin of *Ganoderma lucidum* for bioremediation treatment of wastewater from different types of industry.

2.5 Bioremediation using bioreactor

Bioremediation mainly involves microorganisms for chemical transformations from toxic pollutants to less or non-toxic by products from its selective cell's adsorption and/or consuming capabilities of the pollutants that satisfy their nutritional and energy requirements. The application of microbial treatment has been extensively studied for domestic waste, agricultural waste, and industrial effluents. There are also studies on bioremediation of pollution in soils, sediments, and marine environments (Espinosa-Ortiz et al., 2022).

Bioreactors are commonly used to study the treatment of wastewater with microorganisms in a controlled condition. In recent times, the utilization of bioreactors for bioremediation has garnered significant interest owing to its cost-effectiveness, operational feasibility, and commendable efficacy. Microbial bioreactors play a pivotal role in enhancing the circular bioeconomy by efficiently transforming organic waste and renewable resources into valuable commodities, including biofuels, bioplastics, and pharmaceuticals (Wan-Mohtar et al., 2023).

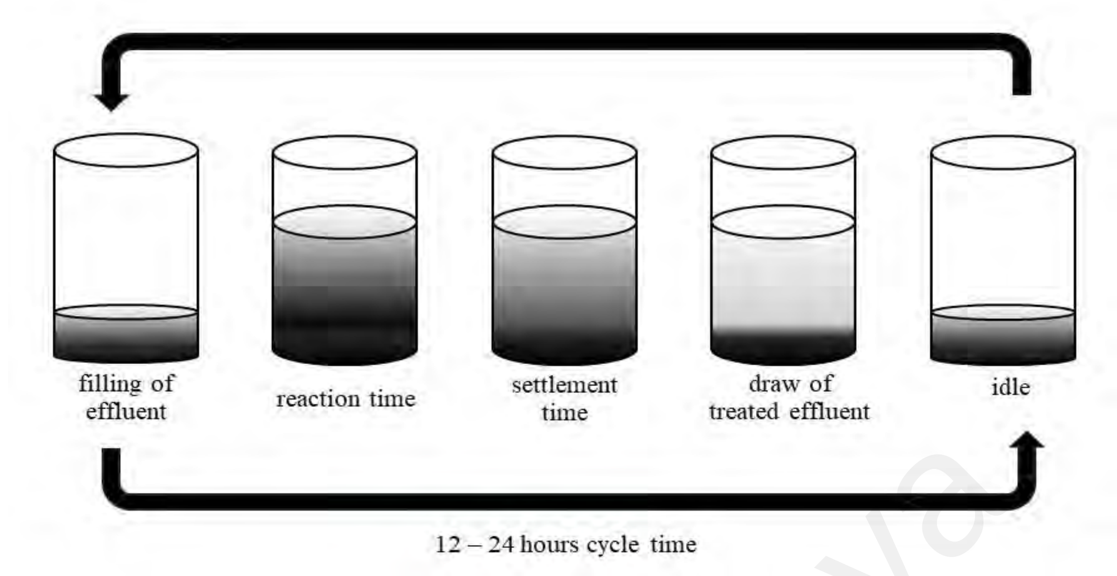


Figure 2.3: Sequencing Batch Reactor (SBR) with five consecutive steps involves (Figure is by Author).

The standard technique for wastewater treatment process that utilizes the activated sludge process is the Sequencing Batch Reactor (SBR). This widely used treatment process involves five consecutive steps, namely filling of effluent, reaction time, settlement time, draw of treated effluent, and idle as per Figure 2.3. These steps are performed in a batch reactor, making the SBR technique a common and effective method for wastewater treatment (Lee and Park, 1999). Another common use is Stirred Tank Reactors (STR) which are simpler in design and easy to operate. To enhance the efficacy of wastewater treatment in the STR, the addition of a specific quantity of active biomass in its clarifiers is imperative. SBR performance was found able to treat molasses-based wastewater COD greater than STR at removal rate of 89% but only about 57% removal rate could be maintained by STR at hydraulic load of 10 days (Budiastuti et al., 2021).

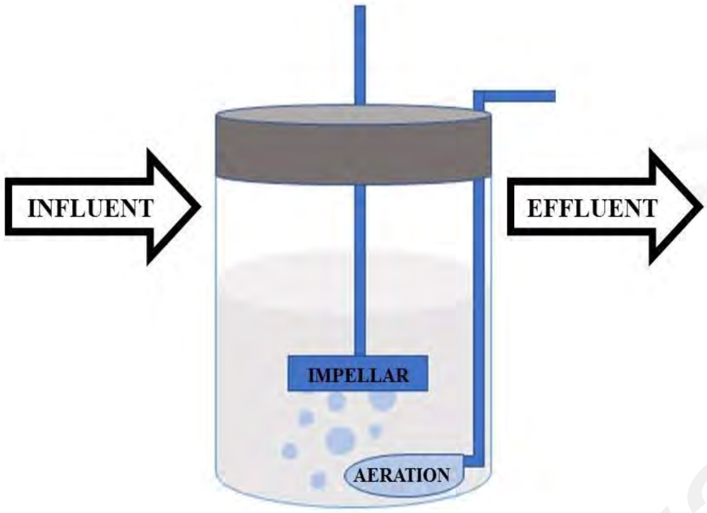


Figure 2.4: The simplest lab scale Stirred Tank Reactor (STR) design (Figure is by Author).

The employment of stirred tank bioreactors for aerobic treatment remains a prevalent method for treating industrial effluents, despite its status as one of the earliest biotechnological applications in industry. It is simple to construct and easy to operate and only uses suspended growth of microbes which is suitable for aerobic and anaerobic treatment processes. Although this process is having some of its inherent limitations, it has been undergone many times of modifications in order to increase its treatment performance and cost effectiveness (Narayanan & Narayan, 2019). Using the stirred batch bioreactor, the initial pH, adsorbent concentration, aeration rate, agitation rate and treatment time variables can be easily manipulated. Figure 2.4 shows an illustration of the simplest lab scale STR design which will be used in this research.

More technologies are including aerobic digesters (AD), continuous stirred tanks reactors (CSTR), up flow anaerobic sludge blanket (UASB) bioreactor, biofilter (BF), microbial fuel cell (MFC), moving bed biofilm reactor (MBBR), packed bed bioreactor (PBB), and membrane bioreactors (MBR) (ElMekawy et al., 2015).

CHAPTER 3: MATERIALS AND METHODS

3.1 Textile dye wastewater sampling collection

The real textile dye wastewater (RTWW) was collected from a private textile factory located in Batu Pahat, Johor, Malaysia. Samples collected were analyzed for initial pH, colour and chemical oxygen demand (COD) parameters using HACH SensION+ pH1 meter and HACH DR900 portable colorimeter. Table 5 shows the real textile wastewater characteristics tested by the third-party laboratory for selected parameters as per Schedule A, Environmental Quality (Industrial Effluent) Regulations 2009 (Malaysia, Department of Environment). The wastewater was stored in an airtight plastic container and is stored in a refrigerator (4 ± 1^0C).

Table 5: The characteristics of the RTWW collected in a textile factory in Batu Pahat, Johor, Malaysia.

Parameter[a]	Value	Standard A	Standard B
pH @ 25°C	7.10 - 8.22	6.0 – 9.0	5.5 – 9.0
Colour Appearances	Red purplish	NA	NA
Colour (Platinum-Cobalt, PtCo)	900.0 - 1116.0	100 ADMI[b]	200 ADMI[b]
Total Suspended Solid (TSS)	39.0	50	100
Biological Oxygen Demand (BOD) at 20^0C	50	20	50
Chemical Oxygen Demand (COD)	326.0 – 1,655.0	80	250

[a]All values except pH and Colour are in mg/L.

[b]ADMI-American Dye Manufacturers Institute.

[c]Acceptable conditions for discharge of effluent containing chemical oxygen demand (COD) for specific trade or industry sectors. Environmental Quality (Industrial Effluents) Regulations 2009 (Seventh Schedule, Regulation 12).

Discharge from such industries must complying the Malaysia regulations of industrial effluent discharge as listed in Table 6. The discharge limit of colour must comply with the Schedule A or B, Environmental Quality (Industrial Effluent) Regulations 2009 (Malaysia, Department of Environment) which is 100 ADMI for Standard A and 200 ADMI for Standard B. The RTWW collected recorded a high color reading of 900 to 1116 PtCo. Colour assessed using PtCo scale are more reliable as this unit measure was widely used throughout the water and wastewater treatment plant particularly in Malaysia.

The COD discharge limit from textile industry specifically defined in Environmental Quality (Industrial Effluents) Regulations 2009 (Seventh Schedule, Regulation 12) (Department of Environment, Malaysia) which are 80 mg/L (Standard A) and 250 mg/L (Standard B) as per Table 6. The collected RTWW has a high COD reading of 326.0 mg/L to 1,655.0 mg/L which is more than regulated discharge limit for textile industry. The high COD reading indicates the presence of a high amount of chemical organic materials contained in the wastewater. These chemically organic materials are hard to break down physically or chemically and are hazardous to the environment (Holkar et al., 2016; Lellis et al., 2019).

Table 6: Acceptable conditions for discharge of effluent containing chemical oxygen demand (COD) for specific trade or industry sectors. Environmental Quality (Industrial Effluents) Regulations 2009 (Seventh Schedule, Regulation 12). Malaysia, Department of Environment.

Trade/Industry	Unit	Standard A	Standard B
Pulp mill	mg/L	80	350
Pulp mill (recycled)	mg/L	80	250
Pulp and paper mill	mg/L	80	300
Textile industry	mg/L	80	250
Fermentation and Distillery	mg/L	400	400
Other industries	mg/L	80	200

3.2 Malaysian *Ganoderma lucidum* mycelial pellets

The Malaysian *Ganoderma lucidum* mycelium pellets (Malaysian GLMP) was cultured and obtained from the Functional Omics and Bioprocess Development Laboratory University of Malaya, Malaysia (Supramani, 2019; Supramani, 2023). For the plate subculture and seed culture, the medium contained 39 g/L of potato dextrose agar (PDA; Sigma-Aldrich, Dorset, UK), whereas the fermentation media contained 30.0 g/L of glucose, 1.0 g/L of yeast, 0.5 g/L of KH_2PO_4, 0.5 g/L of K_2HPO_4, 0.5 g/L of $MgSO_4$, and 4.0 g/L of NH_4Cl. The ideal procedure recommended by WAAQI Wan-Mohtar was followed for all these operations, which were performed at 30 °C and shaken at 100 rpm for 11 days (Wan et al., 2016). The mycelium of the initial seed culture was homogenized in a sterile Waring blender for a duration of 20 seconds, following a period of 10 days, in order to generate additional hyphae tips for enhanced growth.

Subsequently, the mycelium was transferred to the bioreactor, employing the identical media utilized in the first seed culture, and employed as the inoculum for the second seed culture. This process took place within a 500 mL Erlenmeyer flask, containing 200 mL of medium. Figure 3.1 shows the Malaysian *Ganoderma lucidum* (6.A) and in its mycelium state prepared in preparation flasks (6.B). The mycelium pellet has been filtered from the liquid media and is prepared for experimental use as biosorption media.

Figure 3.1: Malaysian *Ganoderma lucidum* (6.A) and Malaysian GLMP in preparation flask (6.B) (Figure is by Author).

3.3 Batch Bioreactor Optimization

Stirred tank bioreactor are common bioreactor design used in fermentation system which consist of cylindrical container with internal mechanical agitation (stirrer) which have advantages for easy to upscale with good fluid and oxygen mixing. Optimization of bioremediation treatment of wastewater using fungi mycelium pallets can be accomplish by inputting various variable factors including initial pH, aeration rate, adsorbent concentration, wastewater dilution ratio and contact time (Zhong, 2011).

One of the major upsets of this type of bioreactor is exerting vast shear forces towards the particles and potentially damaging the particles inside especially in gel form (Nemati & Webb, 2011). Using Malaysian *Ganoderma lucidum* in its mycelium pellets inhibits these upsets as by forcing stronger shear strength will improve the contact of wastewater to the bioadsorbent material's surface. Studies shows that the agitation speed will improve the adsorption rate as it increases. Agitation speed of 50 rpm to 150 rpm has been studied in the treatment wastewater of using fungi (Hossain et al., 2016; Mooralitharan et al., 2023; Pratiwi, Indrianingsih, Darsih, et al., 2017).

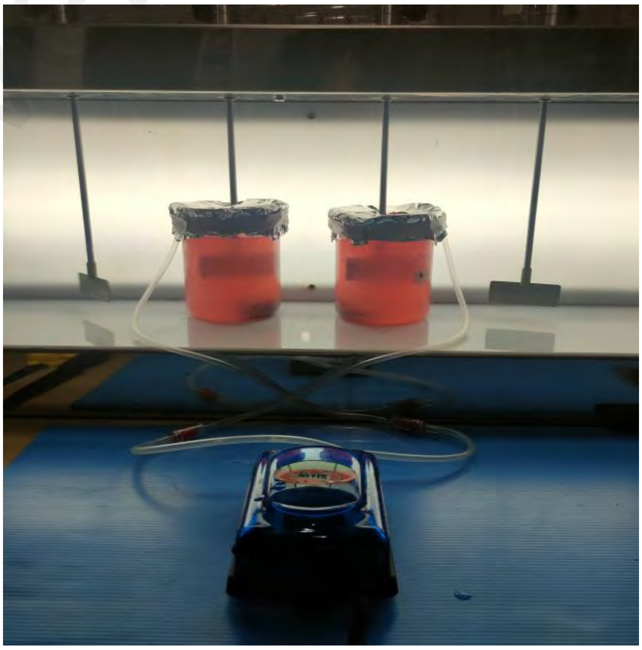


Figure 3.2: Batch bioreactor setup for aerobic wastewater treatment (Figure is by Author).

The agitation speed was preselected at 150 rpm as to provide adequate aeration mixing of real textile wastewater (RTWW) with the Malaysian GLMP to accelerate the contacts of wastewater to Malaysian GLMP cell surfaces. This speed reduces the shear strength forces towards the particles in the batch bioreactor (Jasińska et al., 2015; Manai et al., 2016).

500-mL reactors were filled with 400 mL of RTWW mixed with Malaysian GLMP as per the set-up shown in Figure 3.2. The initial pH of wastewater was adjusted to pH 4 using 0.1-M HCl or 0.01-M NaOH until the desired pH was achieved. The selection of pH 4 is made as per data driven on the preliminary test and align with the other studies (Mohd Hanafiah et al., 2019; Mooralitharan et al., 2023). The Malaysian GLMP was weighed and filled with a sterile spatula in the reactor to simulate the aerobic treatment process at ambient temperature (between 26-34°C). The Malaysian GLMP volumes was varies for 3.5g, 5g and 10 g treated with RTWW volume of 400 mL. The reactor received a continuous supply of aeration at a rate of 4 L/min, thereby establishing an aerobic environment. Further to the experiment, the RTWW was diluted in the ratios of 1:0, 1:4 and 2:3 to distilled water, respectively to determine the effectiveness of treatment on less polluted wastewater source (Gaviria-Arroyave et al., 2018).

Initially, the treatment duration for the first cycle was fixed at 6 hours, but it was subsequently adjusted for subsequent experiments depending on the effectiveness of colour and COD reduction in the following cycle until completion of 72 hours treatment cycle.

3.4 Sampling and analytical analysis

The pH was observed (using the pH meter, HACH SensION+ PH1) and recorded. The pH of the wastewater was assessed at regular intervals (Hour, h) to examine the outset response of fungi towards the real textile wastewater. 5 mL of samples were extracted every 12 hours until completed 72 hours of treatment time by using a clean pipette after 30 minutes of sedimentation.

All the wastewater samples underwent filtration using Whatman filter paper Cellulose Nitrate Membrane Filters with a pore size of 0.45 μM before the analysis, aiming to eliminate the suspended mycelium. Samples collected in clean plastic or glass bottles and were analyzed for colour and COD using standard protocol (HACH, Kuala Lumpur, Malaysia) as soon as possible after collection for the best results.

Samples of mycelium pallets were collected in clean plastic or glass bottles and were analyzed under microscope Leica EZ4 microscope (Leica Microsystems (Switzerland) Ltd.). The examination was carried out on the pre- and post-treatment of the Malaysian GLMP in duplicate.

3.4.1 Microscopic morphology analysis

Morphology analysis examine the fungal structures and features towards the adsorption processes. Mycelium pallets were collected on the 12, 24, 48 and 72-hours of treatment time interval in sample bottles and microscopic images of the mycelium is observed using light emission microscope, Leica EZ4 microscope Leica Microsystems (Switzerland) Ltd. The examination was carried out on the pre- and post-treatment of the Malaysian GLMP towards the real textile wastewater (RTWW).

3.4.2 Analysis of the colour removal percentage

The analysis of colour value was conducted by employing the HACH APHA Platinum-Cobalt Standard Method* (Method 8025), which involved referencing the manufacturer's standard (HACH, Kuala Lumpur, Malaysia). Analysis of optimum result will be based on the percentage removal of colour. The test is done using the HACH DR900 portable colorimeter.

Each sample was withdrawn by using a syringe and was diluted 5 times in a measuring cylinder with a stopper. The diluted sample was transferred into the sample cell and tested using HACH portable colorimeter model DR900.

The colour removal sum is determined by subtracting the initial (C_0) with end of treatment (C_t) colour. The percentage of colour removal for each time interval is calculated by using the Formula 1:

$$\text{Colour removal percentage (\%)} = \frac{(C_0 - C_t)}{C_o} x\ 100 \tag{3.1}$$

3.4.3 Analysis of the COD removal percentage

The concentration of chemical oxygen demand (COD) was determined using a closed reflux technique, which involved a colorimetric method utilizing the HACH vial high-range reactor digestion method. This method, specified under Method 8000, followed the standard protocol provided by the manufacturer (HACH, Kuala Lumpur, Malaysia). The range of COD concentrations measured using this method ranged from 20 to 1500 mg/L. Analysis of optimum result will be based on the percentage removal of COD. The test is done using the HACH DR900 portable colorimeter.

Each sample was withdrawn by using a syringe and was diluted 5 times in a measuring cylinder with a stopper. The diluted sample was transferred into the sample cell and tested using HACH portable colorimeter model DR900.

The COD removal sum is determined by subtracting the initial (COD_0) with end of treatment (COD_t) colour. The percentage of COD removal for each time interval is calculated by using the Formula 2:

$$\text{COD removal percentage (\%)} = \frac{(COD_0 - COD_t)}{COD_o} \, x\, 100$$

(3.2)

3.4.4 Adsorption isotherm

Isotherms, or functions connecting the quantity of adsorbate on the adsorbent, are typically used to characterize adsorption. There exist several isotherm models, such as Langmuir and Freundlich, which can be employed to elucidate the partitioning of authentic colour dyes present in textile wastewater between the liquid and solid phases. The Langmuir and Freundlich isotherms were used in describing the decolorization and COD removal capabilities of Malaysian GLMP toward the real textile wastewater (Zahuri et al., 2023).

Sorption capacity was estimated according to the equation using Formula 3:

$$Q_e = \frac{C_i - C_e}{W}$$

(3.3)

Where: Q_e is the quantity of dye colour uptake from solution by mass of adsorbent (*mg/g*); C_i and C_e are the initial and equilibrium dye colour concentration (*mg/L*), and *W* is the weight of the adsorbent added (*g*).

The Langmuir isotherm model assumes that adsorption occurs in a monolayer on a surface that contains a finite number of adsorption sites that are uniformly distributed. Additionally, it assumes that there is no transmigration of the adsorbate within the surface. Once an adsorption site is occupied, no further adsorption can occur at that site. This implies that the surface reaches a saturation point where the maximum adsorption capacity is achieved.

The representation for Langmuir isotherm referring to Formula 4:

$$\frac{C_e}{Q_e} = \frac{1}{K_L Q_m} + \frac{\text{Ce}}{Q_m} \tag{3.4}$$

The linear relationship between the specific adsorption (C_e/Q_e) and the equilibrium concentration (C_e) provides evidence that the adsorption process adheres to the Langmuir model. The determination of the constants K_L and Q_m, representing the adsorption energy and maximum adsorption capacity respectively, is accomplished by analyzing the slope and intercept of the graph.

The Freundlich isotherm presented, which is an empirical approach used to describe the adsorption process. The quantity adsorbed per unit of adsorbent at equilibrium (mg/g) is denoted by Q_e, while C_e represents the equilibrium concentration (mg/L). The parameters K_f and n are also included in the model, which are dependent on the adsorbate and adsorbent, respectively. The representation for Freundlich isotherm referring to Formula 5:

$$Q_e = K_f C_e^{1/n} \tag{3.5}$$

The dependent constants K_f and $1/n$ found by linear regression, Formula 6:

$$\ln Q_e = \ln K_f + \left(\frac{1}{n}\right) \ln C_e \tag{3.6}$$

The Freundlich constants, denoted by K_f and n, represent the adsorption capacity and intensity, respectively. To determine the Freundlich equilibrium constants, the plot of ln Q_e versus ln C_e was utilized.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Microscopic morphological observations

Figure 4.1 demonstrates a view of features of Malaysian GLMP, which in its initial shape (0-hour) has a round shape yellowish white colour with size of less than equal or less than 2 mm in diameter.

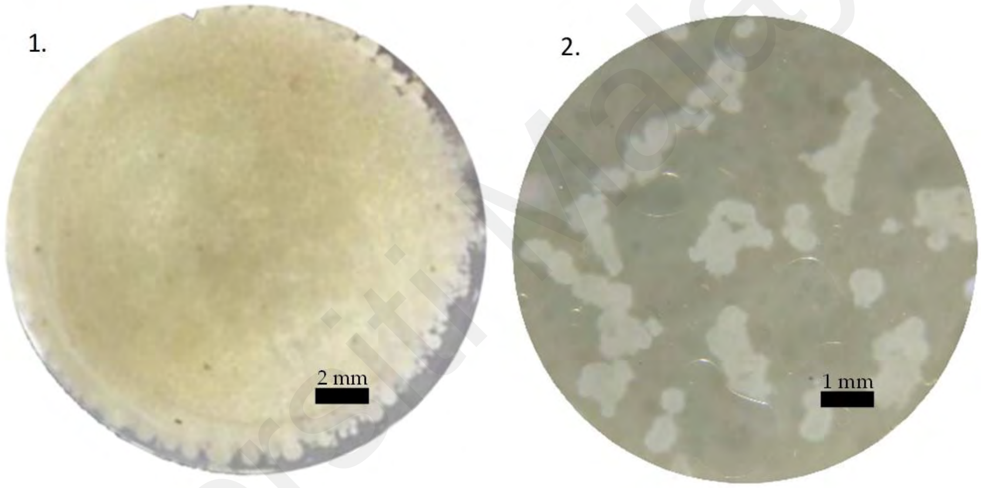


Figure 4.1: View of Malaysian *Ganoderma lucidum* mycelium pallets 1. Malaysian GLMP in its initial shape under naked eye observation 2. Initial shape of the Malaysian GLMP under microscopic view (30X magnification).

The observation of pigmented components on the dye treated Malaysian GLMP in contrast to the control Malaysian GLMP as per shown in Figure 4.2, suggests the occurrence of adsorption. The process of decolorization is facilitated by the physical attachment of dyes to specific membrane receptors located on the surface of the strain (Chen & Ting, 2015).

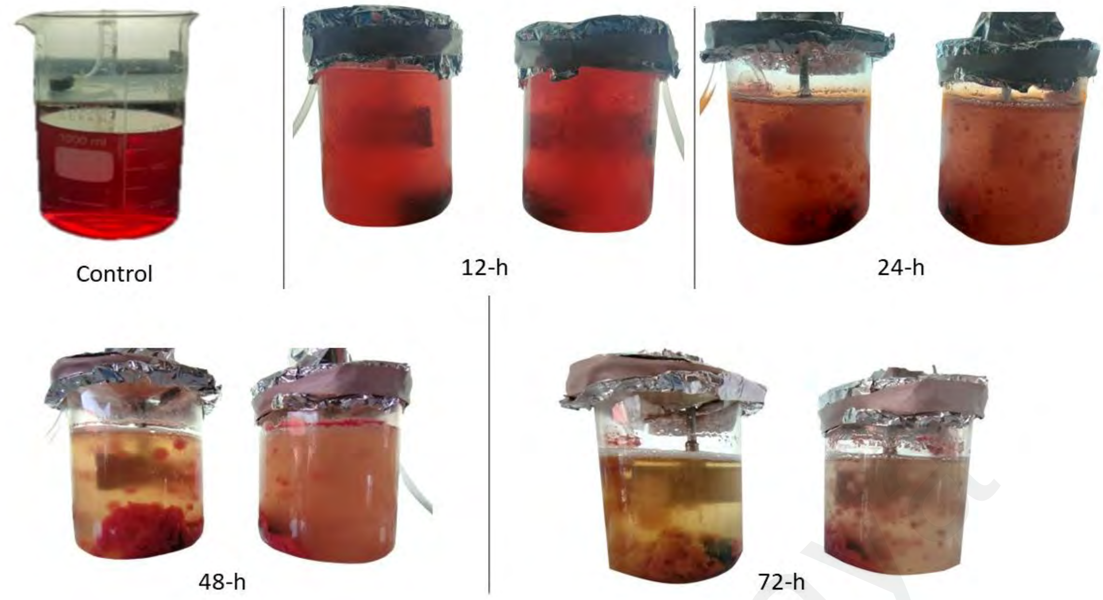


Figure 4.2: Real textile wastewater (RTWW) treated with Malaysian GLMP in initial pH 4 at wastewater dilution of 1:4 (left) and 2:3 (right). Observation was made on time interval hours (h) of Control 0, 12, 24, 48, and 72-h of treatment.

The present study utilized a dissecting microscope (DM) to examine and delineate the surface morphology and fundamental physical properties of the adsorbent surface of Malaysian GLMP. This approach facilitated the monitoring of the adsorption process, including its natural occurrence, degree of adsorption capacity, and associated cellular changes. Figure 9 shows the wastewater decolorization towards the Malaysian GLMP and reducing RTWW colour shows that there is colour adsorption occurs during the treatment (Przystas et al., 2018). The DM images of dye treated Malaysian GLMP were displayed in Figure 4.3 and Figure 4.4, respectively.

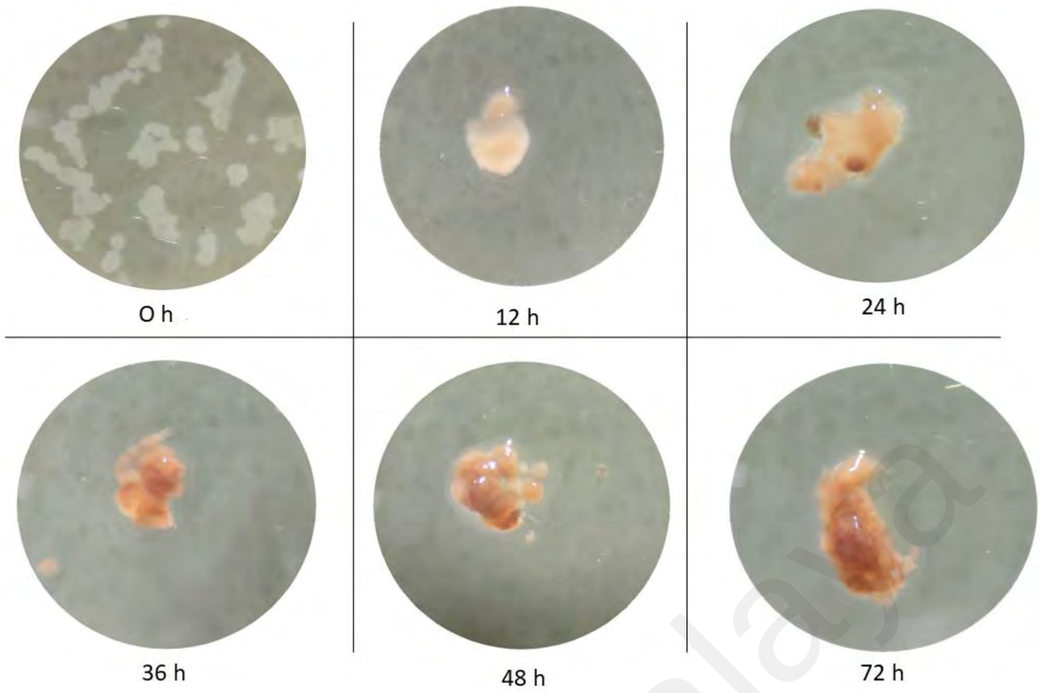


Figure 4.3: *Ganoderma lucidum* mycelium pallets under microscopic images using dissecting microscopes, Leica EZ4. 1:4 dilution. Observation was made at time intervals hours (h) of 0, 12, 24, 48, and 72-h of treatment.

The decolorization process comprises two main steps: the initial biosorption of the dye onto the fungal mycelium, and the subsequent enzymatic degradation leading to the formation of colourless end products. Biosorption is facilitated by the interaction between the reactive groups within the dyes and the active sites located on the surface of the fungi (Karim et al., 2020). An increased ratio of fungi in relation to the surface area leads to enhanced physical and enzymatic interactions with the surrounding environment (Manai et al., 2016). The other mechanisms are the fungi secrete extracellular enzymes, including laccase, manganese peroxidase, and lignin peroxidase, into the medium. These enzymes can break down the chromophoric material present in the dyes, resulting in decolorization. This adsorption process can contribute to the decolorization of dye wastewater (Espinosa-Ortiz, 2021).

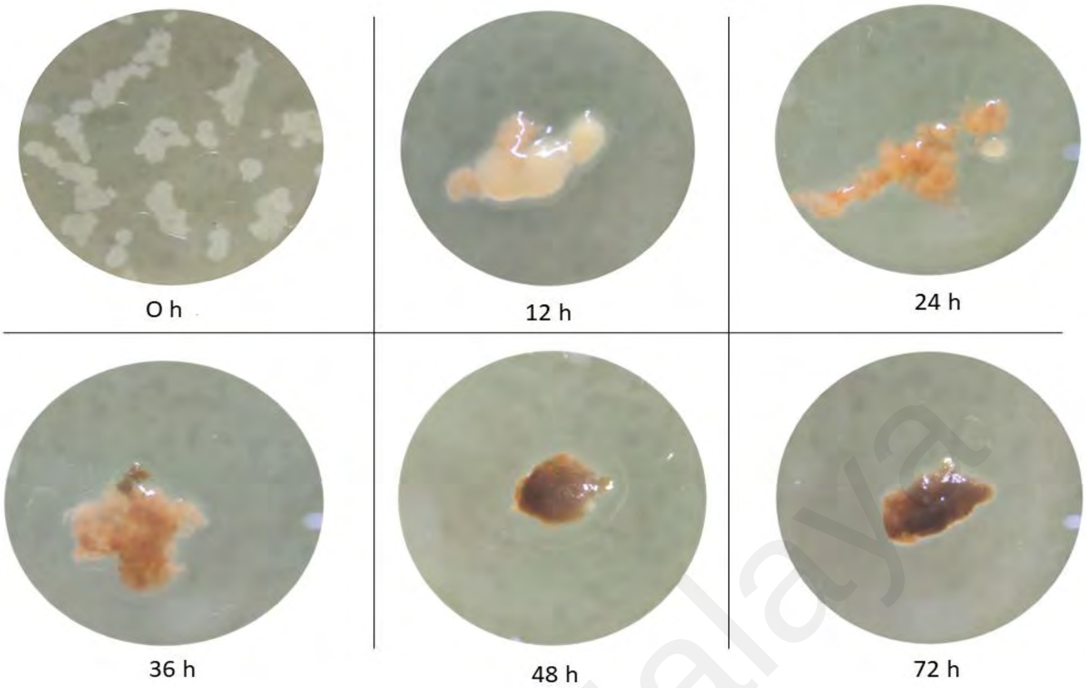


Figure 4.4: Malaysian *Ganoderma lucidum* mycelium pallets under microscopic images using dissecting microscopes, Leica EZ4. 2:3 wastewater dilution. Observation was made at time intervals of 12, 24, 48, and 72-h of treatment.

Figure 4.3 and 4.4 shows that the cell of Malaysian GLMP absorbing the RTWW which concluded the degree of its adsorption capabilities and cell changes. Mycelium of fungi has notable adsorbing-like functional properties, where its cell walls can constitute organic and inorganic materials as its food source hence made it conceivable to be use as biosorbent for pollution control. The active groups on the cell walls of the fungal mycelium can be bound by the molecules of dyes through either van der Waals forces or chemical mechanisms (Thampraphaphon et al., 2022).

4.2 Decolorization effect of the adsorbent concentration

The impact of the concentration of Malaysian GLMP on its ability to decolorize the RTWW in the bioreactor is a crucial factor that significantly affects its effectiveness. Figure 12 demonstrates that varying volumes of Malaysian GLMP (0g, 3.5g, 5g, and 10g) significantly influenced the percentage removal of dye during the treatment with undiluted RTWW (Przystaś et al., 2018; Mohd Hanafiah et al., 2022).

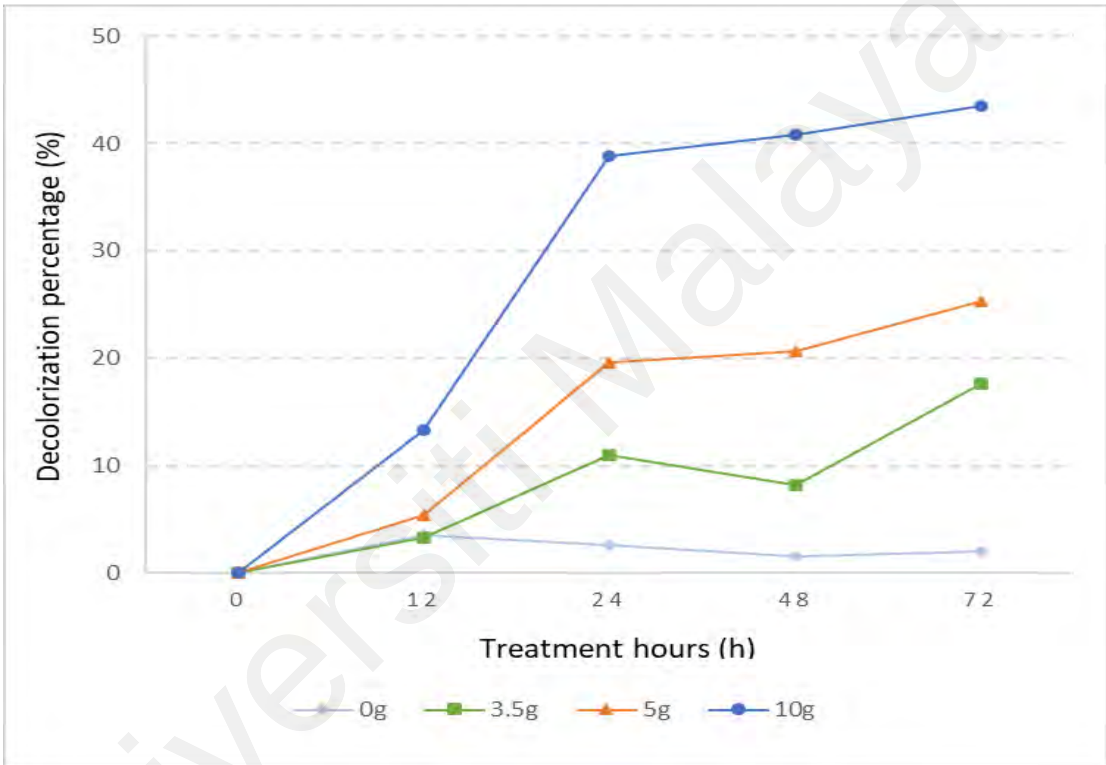


Figure 4.5: The decolorization percentage of RTWW based on treatment with different Malaysian GLMP adsorption concentrations in gram toward undiluted and unadjusted pH of wastewater.

Figure 4.5 illustrates a positive correlation between the decolorization percentage and the adsorbent concentration, wherein the decolorization percentage increases as the adsorbent concentration rises. The maximum decolorization percentage was observed at an adsorbent concentration of 10g. Consequently, this concentration was selected for the subsequent adsorption experiments.

The concentration of Malaysian GLMP has a significant impact on the adsorption of dyes during the treatment process. Higher concentrations of Malaysian GLMP lead to a greater percentage of decolorization and reduction in COD. A study have been conducted to assess the decolorization efficiency of various dyes using immobilized fungal biomass. The quantity of fungal biomass used as an adsorbent was found to influence the efficiency of decolorization. The study utilized different strains of fungi immobilized on various solid supports and observed the decolorization of two distinct classes of dyes. The results demonstrated the effectiveness of immobilized fungal biomass in decolorizing the dyes, with the quantity of biomass used playing a crucial role in the efficiency of the process (Przystaś et al., 2018).

The augmentation of the adsorbent dose resulted in a corresponding augmentation in the accessibility of adsorption sites and pore surface areas, thereby facilitating the infiltration and adsorption of dye molecules (Abdulsalam et al., 2020). This suggests that a higher quantity of fungal adsorbent can enhance the decolorization process by providing more adsorption sites for the dye molecules.

4.3 Decolorization effect of different initial treatment pH

According to the results on the decolourisation percentage of RTWW based on initial pH treatment with 10g of Malaysian GLMP towards undiluted wastewater, Figure 4.6 determine the optimum initial treatment pH of 4 favorable to the Malaysian GLMP which the maximum colour percentage removal recorded at 61.55% compared to maximum percentage removal of 46.28% of pH 6. The pH level observed initially ranged from pH 7.10 to pH 8.22 without any adjustments. Subsequently, the procedures were conducted based on this pH level, as it was determined that the highest removal percentage was achieved through acidification at pH 4.

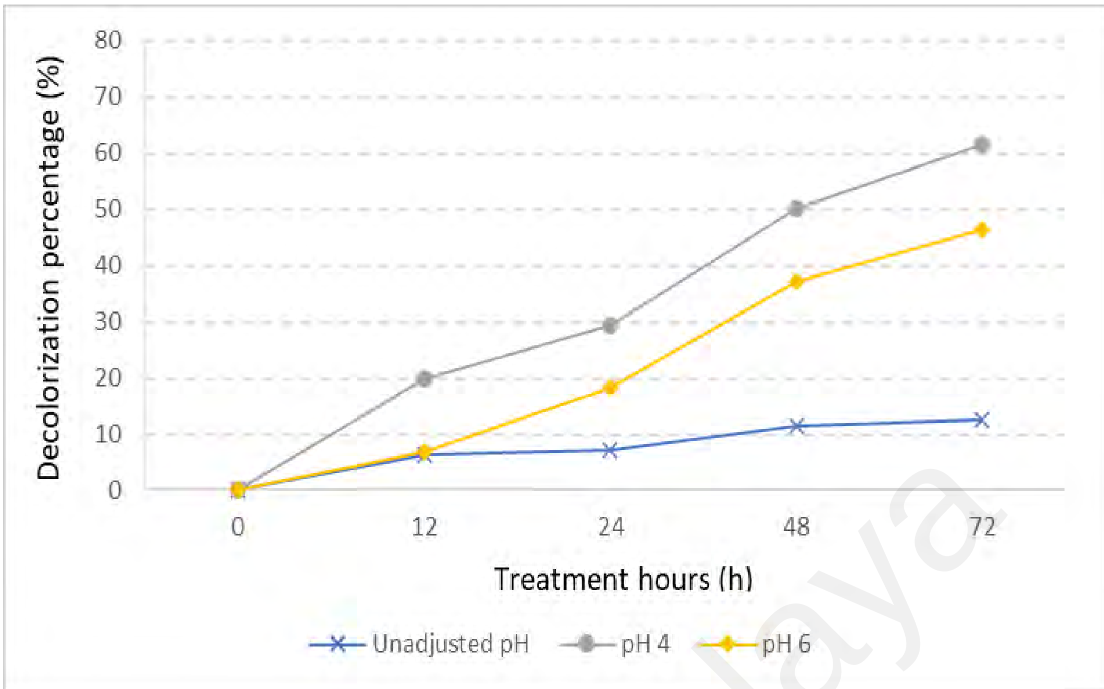


Figure 4.6: The decolorization percentage of RTWW based on initial pH treatment with 10g of Malaysian GLMP toward undiluted wastewater.

RTWW treatment with Malaysian GLMP have highest decolorization with the initial treatment pH of 4 as shown in Figure 13. This was found and aligned with findings by Zarimah Mohd Hanafiah (2019) where fungi were known to be most efficient at pH 4. According to Tony Hadibarata (2013) and Li Wang (2019), fungi can grow and produce enzymes at a pH between 3 and 5, which is the ideal range for treatment of wastewater with fungi and increase in pH will decrease the decolorization capabilities of fungi. The best pH for dye decolorization is largely dependent on the kind of fungus, dyes, medium, and environment factors (Mostafa et al., 2019). Mycelium has demonstrated a diverse array of application conditions, encompassing temperature and pH ranges, that enable it to sustain a substantial processing capacity for different categories of wastewaters, such as dye wastewater (Guo et al., 2020).

4.4 Effect of wastewater dilution

Based on the findings presented in Figure 4.7, the highest percentage of colour removal was observed after 72 hours of treatment at a dilution ratio of 1:4 (wastewater: distilled water). In terms of dye decolorization, the greatest percentage of removal was achieved at 77.24% and 42.15% for wastewater dilutions of 1:4 and 2:3, respectively, with an initial pH treatment of 4. These results suggest that the optimal conditions for effective dye removal involve a specific dilution ratio and pH level.

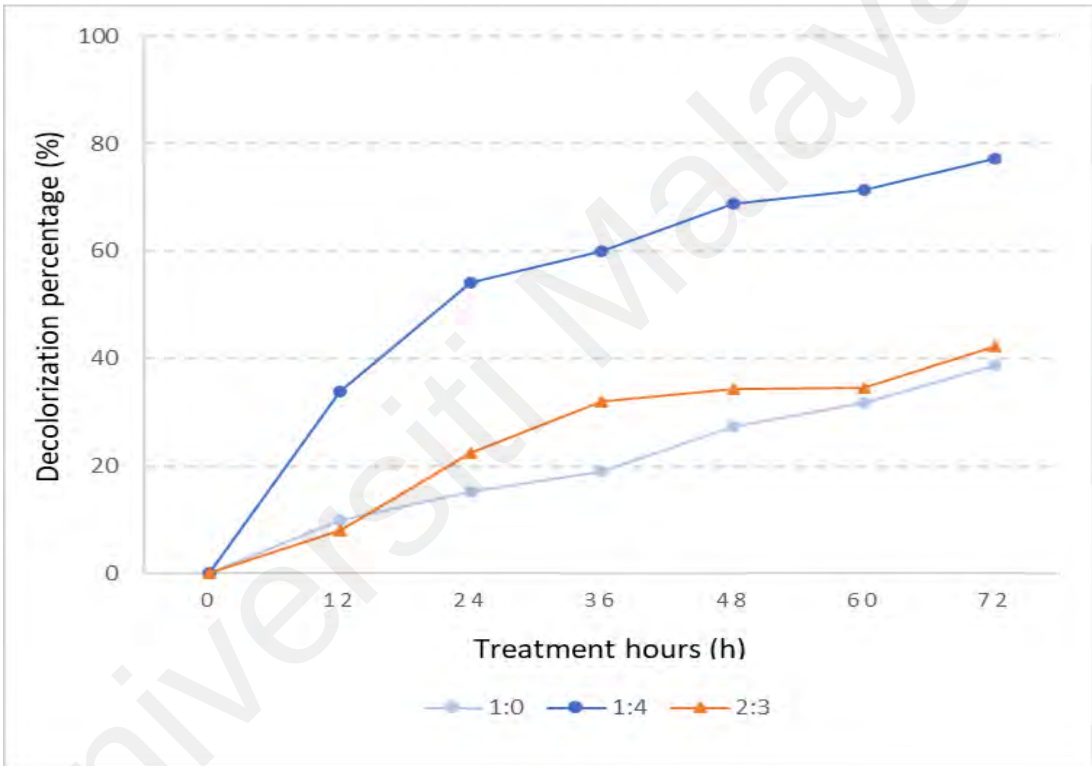


Figure 4.7: The decolorization percentage of RTWW based on wastewater dilution of 1:0 (undiluted wastewater), 1:4 and 2:3.

The treatment of dye-containing wastewater achieved effectively through the process of dilution. Dilution entails the mixing of the initial wastewater with a larger volume of clean water, which results in a reduction of the concentration of dyes and other pollutants present in the wastewater. This reduction in concentration facilitates the removal of dyes through various treatment processes, thereby enhancing the overall effectiveness of the treatment process.

The significance of dye wastewater concentration outweighs the influence of agitation speed when considering the remediation of textile dye wastewater (Selvakumar et al., 2013).

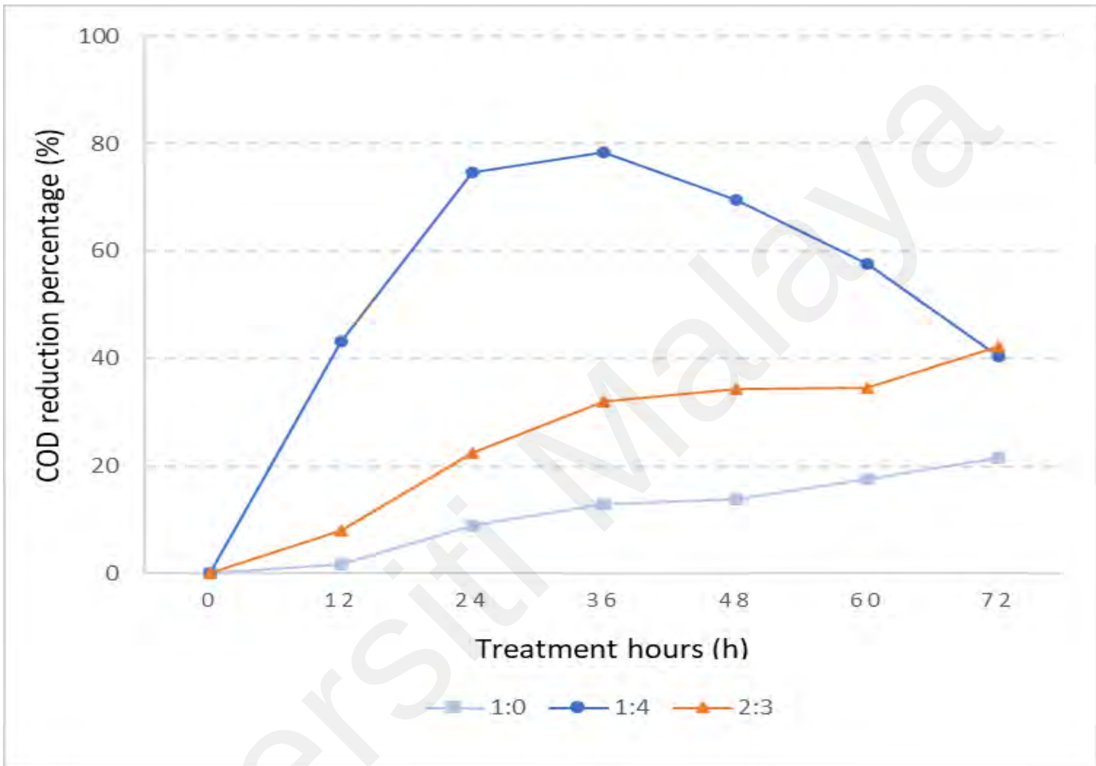


Figure 4.8: The COD reduction percentage of RTWW based on wastewater dilution of 1:0 (undiluted wastewater), 1:4 and 2:3.

The decolorization percentage was high within 72 hours of treatment at 1:4 wastewater dilution as shown in Figure 4.7 compared to undiluted wastewater in and 2:3 wastewater dilution in. COD reduction capabilities were also observed maximum in treatment time of 36 hours as shown in Figure 4.8 of 1:4 wastewater dilution compared to others. Dilution can help in reducing the initial concentration of pollutants, including colour and COD, in wastewater. Lower concentrations of pollutants may be easier to treat, and dilution can potentially enhance the performance of the treatment processes, such as biological treatment or chemical treatment by reducing the inhibitory effects of the pollutants towards microorganisms, leading to improved decolorization and COD reduction.

The reduction of the concentration of toxic dyes in wastewater can be achieved through dilution, thereby rendering the effluent less detrimental to the Malaysian GLMP. This, in turn, leads to a decrease in the treatment time required to address a particular pollutant.

Adsorption entails the binding of dye molecules to a solid substrate, such as activated carbon or nanomaterials. This method is advantageous due to its low initial cost, generation of non-hazardous by-products, and ability to eliminate dyes from even low concentration solutions (Ruan et al., 2019). Dilution has the potential to enhance the interaction between the dye molecules and the adsorbent, thereby augmenting the effectiveness of the adsorption process.

While dilution can indeed facilitate the easier treatment of textile wastewater, it is crucial to maintain a delicate equilibrium. Excessive dilution may result in excessive water consumption, increased treatment volumes, and higher operational costs. Therefore, careful consideration should be given to finding an optimal dilution ratio that maximizes treatment efficiency while minimizing associated drawbacks.

4.5 Colour percentage removal

Based on the findings presented in Figure 4.7, observed that the highest percentage of colour removal achieved within a period of 72 hours upon administering 10g of Malaysian GLMP. In the context of decolorizing dye in RTWW treatment, the maximum percentage of removal recorded at 77.24% when treated with 10g of Malaysian GLMP, with an initial treatment pH of 4 and a wastewater dilution ratio of 1:4.

The high surface area and porous structure of fungi mycelium improve their capacity to adsorb dye molecules (Wang et. al., 2019; Mohd Hanafiah et. al., 2019). Due to the mycelium's large surface area, more dye molecules can interact with the cell wall's binding sites. The adsorption capacity of mycelium can be ascribed to its extensive surface area, facilitating the binding of dye molecules.

The larger the surface area of mycelium, the greater the quantity of dye molecules it can adsorb, thereby enhancing the efficacy of wastewater treatment. The physical structure of the adsorbent, encompassing its overall charge, charge distribution, pore size, and accessible surface area, emerges as the pivotal determinant in the process of adsorption (Haidukowski et al., 2019).

The effectiveness of the biosorption process can be influenced by various factors such as the concentration of dye at the beginning, duration of contact, amount of mycelium used, and the existence of other ions or chemicals in the solution. Competitive adsorption between colours with other chemicals might occur. The amount of decolorization increases as the concentration of the initial textile dye wastewater decreases (Selvakumar et al., 2013). Decolorization is mostly caused by dyes adhering to microbial cell surfaces (Singh, 2017).

4.6 COD percentage reduction

In Figures 4.8, the chemical oxygen demand (COD) of the RTWW was examined to determine the adsorbent's affinity for various compounds found in textile effluent. The utilization of a small quantity of Malaysian GLMP for treatments may lead to the buildup of metabolic wastes within its cells during the initial stage, consequently inducing cell death and subsequently elevating the COD of the RTWW. As the volume of Malaysian GLMP is increased, the cells will adsorb and reduces the COD. The maximum reduction percentage was determined at 78.32% of treatment with wastewater dilution ration of 1:4, 10g of Malaysian GLMP at 36-hours of similar treatment. The efficacy of the Malaysian *Ganoderma lucidum* inoculum in the degradation of COD and ammonia was investigated in a recent study. The results revealed a remarkable percentage elimination ranging from 95% to 100% within a treatment period of 30 hours when applied to synthetic domestic wastewater (Mooralitharan et al., 2023).

The decline in COD reduction observed after a 36-hours treatment period may be attributed to the partial suppression of microbes responsible for the biodegradation of organic matter. This observation suggests that the susceptibility of microbes to their surrounding environment is a plausible explanation for the reduction in COD percentage (Khouni et al., 2012). The increasing COD reduction of controlled wastewater (without adsorbent) was increased due to the acidification effects. Research have found that COD values of wastewaters reduce as pH decreases from its starting value and further decrease when the treatment time is extended (Yang et al., 2021). As the pH rose from 1 to 3, the percentages of COD that were removed increased. At pH 3, a substantial COD decrease was attained (Birgani et al., 2016).

4.7 Langmuir and Freundlich adsorption isotherms

The data obtained from the experiment was subjected to analysis using the Langmuir and Freundlich isotherm models. This analysis aimed to determine the most suitable adsorption model of the coloured textile wastewater treated with 10g of GLMP, as indicated in Table 7.

Langmuir isotherms graph in Figure 4.9 and Figure 4.11 shows the variation of adsorption (Ce/Qe) against the equilibrium concentration (Ce) for adsorption of RTWW colour onto Malaysian GLMP for 1:4 and 2:3 wastewater dilution respectively. Figure 4.10 and Figure 4.12 shows the Freundlich isotherm representing variation of ln Qe with respect to ln Ce for adsorption of RTWW colour onto Malaysian GLMP for 1:4 and 2:3 wastewater dilution.

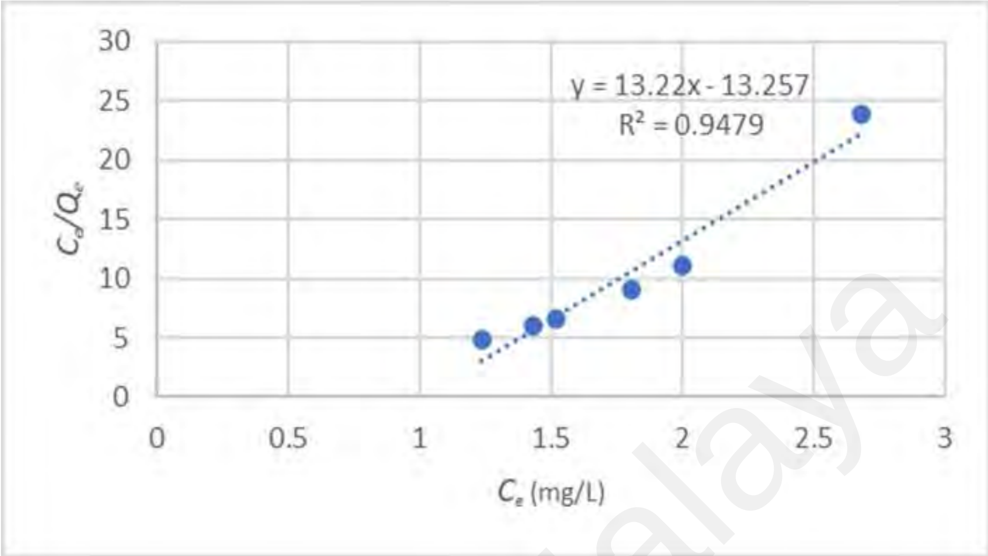


Figure 4.9: Langmuir isotherm showing the variation of adsorption (Ce/Qe) against the equilibrium concentration (Ce) for adsorption of RTWW colour onto Malaysian GLMP for 1:4 wastewater dilution.

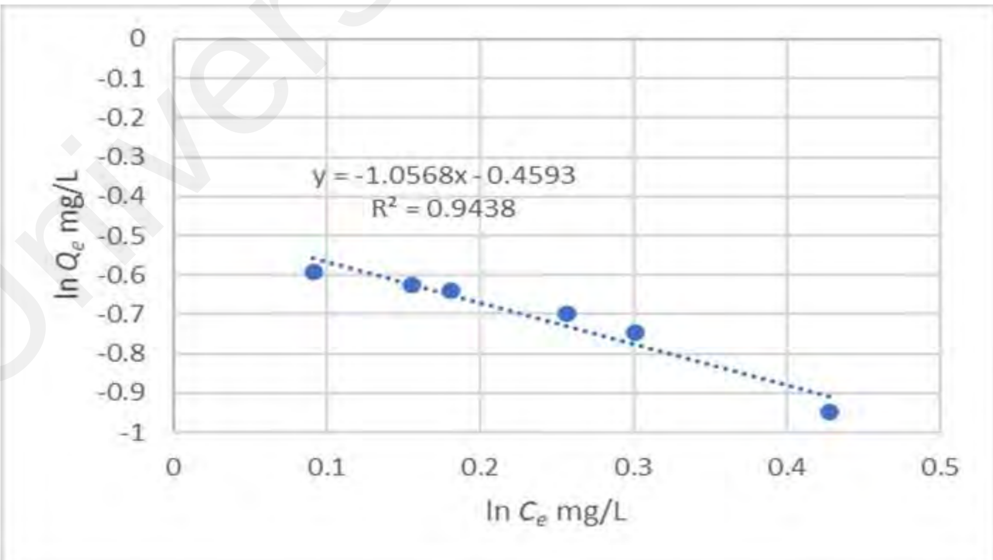


Figure 4.10: Freundlich isotherm representing variation of ln Qe with respect to ln Ce for adsorption of RTWW colour onto Malaysian GLMP for 1:4 wastewater dilution.

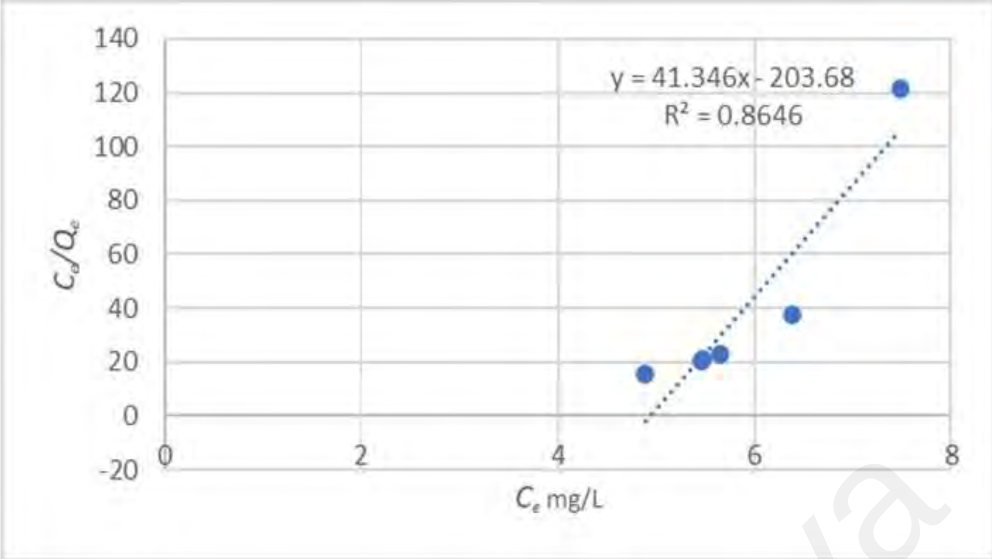


Figure 4.11: Langmuir isotherm showing the variation of adsorption (Ce/Qe) against the equilibrium concentration (Ce) for adsorption of RTWW colour onto Malaysian GLMP for 2:3 wastewater dilution.

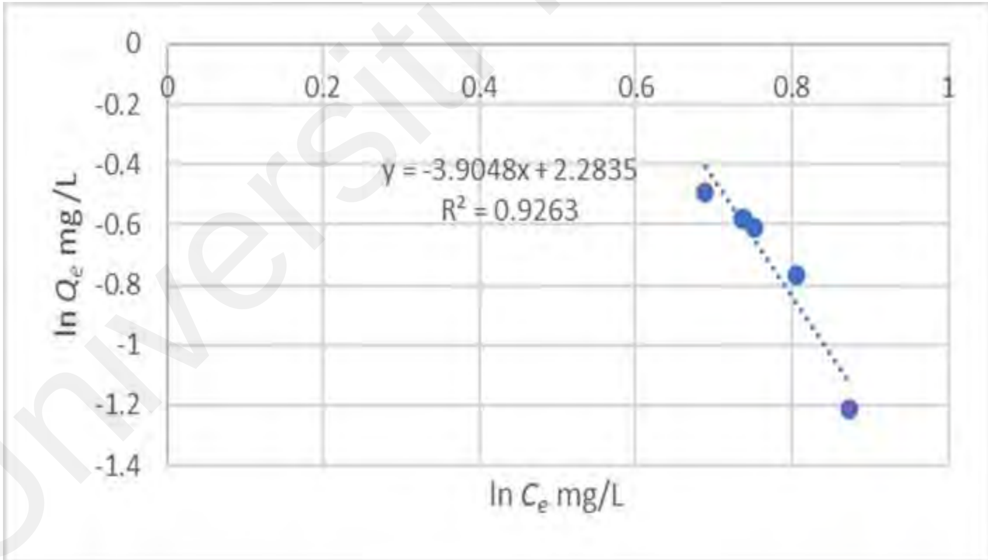


Figure 4.12: Freundlich isotherm representing variation of ln Qe with respect to ln Ce for adsorption of RTWW colour onto Malaysian GLMP for 2:3 wastewater dilution.

Table 7. Langmuir and Freundlich adsorption isotherms for the decolorization of RTWW at different wastewater dilution ratios. (Experimental conditions: adsorbent dosage = 10.0 g per 400 mL, mixing rate = 150 rpm, Aeration rate = 4 L/min, Temperature= Ambient).

Isotherms / Adsorbates	Langmuir Isotherm			Freundlich Isotherm		
	Q_m (mg/g)	K_L (L/mg)	R^2	K_f	n	R^2
1:4 Dilution	0.0866	2.6820	0.9479	0.0294	0.9463	0.9438
2:3 Dilution	0.0257	0.3165	0.8646	0.0091	0.2561	0.9263

The equilibrium isotherms of different biomass-derived adsorbents for the purpose of removing dye from wastewater were analyzed using Langmuir and Freundlich models. The obtained Langmuir and Freundlich adsorption constants and correlation coefficients (R^2) are presented in Table 7, which were utilized to determine the most suitable model for colour adsorption and COD reduction.

Results revealed that the colour adsorption onto Malaysian GLMP was fitted for both Langmuir and Freundlich adsorption isotherm for wastewater dilution of 1:4 with R^2 of 0.9479 and R^2 of 0.9438 respectively. For 2:3 wastewater dilution, Freundlich adsorption isotherm are more favorable, R^2 of 0.9263 compared to Langmuir, R^2 of 0.8646.

According to the Freundlich equation in its linear form, the nature of adsorption can be determined based on the value of the parameter n. When n=1, the adsorption process considered to be linear. On the other hand, if n<1, it indicates chemical adsorption, while n>1 suggests physical adsorption. The value of n represents the degree of nonlinearity between the concentration of the solution and the adsorption process. In the specific case of the experiment, the value of n was found to be 0.9463 for a wastewater dilution ratio of 1:4, and 0.25610 for a dilution ratio of 2:3. The fact that n<1 in both cases implies that the colour was chemically adsorbed onto the Malaysian GLMP.

4.8 Comparison of the current work on adsorption using GLMP toward decolorization and COD reduction

The comparison of wastewater adsorption utilizing Malaysian GLMP was conducted in relation to other substances. The adsorption of dye and COD removal using *Ganoderma lucidum* was compared with that using other dyes, as shown in Table 8, with regards to the parameters of decolorization and COD reduction. Fungi assumes a significant role in tackling present and future sustainability challenges by facilitating various processes, including the degradation of lignocellulose, the generation of biofuels, and the remediation of biomass.

Due to their distinctive enzymes and environmentally friendly characteristics, fungi assume a crucial function in the advancement of sustainable resource utilization and remediation approaches. The utilization of fungi for treatment purposes offers numerous advantages compared to bacterial treatments, with one notable advantage being their capability to degrade intricate pollutants effectively (Wan Mohtar et al., 2022).

Table 8. Comparison of current studies on adsorption using GLMP toward wastewater decolorization and COD reduction.

Type	Compound Removed	Treatment Time	COD Reduction	Colour Reduction	References
Malaysian GLMP	Real Textile Wastewater (RTWW)	72 hours 36 hours	- 78.32%	77.24% -	This study
Wild-Serbian *Ganoderma lucidum* mycelium	Synthetic domestic sewage	120 hours	96.00%	NA	(Mohd Hanafiah et al., 2019)
***G. lucidum* mycelium**	Paper Mill Effluent	15 to 18 days	98.00%	94.00%	(Perumal et al., 2000)
***G. lucidum* mycelium**	Textile Dye Wastewater	120 hours	81.40%	91.30%	(Selvakumar et al., 2013)
***G. lucidum* mycelium**	Batik Dye Effluent - Naphtol Black	30 days	81.03%	60.53%	(Pratiwi, Indrianingsih, & Darsih, 2017)

Table 8, continued.

Type	Compound Removed	Treatment Time	COD Reduction	Colour Reduction	References
G. lucidum **supported on PET-coated SBS paper**	Remazol Brilliant Blue R	30 days	NA	96.00%	(Rainert et al., 2021)
Malaysian *Ganoderma Lucidum Inoculum*	Synthetic domestic Wastewater	30 hours	>95.00%	NA	(Mooralitharan et al., 2023)

* NA denotes not available.

This study showed that the decolorization rate achieved 77.24% within 72 hours. of treatment using Malaysian GLMP as primary adsorbent. Based on comparison on current works on adsorption of dyes using GLMP, this study recorded shorter treatment time compared to other studies using real dye containing wastewater.

COD reduction of RTWW recorded at 78.32% within 36 hours of treatment which is lower than the other studies. This difference of study results was influenced by RTWW wastewater individual characteristic is where the textile production factories are usually using many types of dyes and many toxin chemicals present.

4.9 Limitations, challenges, and future strategies of GLMP decolorizations

The sustainable and eco-friendly treatment approaches are mostly showing a great potential to be further the studies and implemented in the industries. However, addressing the limitations and challenges of GLMP decolorization and future strategies as per illustrate in Figure 4.13 aims to further enhance its efficiency and sustainability approach.

- Composition and characteristics of effluent

GLMP's efficacy in decolorization and COD reduction of diverse industrial effluents and wastewater may be limited. The efficiency of decolorization is contingent upon the specific composition and characteristics of the effluent. Additionally, the growth of GLMP may be impeded by the toxicity present in certain industrial effluents and wastewater, thereby constraining its capacity to decolorize and reduce COD levels in the effluent.

According to Zarimah Mohd Hanafiah (2019), the accumulation of metabolic waste during the treatment induced cell death, which is why GLMP removal performance was less effective.

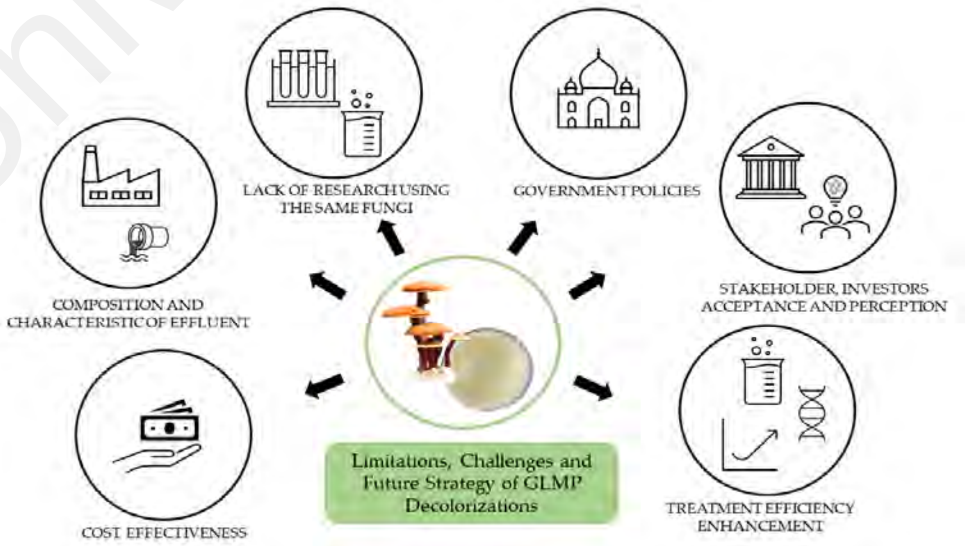


Figure 4.13: Limitations, and future strategies for Malaysian GLMP decolorizations.

- Cost effectiveness

The process of optimizing the conditions for GLMP decolorization and COD reduction can be time-consuming and require a significant amount of experimentation which may lead to cost of producing GLMP on a large scale can be expensive, and the process may not be economically feasible for some industries.

In a study conducted by Wan-Mohtar (2016) found that different method of production of exopolysaccharide of *Ganoderma lucidum* produce different results where shake flask produces 1.3-fold more with only 6 days of total fermentation time for repeated batch fermentation compared to 32 days for batch culture in a bioreactor. This research revealed a shorter production time of producing mycelium pallets.

- Treatment efficiency enhancement

In the realm of application, the utilization of immobilized fungal mycelium has demonstrated efficacy in the remediation of dye wastewater. The immobilized mycelium has exhibited a notable processing capacity for diverse forms of wastewaters, encompassing dye wastewater. Furthermore, it has the ability to sustain a heightened level of activity even following successive rounds of wastewater treatment, thereby enabling uninterrupted treatment processes. (Guo et al., 2020).

Advances 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 the efficiency of decolorization, particularly for effluents that are difficult to degrade. Immobilization of the 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 colour and COD as a study recorded high color removal percentage of 98.7% and COD reduction percentage of 66.7% using Activated Zeolite in treating real textile wastewater (Zahuri et al., 2023).

The enhancement of process parameters, including temperature, pH, and agitation, through continuous optimization can result in heightened efficacy 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 pallets for textile wastewater treatment. This lack of understanding can hinder the development of appropriate policies and guidelines for implementation.

The absence of 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 face constraints due to insufficient awareness and comprehension of the potential advantages and efficacy associated with the utilization of mycelium pallets for bioremediation. Consequently, this dearth of understanding may lead to inadequate backing, financial resources, or motivational factors for the execution of these technological advancements.

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.

- Stakeholder, investors acceptance and perception

The use of mycelium pallets for wastewater bioremediation may be met with skepticism or resistance from the stakeholder and investors due to unfamiliarity or misconceptions about the technology. This could create challenges in gaining their trust and support for scaling up bioremediation projects which need significant infrastructure investments and operational costs.

4.10 Sustainable Development Goals and Malaysian Policy Linkage

The United Nations' adoption of the Sustainable Development Goals (SDGs) in 2015 signified a momentous global commitment to addressing urgent socio-economic, environmental, and developmental challenges. Comprising 17 interconnected goals and 169 targets, these SDGs establish a comprehensive framework aimed at achieving sustainable development on a global scale by the year 2030. Malaysia has duly recognized the significance of the SDGs as a guiding framework for its own national development, as evidenced by its inclusion in various national policy documents. The process of aligning Malaysia's development plans with the SDGs commenced with the 11th Malaysia Plan (11MP) for the period of 2016-2020, followed by the Mid-Term Review (MTR) of the 11MP for the years 2018-2020. Subsequently, the 12th Malaysia Plan (2021-2025) and the 13th Malaysia Plan (2026-2030) were formulated in accordance with the 2030 Agenda for Sustainable Development. Additionally, Malaysia has developed a

Sustainable Development Goals Roadmap and the National Sustainable Development Goals Blueprint (2016-2030) to further support its commitment to the SDGs.

The United Nations has identified several Sustainable Development Goals (SDGs) that are crucial for the well-being of our planet and its inhabitants. Among these goals, SDG 6 emphasizes the importance of providing clean water and sanitation for all people promoting better health of humankind as per SDG 3. SDG 11 aims to ensure inclusive, secure, resilient, and sustainable urban and human settlements through the promotion of sustainable cities and communities while using GLMP as bioadsorbent cultivated from natural source will ensure sustainable consumption and production fulfilling the SDG 12. Additionally, SDG 15 seeks to safeguard, restore, and encourage the sustainable utilization of land-based ecosystems, manage forests sustainably, reduce desertification, halt and reverse soil degradation, and halt biodiversity decline. Finally, SDG 14 focuses on ensuring inclusive, secure, resilient, and sustainable urban and human settlements towards the oceans, seas, and marine resources. These SDGs are critical for the achievement of a sustainable future for all.

Figure 4.14: Sustainable development goals and Malaysian policy linkage.

Malaysia, like other countries, is faced with potable water scarcity and wastewater management challenges, particularly in urban areas. One of Malaysia's ways to meet SDGs as per Figure 4.14 is by implementing innovative and sustainable solutions, such as fungal based treatment systems. White-rot fungi, on the other hand, are the most economical method of treating water contains chemical and have the ability to remove pathogenic microbes such as *E.coli* in water (Wan Mohtar et al., 2022).

Fungal bioremediation can be applied as pre-treatment of potable water treatment plant to reduce and eliminate pollutants before the next treatment processing stage, thus enhancing the treatment design capabilities in treating more type of pollutants and reducing cost of chemicals used.

In addition to enhancing the quality of discharged wastewater, the implementation of fungal wastewater treatment methods can effectively enhance the treatment of both domestic and industrial wastewater. This approach aids in minimizing the release of harmful pollutants into water bodies, thereby contributing to the preservation of clean water resources and the conservation of marine ecosystems. Furthermore, the solid waste generated from water and wastewater treatment processes can be safely repurposed as soil fertilizer and soil stabilizer. This is due to the potential presence of beneficial enzymes within the waste, which exempts it from being classified as scheduled waste.

Malaysia's policy linkage towards the usage of fungal-based wastewater treatment technologies demonstrates a proactive approach to address water-related challenges while aligning with United Nation's Sustainable Development Goals (SDGs). Current available studies provide valuable insights into the potential of fungal-based solutions in the Malaysian context, highlighting their role in achieving these sustainability goals.

4.11 Sustainable prototype system integration as pre-treatment of a conventional potable water treatment plant in Malaysia

The integration of the fungal Malaysian GLMP treatment prototype as a pre-treatment process to conventional potable water treatment systems as per illustrated in Figure 4.15, signifies a strategic approach to advancing sustainable water treatment practices.

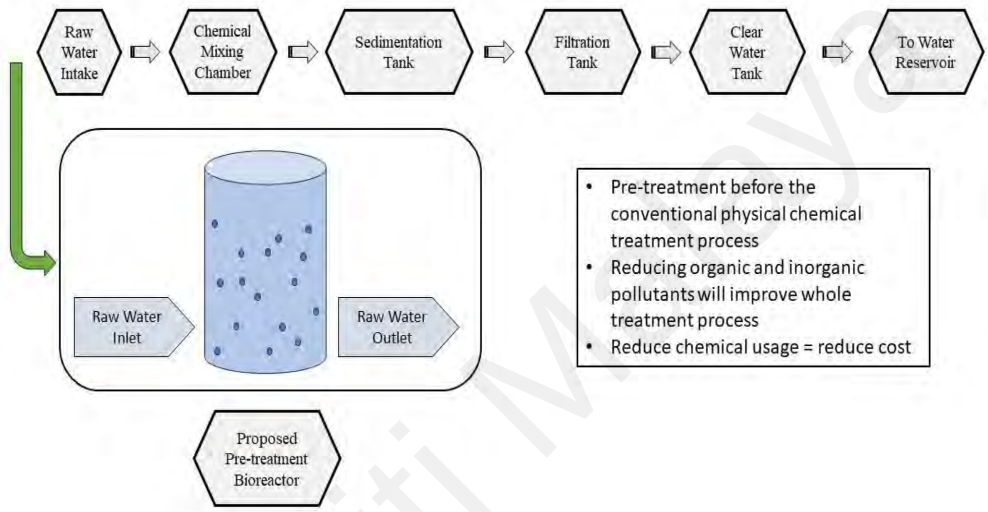


Figure 4.15: The integration of Malaysian GLMP as pre-treatment process to conventional potable water treatment plant systems.

The primary objectives of integrating the fungal Malaysian GLMP treatment prototype as a pre-treatment process to conventional potable water treatment systems includes to effectively reduce specific contaminants and organic compounds in the raw water feed, thereby enhancing the overall water quality entering the conventional treatment process, to decrease reliance on traditional chemicals in subsequent treatment stages, resulting in cost savings and environmental benefits and help achieve sustainability goals by improving energy efficiency and minimizing the environmental footprint of water treatment operations.

The integration approach includes the following key steps: Design and implementation of a specific pre-treatment phase in the water treatment plant using purpose-designed bioreactors optimized for the growth of Malaysian GLMP fungi, Insertion of cultivated Malaysian GLMP fungi biomass into pre-treated bioreactors for contaminant removal phase, Utilization of collected data to optimise operational parameters to ensure maximum contaminant removal performance during pre-treatment phase, Rigorously test under operational conditions to confirm its effectiveness, and providing training to operator in ensuring the system being managed effectively.

CHAPTER 5: CONCLUSION

For centuries, *Ganoderma lucidum* has been utilized in traditional medicine due to its numerous health benefits. Recently, there has been an increasing interest in the potential application of GLMP for the reduction of COD and decolorization of various industrial effluents and wastewater. The bioremediation of real textile wastewater's colour and COD through the utilization of Malaysian GLMP has shown great potential in addressing the challenges posed by the industries. The potential usage of Malaysian GLMP using batch bioreactor for the decolorization and COD reduction of industrial effluents and wastewater is evident. The utilization of 10g of Malaysian GLMP volume, initial treatment pH of 4, wastewater dilution of 1:4 (wastewater: distilled water), 150 rpm of agitation speed, and aeration rate of 4 L/min has been found to yield the highest removal of RTWW colour and COD under unsterile conditions, as supported by the experimental data.

The use of Malaysian *Ganoderma lucidum* in its mycelium pellets forms provides a sustainable and environmentally friendly solution for the treatment of textile wastewater using a batch bioreactor, with the potential to significantly reduce colour intensity and organic pollutants. This natural and environmentally friendly wastewater treatment method is aligned with United Nation's Sustainable Development Goals.

However, it is important to acknowledge the existing challenges that must be addressed. 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 real textile wastewater.

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