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Phytoremediation of Remazol Brilliant Blue R Dye using Different Types of Aquatic Plants

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Anthraquinone dyes such as Remazol Brilliant Blue R (RBBR) has a toxic characteristic due to its aromatic structure and known as the second largest dye used in the textile industry. This study aimed to investigate the remediation potential of various aquatic plants towards anthraquinone dye. In this study, RBBR dye was treated with *Pistia* sp., *Eichhornia* sp., and *Salvinia* sp.. The optimisation studies were carried out using different pH value (5, 7, and 9), fresh biomass (20, 40, and 60 g), and dye concentration (10, 20, 30 m/L). Findings after optimisation demonstrated that 60 g of fresh biomass, 10 mg/L of initial dye concentration, and pH 7 were the favourable conditions of *Pistia* sp. in degrading RBBR dye. The UV-Vis analysis showed a significant difference (p-value <0.05) in the absorption spectra before and after treatments, indicating the breakdown of the dye molecules. FTIR analysis demonstrated the difference between the peak numbers of functional groups before and after the root of *Pistia* sp. plants were exposed to dye. Besides, the plant cell analysis showed the accumulation of RBBR dye in the plant cell. Meanwhile, the phytotoxicity test showed that the germination of *Vigna radiata* with the treated water sample was higher compared to the untreated RBBR dye. This study revealed that *Pistia* sp. exhibited the highest efficiency in degrading RBBR dye, with 93.4% decolourisation efficiency.

Keywords: Anthraquinone; biosorption; biodegradation; phytoremediation; toxicity

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Dyes can be divided into two categories, which are synthetic and natural dyes. However, as the demand for dyes grows rapidly, synthetic dyes derived from petrochemical compounds have been developed to meet the demand [1]. The extreme uses of dye in industries such as food, textile fibres, leather, paper, tannery, pharmaceuticals, and cosmetics have been a source of environmental concern. Precisely, the wastewater discharged from the industries affects primary clean water sources, such as lakes and rivers [2].

Dyes discharged from the sewage negatively impact living organisms as they are highly carcinogenic, mutagenic, and toxic, which has potential in disturbing the ecosystem [1]. In addition, the water contaminated with dyes increases the death rate of aquatic life as light and oxygen cannot penetrate the water properly [3]. Humans are generally prone to be affected and exposed to diseases caused by contaminated water, such as cholera, diarrhoea, typhoid, and polio. This may continue indefinitely if wastewater from industries is not effectively treated [4].

Various methods have been explored for the remediation of dyes, including UV-light, flocculation, carbon absorption, electrocoagulation, and ultrafiltration. However, these methods are costly, time-consuming, and not eco-friendly. In addition, the approaches are ineffective as they generate high amount of sludge, produce toxic byproduct, and require further disposal [5]. Remazol Brilliant Blue R (RBBR) is categorised as a highly carcinogenic and recalcitrant pollutant that can harm both vegetative and aquatic life. RBBR is also classified as a reactive dye with poor degree of fixation, and it is not easily degraded due to its stable chemical structures. As a result, it is difficult to degrade by physical or chemical methods, necessitating a biological method to remediate the dye without harming the ecosystem [6].

Phytoremediation is one of the biological techniques used in the dye treatment process, in which plants are used as biosorbents to absorb pollutants, such as dye and heavy metals. Aquatic plants contain carboxylic and sulfonate groups, providing a high metal binding capacity as an adsorbent [7]. Moreover, each aquatic plant species has different abilities in decolourising pollutants due to various factors, such as dye structure, dye concentration, pH value, and enzymatic reactions [8]. Several plants, such as *Azolla*

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pinnata and *Lemna minor* have been proven to absorb dye in remediating azo dyes. The use of plants to degrade dyes is one of the most practical methods, as it is environmentally friendly, inexpensive, and effective [2].

This study discovered an alternative way to treat dye pollution through the biological method, which is phytoremediation, that is environmentally friendly. Since it is considered a new technology, the application of aquatic plants for this technique is still in the early stages and has limited exploration on a large scale, especially in Malaysia [9]. This has led to the importance of this study to conduct further research about the efficiency of the aquatic plants in degrading dye in a clear view. Thus, exploring and understanding more about the optimisation study, which maximises the efficiency of different aquatic plants, particularly *Pistia* sp., *Eichhornia* sp., and *Salvinia* sp., in removing dye are necessary.

MATERIALS AND METHODS

Collection and Preparation of Aquatic Plants

The aquatic plants were purchased from the local shop. Then, plants (*Eichhornia* sp., *Pistia* sp., and *Salvinia* sp.) were washed with distilled water to remove all dirt and impurities to get a clean sample of plants [10].

Preparation of Dyes

The stock solution (20 mg/L) of RBBR was weighed accurately without further purification and mixed with 1 L of distilled water to achieve the 20 mg/L concentration [11].

Dye Decolourisation Activity

Firstly, 40 g of aquatic plants (*Eichhornia* sp., *Pistia* sp., and *Salvinia* sp.) were placed in contact with 250 mL of RBBR dye solution at a concentration of 20 mg/L, respectively. The solution was mixed using an orbital shaker under constant agitation at 90 rpm for approximately 10 days. The decolourisation activity was analysed using UV-Vis spectrophotometer at 590 nm wavelength using the following formula [5]:

$$Decolorization\ efficiency\ (DE, \%) = \frac{Initial\ absorbance - Final\ absorbance}{Initial\ absorbance} \times 100\%$$

Optimisation Studies

Then, the optimisation of the study was conducted to establish the optimum conditions in the dye decolourisation activity. The decolourisation activity was observed in a specific condition based on the variability of pH value (5, 7, and 9), fresh biomass (20, 40, and 60 g), and dye concentrations (10, 20, 30 mg/L) [12].

Dye Decolourisation at Optimal Conditions

The dye removal efficacy was further evaluated under the combination of optimal conditions derived from the OFAT test. The optimised conditions were implemented to determine the best conditions for plants to remediate dyes. Fresh biomass was first generated and used for the decolourization study as described earlier. After 32h, 3 ml of dye supernatant was collected and subsequently used for absorbance reading. The decolourization efficiency of RBBR dye was then calculated [12].

UV -Vis Analysis

UV-Vis spectrophotometer was used to evaluate the dye removal by comparing the absorbance peak of the treated and untreated dyes. The RBBR (treated and untreated) was monitored at 590 nm maximum wavelength [13].

FTIR Analysis

Fourier-transform infrared (FTIR) spectroscopy was performed to analyse the presence of functional groups in dye before and after treatments. The roots of *Pistia* sp. were dried in an oven at 45ºC for 24 hours before being ground to fine powder. The spectral range of the FTIR was set up at 4000–650 cm^{-1}, with a resolution of 4 cm^{-1} and a scan speed of 2 mm/s [14].

Plant Cell Analysis

Plant cell analysis was conducted using a light microscope to analyse the changes on the part of the plant (leaf) before and after the plant was exposed to RBBR dye [7].

Phytotoxicity Study

The phytotoxicity test was conducted by observing the germination of *Vigna radiata* (mung bean) after being exposed to the pre-treated (0 h) and treated (32 h) RBBR dye. Ten seeds were used for every petri dish containing filter paper. Then, 5 ml of distilled water, pre-treated RBBR dye, and post-treated RBBR dye were applied on every petri dish daily, for 7 days [5]. The seed germination (%) rate was determined using this formula [5]:

$$Germination\ \% = \frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds} \times 100\%$$

Statistical Analysis

Statistical data was analysed with one-way Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS).

RESULTS AND DISCUSSION

Decolourisation Efficiency (DE) of RBBR by *Eichhornia* sp., *Pistia* sp., and *Salvinia* sp.

This study demonstrated the decolourisation efficiency of RBBR dye using different types of aquatic plants consisting of *Eichhornia* sp., *Pistia* sp., and *Salvinia* sp.. Figure 1 shows that *Pistia* sp. performed the most rapid decolourisation when it was applied to RBBR dye for 10 days, followed by *Eichhornia* sp., and *Salvinia* sp.. The dye removal efficiency was achieved with 71% of DE by *Pistia* sp., 64% of DE by *Eichhornia* sp., and 51% of DE by *Salvinia* sp.. The removal of dye achieved by *Pistia* sp. was significantly higher compared to others, which could be influenced by the enzymatic activities of the plant roots upon exposure to dye stresses [14]. An earlier study stated that the deterioration of dye by plants is influenced by the activation of the peroxidase and oxidoreductase (catalase, azo-reductase, and laccase) that has been triggered by the physiological stress that happened to the plant [11]. This proved that the removal efficiency of RBBR dye had triggered the enzymatic activity of *Eichhornia* sp., *Pistia* sp., and *Salvinia* sp.. Furthermore, the phenol and flavonoid compounds from the plants may also contribute to the dye removal activity by acting as an antioxidant that neutralises free radicals and reactive oxygen, thus increasing the performance of *Pistia* sp. to effectively remove the dye [15].

The highest DE of *Pistia* sp. could be attributed to its high amount of biomass consisting of carbohydrate, fat, protein, and raw fibers. The high contents of carbohydrate and protein in plant contribute to the stimulation of its growth and production of enzymes. Besides, *Pistia* sp. could also resist a highly toxic environment due to its larger root surface that could trap the particles of dyes [16].

A study by Mustafa & Hayder recorded that *Pistia* sp. showed the highest efficiency in the accumulation of dye and heavy metals compared to *Eichhornia* sp. and *Salvinia* sp. since it can tolerate high concentrations of heavy metals [2]. A similar finding by Madikizela et al. showed that *Pistia* sp. has the highest efficiency in the absorption of dyes (Direct Blue, Congo red, and Malachite green) and heavy metals (Copper and Zinc) [17]. This proved that *Pistia* sp. could act as the best hyperaccumulator, resulting in the most rapid removal efficiency in RBBR dye [18].

Effect of Biomass on Decolourisation of RBBR by *Pistia* sp.

Figure 2 shows that there were significant differences in the removal activity of RBBR dye when different biomass (20, 40, and 60 g) was put into contact with 20 mg/L RBBR dye. The highest efficiency was achieved by 60 g fresh biomass of *Pistia* sp., with nearly complete decolourisation (98%), which was significantly higher compared to 40 g biomass (81%) and 20 g biomass (55%). The increasing amount of biomass increases the tolerance of *Pistia* sp. with textile wastewater, allowing them to remediate the dye with greater efficiency [19]. The higher the amount of fresh biomass in contact with dye, the faster the dye molecules were broken down, owing to plants' high metabolic rate and enzyme activity [3].

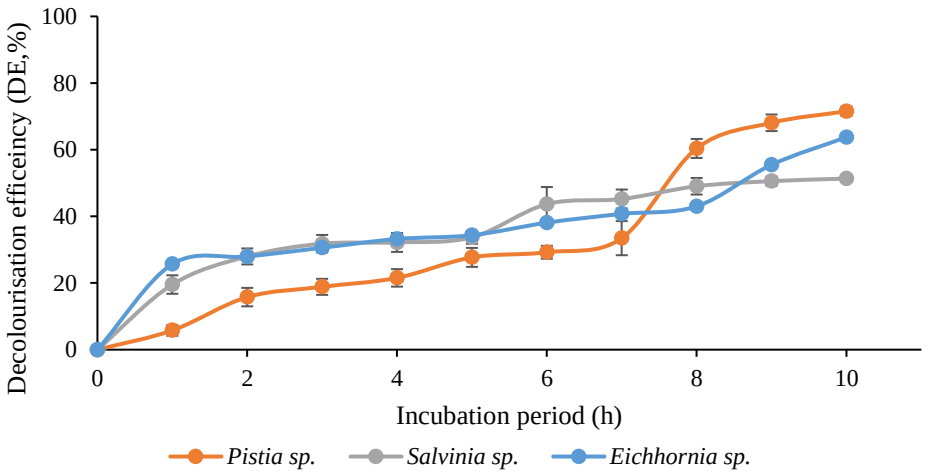

Figure 1. Decolourisation activity of *Eichhornia* sp., *Pistia* sp., and *Salvinia* sp. when placed in contact with RBBR dye.

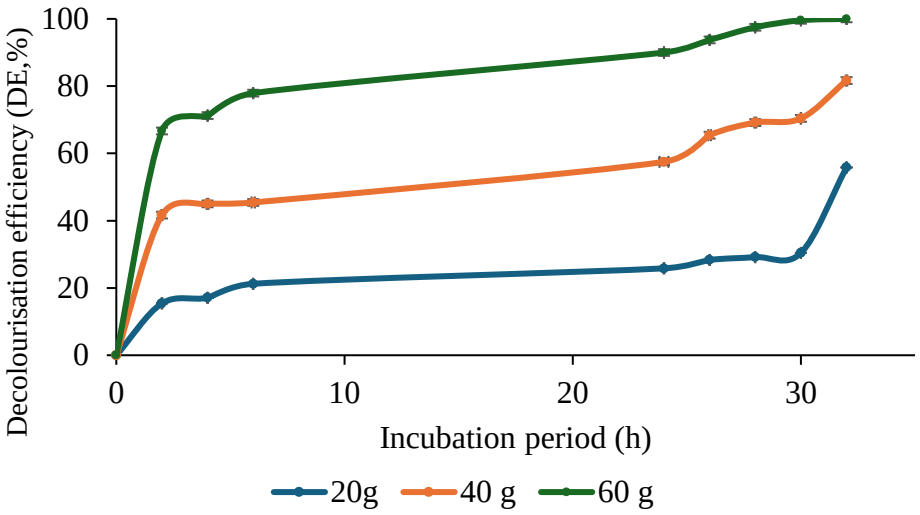

Figure 2. Decolourisation of RBBR dye with difference biomass (20, 40, and 60 g) within 32 h of incubation period.

Effect of Initial Dye Concentration on the Removal Efficiency of RBBR Dye by *Pistia* sp.

Figure 3 presents 10 mg/L initial dye concentration achieved the highest decolourisation activity (88% of DE), which was significantly higher compared to 20 mg/L and 30 mg/L, which recorded 53% and 45%, respectively. From the observation, it could be concluded that the higher concentration of initial dye led to the lower removal efficiency due to the limited capacity of the adsorbent to absorb the dye as there is high mass transfer driving force caused by the high initial dye concentrations [1]. High concentration of initial dye concentrations could lead to low removal efficiency due to the limited capacity of the plants to absorb the dye. This phenomenon indicated the saturation of the metabolic pathways of *Pistia* sp. since the capacity of the plants has been limited by the excess amount of dye molecules [1]. Moreover, high initial dye concentration resulted in high toxicity of the dye, which affected the viability of the plants. The exposure of the plants to the highly toxic environment affects their metabolic process, which could disrupt the normal functioning of cellular enzymes of the plants. Thus, the plant uptake is reduced, resulting in slower rate of dye degradation [20]. A similar study also stated that 10 mg/L of RBBR achieved the most effective photodegradation reaction by rambutan leaves [6].

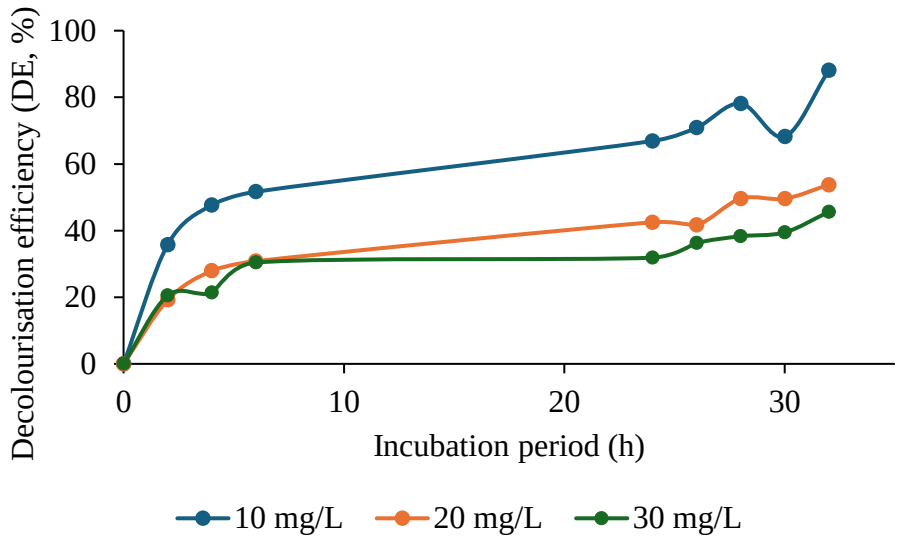

Figure 3. Decolourisation of RBBR dye at different initial dye concentrations (10 mg/L, 20 mg/L and 30 mg/L) with 32 h incubation period.

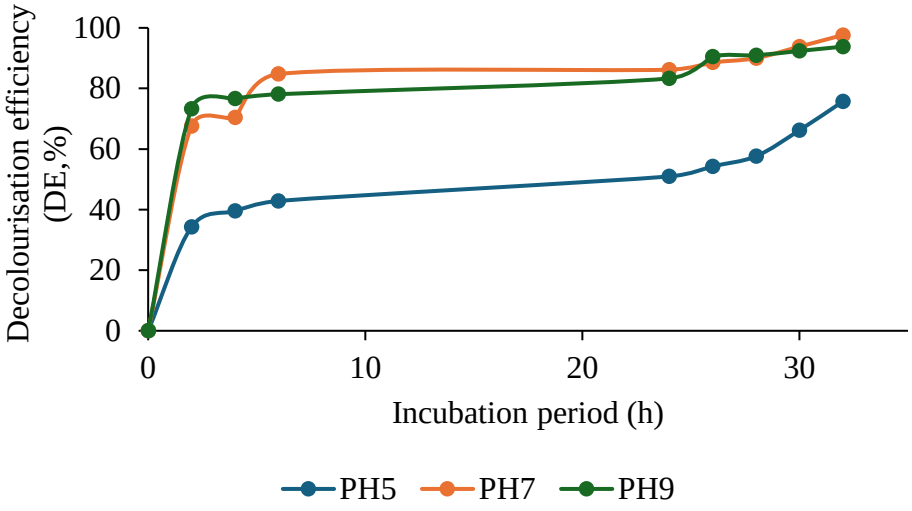

Figure 4. Effects of different pH (5, 7 and 9) on the decolourisation percentage of RBBR dye.

Effects of Different pH on the Removal Efficiency of RBBR Dye by *Pistia* sp.

Figure 4 shows that pH 7 had the highest decolourisation activity (97% of DE), which was significantly different from pH 9 (93% of DE) and pH 5 (75% of DE). Essentially, the optimum pH for *Pistia* sp. to work in the best condition is between the range of 6 to 7 pH values. However, from the 4.4 Figure, when the *Pistia* sp. was put in contact with RBBR dye, pH 9 could also be chosen as the best condition since its decolourisation efficiency was above 90%, but the alkalinity condition of dye could lead to the wilted and decayed condition of *Pistia* sp. after it was exposed to dye in 32 h of the incubation period [11]. It could be predicted that *Pistia* sp. can adapt to alkaline conditions and change its physiological activity to survive at an elevated level of pH.

Moreover, previous findings on *Pistia* sp. stability by Arise et al. recorded that *Pistia* sp. showed higher leaf peroxidase activity when it was placed between the pH range of 6–6.5 [21]. A similar finding reported that the pH changes led to the maximal adsorption between Indosol Dark Blue GL dye and water hyacinth leaves due to the higher activity of electrostatic attraction. This occurs because of structural changes in the adsorbents and adsorbate that cause them to be attracted to one another [1]. It could be proven that structural changes in anionic RBBR dye molecules could be changed by the protonation process that occurred at certain pH, attracting them to the negatively charged surface of *Pistia* sp..

Decolourisation of RBBR Dye in Optimised Condition by *Pistia* sp.

The best parameters for RBBR dye showed that 93% of DE was achieved when optimised conditions were applied (Figure 5). The incubation period for the combined optimised condition remained the same (32 h). However, the decolourisation percentage achieved was significantly higher compared to non-optimised conditions (18% of DE). The optimised condition allowed the biosorbent which is *Pistia* sp. to work in the best condition to continuously remove RBBR dye. This study demonstrated that the favourable conditions of *Pistia* sp. could lead to better growth and higher enzyme secretion, resulting in the higher decolourisation efficiency of RBBR dye [22]. Previous study about optimisation studies such as pH, temperature, enzyme activity and initial dye concentration were also conducted on the two different white rot fungi such as *C. versicolor* and *P. ostreatus*, to determine the best white rot fungi in decolourizing RBBR dye effectively [23]. Therefore, optimisation study is a crucial method to discover the most productive way to maximize the decolourization efficiency of RBBR dye since it could save time, cost and energy effectively.

UV-Vis Analysis

The changes in the RBBR's dye structures could be examined by UV-Vis spectrophotometer. Figure 6 shows that *Pistia* sp. has the potential to remediate RBBR dye. The untreated RBBR dye (control) showed the highest peak at 590 nm, while the treated RBBR dye showed a reduction of the peak after a 32h incubation period. These two peaks showed a significant difference in the decolourisation of RBBR dye before and after treatments. The reduction of a major absorption peak implied the occurrence of the biodegradation process following exposure to *Pistia* sp., indicating that the molecules of RBBR dye were broken down by the metabolic process of the plant [5]. The chromophore group in the dye could influence the change in the wavelength, and the magnitude of the wavelength could be influenced by the visible light

that appears when the white light passes through a certain medium [24]. Thus, the peaks indicated that RBBR dye absorbed the strongest light at 590 nm. A study done by Samthishkumar et al. also recorded a similar wavelength for the adsorption of RBBR dye using *Jatropha curcas* [15].

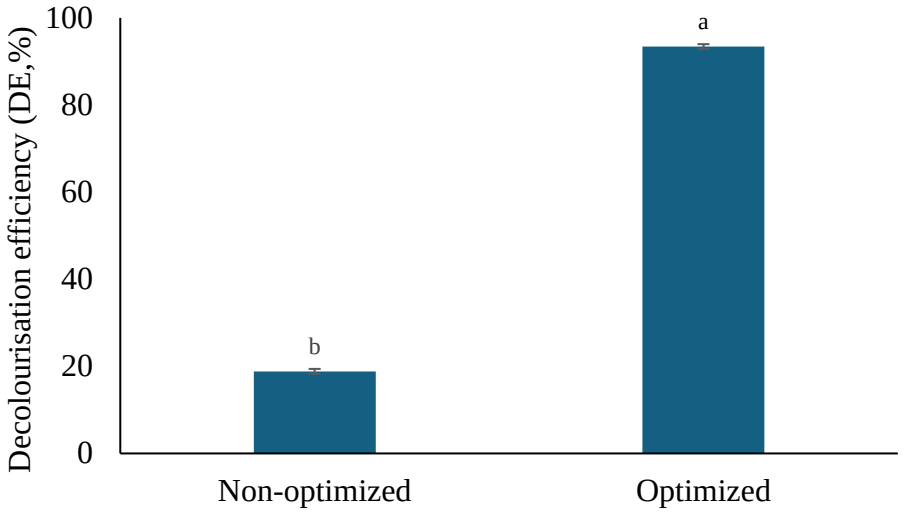

Figure 5. Decolourisation efficiency of RBBR by *Pistia* sp. using optimised conditions and non-optimised conditions.

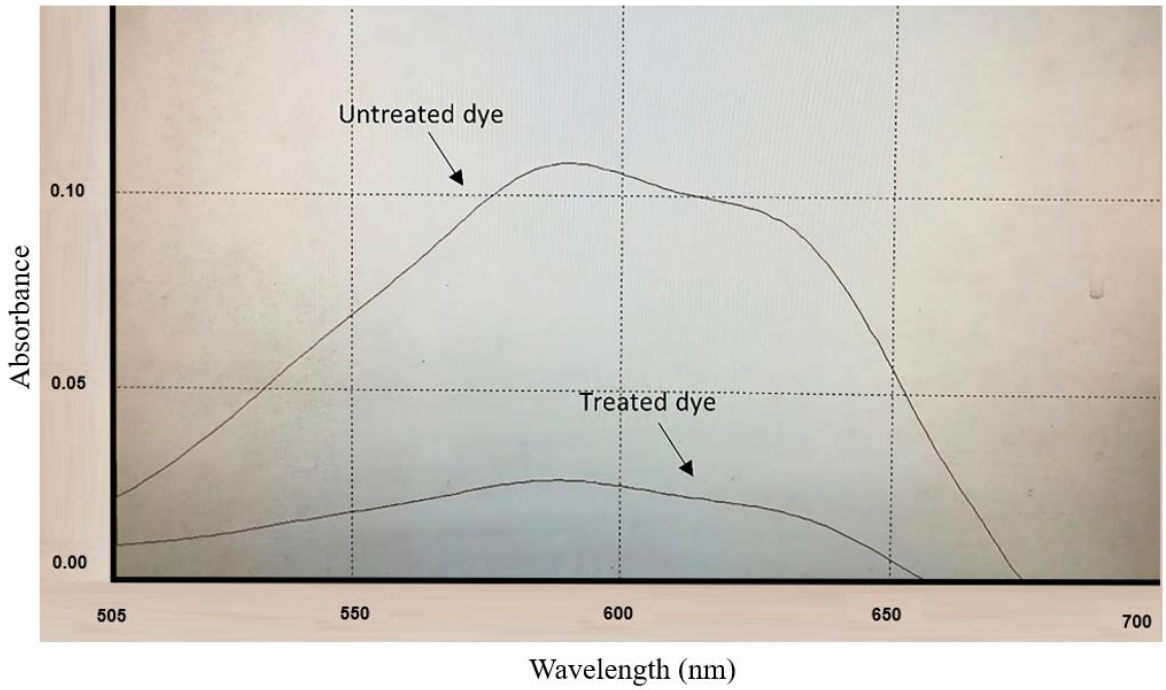

Figure 6. UV-Vis spectrum analysis of before (untreated) and after (treated) decolourisation of RBBR dye using optimised conditions.

Table 1. List of functional groups present in *Pistia* sp. before and after treatments.

Untreated (cm^{-1})	**Treated (cm^{-1})**	**Suggested assignment**
2919.65	2919.34	N-H stretching
1621.72	1621.19	C=C stretching
1317.74	1317.82	C-N stretching
1055.90	1054.59	C-O stretching
-	1033.19	S=O stretching
782.01	782.40	C-H bending
-	684.43	C-H bending

FTIR Analysis

Table 1 shows FTIR analysis of *Pistia* sp. before and after the root was exposed to RBBR dye. The roots of the plants were chosen as a sample for the FTIR analysis as they are the only part that was submerged into RBBR dye properly [14]. A similar study by Madikizela et al. reported that the removal of the Methylene blue and Victoria blue dyes was due to the capability of plant roots to trap the dye molecules effectively [17]. Findings in this study revealed the presence of 5 peaks before dye treatment, consisting of carbonyl and amino groups. Similar findings were recorded for the functional groups in *Pistia* sp., such as carboxylic, hydroxyl, carbonyl, amino groups, and amines [16]. This study discovered a significant difference in the functional group, with five peaks shifted and two new peaks detected on the surface of *Pistia* sp. after exposure to RBBR dye.

After being exposed to dye, new peaks were observed at 1033.19 (S=O stretching) and 684.43 (C-H bending), indicating the absorption occurrence of RBBR dye into *Pistia* sp. In addition, there were slight changes in the absorption peak at 2919.65 (N-H stretching), 1621.72 (C=C stretching), 1317.74 (C-N stretching), 1055.90 (C-O stretching), and 782.01 (C-H bending).

The appearance of the new functional groups at the root's surface after being exposed to dye indicated the presence of the dye molecules due to the root absorption during the contact time. Meanwhile, the shifting peak could be attributed to the structural changes at the outer layer of the roots, leading to changes in its peak. The attraction of the biosorbent and the dyes is due to the electrostatic attraction between the cations dyes and negatively charged biosorbent sites [25]. A similar finding was observed in a previous study when *J. curcas* pods were exposed to RBBR dye, where 5 new peaks were detected, and 6 peaks shifted after RBBR dye was treated [26].

Plant Cell Analysis

Figure 8 shows the changes in the structure of *Pistia* sp. before and after exposure to RBBR dye. When the plant cell was observed for microscopy analysis, a blue spot was observed, indicating that the leaves of *Pistia* sp. were also involved in the absorption of RBBR dye (Figure 9). *Pistia* sp. was in good condition prior to RBBR dye exposure, but the plant cell structure was disrupted after exposure. This disrupted structure proved that *Pistia* sp. has effectively removed the dye via biosorption. However, the absorption of RBBR by the leaves was less than roots, as the roots had been completely submerged in the dye and had greater biochemical uptake of nutrients and pollutants [7]. Despite the accumulation of RBBR dye, plant cells exhibited different structures compared to before dye exposure, demonstrating the effect of dye toxicity. A similar study by Ahila et al. also reported that there was an accumulation of reactive red dyes in *P. stratiotes* after 72 h of exposure [11].

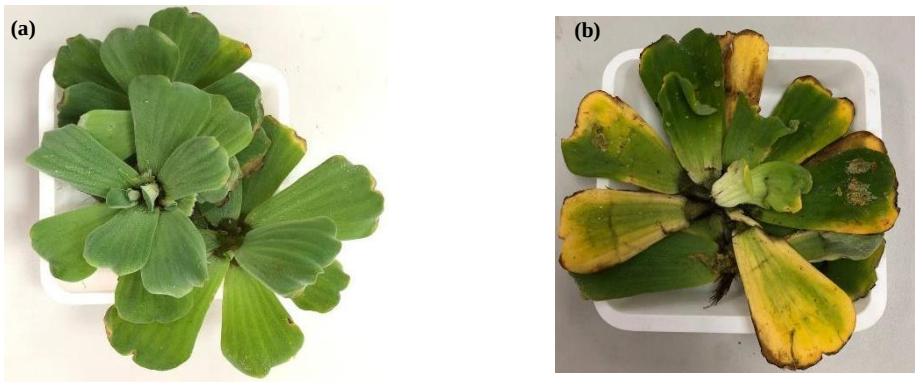

Figure 8. Structure of *Pistia* sp. (a) before and (b) after 32h dye exposure.

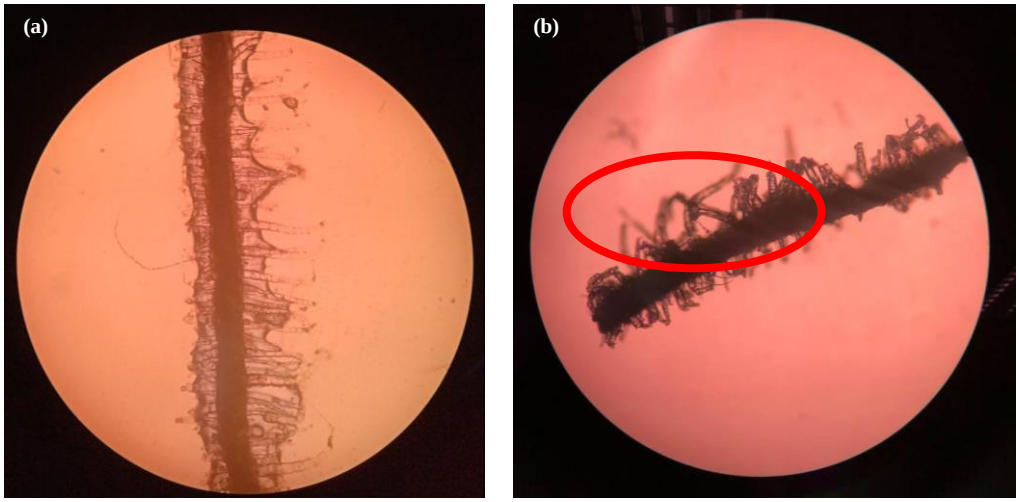

Figure 9. The cell of *Pistia* sp. leaves before **(a)** and after **(b)** 32 h exposure to RBBR dye.

Phytotoxicity Study

The phytotoxicity test proved that the treated RBBR dye has lower toxicity compared to the untreated RBBR dye. The length of the *Vigna radiata* shoots after the exposure was higher compared to the untreated RBBR dye, indicating that the toxicity of the dye has been reduced due to the remediation process (Figure 10). The maximum lengths attained by distilled water, the untreated RBBR dye, and the treated RBBR dye were 15, 10, and 13.5 cm, respectively. However, there was no significant difference (p-value >0.05) between the germination of *Vigna radiata* in the treated dye and distilled water, which served as the control in this experiment. The germination rate of *Vigna radiata* following exposure to distilled water and the treated RBBR was 100%, whereas it was 80% when exposed to the untreated RBBR. This result suggested that *Pistia* sp. still has the potential to act as a good biosorbent in remediating RBBR dye. A similar study conducted also discovered that the germination of wheat seeds using treated wastewater by *Bacillus* sp. and *Pseudomonas* sp. achieved higher germination percentage of 72% and 75%, respectively, when compared to the untreated wheat seeds, which only achieved a 50% germination rate [27]. Adenan et al. also found that seed germination of *V. radiata* in the treated dyes (Malachite green and Congo red) by live cell S. *bacillaris* was significantly higher than in the untreated dyes [5].

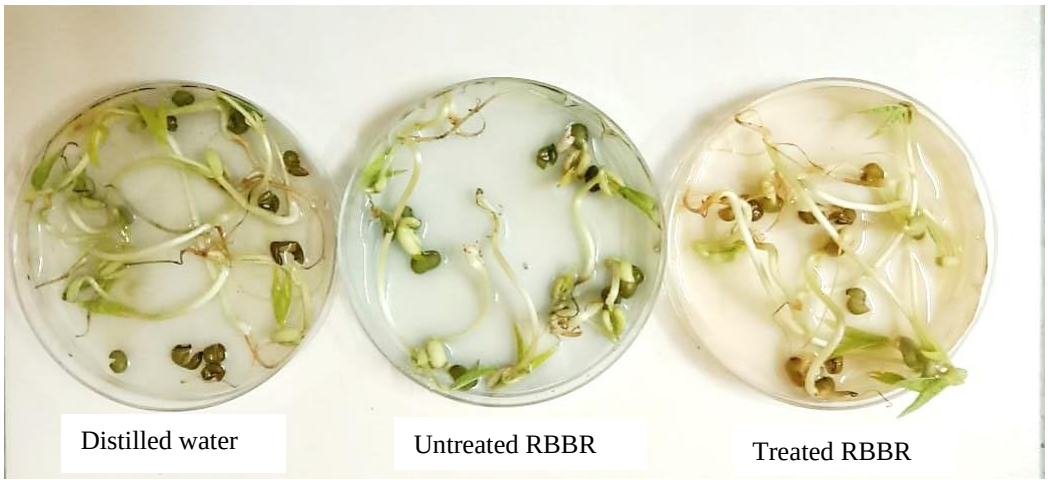

Figure 10. Germination of *Vigna radiata* (mung beans) using sterile distilled water, untreated and treated RBBR dye.

CONCLUSION

This study demonstrated that different aquatic plants have different capacities in remediating RBBR dye. *Pistia* sp. exhibited the highest potential in decolourising RBBR dye at the optimised conditions, revealing the best parameter and condition that could help accelerate the removal activity. The establishment of the RBBR removal was also proven by the occurrence of biodegradation (UV-Vis) and biosorption (FTIR) mechanisms by *Pistia* sp. The observation of plant cell biomass also revealed that *Pistia* sp. could withstand the toxicity of RBBR dye, resulting in a significant reduction in the toxicity of the treated dyes. This study proposes *Pistia* sp. as a promising agent for detoxifying dye pollutants and can be further investigated for the removal of other pollutants.

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