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Self-Immobilization of *Rhizopus oligosporus* on Shrimp Shell Chitin Matrix for *Troso* Fabric Dye-Contaminated Wastewater Adsorption

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Abstract

The *Troso* fabric industry in Indonesia contributes significantly to the national economy but also produces liquid waste containing chemical dyes. Liquid waste from the *Troso* weaving industry exceeds wastewater quality standards, including BOD, COD, and pH, and can pollute the environment if not managed properly. Chemical and physical methods for wastewater removal in dyeing have already been used, but still have limitations, such as being expensive and causing environmental side effects. Therefore, the biological method is preferred to overcome these problems. The present study aims to develop a *Rhizopus oligosporus* mycelium immobilization system using chitin from shrimp shells and to test its adsorption capacity for removing a dye agent (direct yellow) from *Troso* fabric dye-contaminated wastewater. The immobilization was performed using both solid-state and submerged techniques in potato dextrose broth (PDB). The one-factor-at-a-time (OFAT) method was used to determine the optimal pH and the optimal number of biosorbents for efficient dye removal. The results showed that the optimal immobilization system was the solid-state technique, which yielded a well-developed hyphal structure on the chitin surface with a cell-loading capacity (CLC) of 1.19 $g_{biomass}/g_{matrix}$. In the adsorption capacity (qe) assay, the results showed that pH 7.0 and 3 bio-balls application provided the highest adsorption capacity of 122.608 mg/g and 64.73% color removal. This study shows the potential use of immobilized chitin as a biosorbent for effectively treating textile wastewater.

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INTRODUCTION

Various industries have grown rapidly over the years. The leading industry sector in Indonesia is the textile industry, which contributes significantly to the national economy through job creation and foreign exchange earnings (Rachel et al., 2025). Central Java is one of the contributors to the batik industry, which includes the *Troso* woven fabric industry. *Troso* woven fabric is a distinctive woven fabric from Jepara, located in the village of *Troso*, Pecangaan District, Jepara Regency, Central Java (Anisah & Na'am, 2021).

Over the years, several rivers in Jepara have become dumping sites for textile waste. Liquid waste from the *Troso* weaving industry is generated by the dyeing process for *Troso* fabric/thread. The waste produced is in the form of chemical dye waste (Nugraini et al., 2022). Based on test results conducted by Fathina (2024), *Troso* weaving liquid waste has a Biological Oxygen Demand (BOD) of 215.51 mg/L, a Chemical Oxygen Demand (COD) of 33,914 mg/L, lead metal of 5.71 mg/L, and a pH of 11.0. The test results for BOD, COD, and pH have exceeded the wastewater quality standards for textile businesses and/or activities under the Indonesian Wastewater Quality Standards. Therefore, further treatment is needed before wastewater is discharged into rivers. Direct discharge of liquid waste into water bodies without proper management will harm the environment, including the soil and water around the outfall or waste-disposal site (Mulyadi et al., 2022). This is especially true for textile wastewater, due to the presence of synthetic dyes commonly used in the batik industry. Synthetic dyes used in the batik-making process are difficult to break down, resulting in wastewater containing zinc, chromium, and other heavy metals that are harmful to the environment (Purwaningrum, 2024).

Common wastewater management methods to remove dyes from batik wastewater include coagulation, flocculation, photocatalytic degradation, and adsorption (Utami, 2024). The adsorption method is often used because it is a simple, highly efficient process that is safer for the environment than other methods (Sitohang et al., 2022). Adsorbents can be made from natural, environmentally safe materials, commonly known as biosorbents. Biosorbents are made from porous materials and are readily available at low cost (Purwaningsih et al., 2017). One potential biosorbent from natural sources is chitin from shrimp shells. Research on chitin from shrimp shells as biosorbents has been widely conducted. For example, a study of Fabbricino & Pontoni (2016) showed that shrimp shells were used as biosorbents for dyes from textile wastewater. The results of adsorption testing with shrimp shells chitin showed an absorption efficiency of around 85-95%. Another material commonly used as a biosorbent is fungal cells; their cell walls consist of chitin, glucan, polysaccharides, and extracellular protein structures that can bind metals and dyes (Rahmaniah, 2019).

Rhizopus oligosporus is a fungal species with mycelium composed of functional groups such as –OH (hydroxyl), –COOH (carboxyl), and $–NH_2$ (amino), which can absorb heavy metals (Aznur et al., 2022). This fungus is commonly used as a biosorbent because it is easy to cultivate and is a Generally Recognized as Safe (GRAS) microorganism (Widianita, 2023). Previous researchers have reported the use of *Rhizopus* fungi as a biosorbent for textile dyes, particularly reactive azo dyes. Research on the optimization of reactive dye biosorption from textile waste by *Rhizopus arrhizus* biomass through batch

experiments showed that this biomass is effective and economical as a biosorbent, with FTIR analysis confirming the role of amino and carboxylate groups on the cell wall in dye binding (Salvi & Chattopadhyay, 2017). Another study reported that dead *R. arrhizus* NCIM 997 biomass removed up to 99.60% of the dye, indicating that dead fungal biomass is stable, high-capacity, and low-cost, making it a potential alternative to conventional adsorbents (Saraf & Vaidya, 2016). Additionally, up-flow packed-bed column studies using dry biomass of *Rhizopus oryzae* (MTCC 262) for the reactive azo dye Reactive Blue 4 achieved a removal efficiency of around 71.81%. They confirmed the involvement of amino and carboxylate groups on the mycelial surface, reinforcing the potential of dry *Rhizopus* biomass as a stable and applicable biosorbent for textile waste treatment (Bagchi et al., 2022).

Fungi are commonly employed as biosorbents through biomass activation and immobilization (Rahmayanti, 2006). Immobilization is the physical binding of enzymes or cells to a specific support material that retains catalytic activity and increases stability (Dwiyanti & Kuswytasari, 2016). According to Rohmah et al. (2022), immobilization is a technique for binding cells, in which the cells' activity must persist. It is an essential technique in bioprocessing to improve microbial performance and wastewater treatment by increasing cell density, isolating cells from external environmental influences, and separating cells from the medium for reuse (Ogawa et al., 2024). Generally, immobilization techniques are carried out with biomass because the process is easy, environmentally friendly, and inexpensive (Liu et al., 2022).

The immobilization of *R. oligosporus* as a biosorbent has been extensively studied to improve its stability and ease of separation (Muthu & Khadir, 2021). Common immobilization techniques include entrapment in alginate or polyvinyl alcohol (PVA) matrices and cell attachment to solid matrices such as polyurethane foam or other polymeric materials. In entrapment, the biomass is mixed with a polymer solution and then formed into beads/gels so that it is mechanically stable and reusable, but often experiences a decrease in biosorption capacity because the diffusion of dyes to the active sites of the cell wall is inhibited by the matrix (Saraf & Vaidya, 2017). Conversely, adsorptive immobilization/direct cell attachment does not add a barrier layer outside the cell wall; thus, the amino and carboxylate groups remain exposed, and electrostatic interactions with anionic dyes occur more efficiently, while still providing ease of separation and reuse of biosorbents in a continuous column system (Fibriana et al., 2025). Therefore, the use of chitin as a matrix for immobilizing *R. oligosporus* biomass through the direct cell contact technique is promising to be explored. The objective of this study is to develop a system for immobilizing mycelium on chitin and to optimize the adsorption process of *Troso* dye-contaminated wastewater by immobilizing fungal biomass on shrimp shell chitin.

METHODS

Materials

R. oligosporus was purchased from the commercial tempeh starter culture Raprima (Aneka Fermentasi Indonesia Co., Ltd., Indonesia). Potato Dextrose Broth (PDB) medium was obtained from Micromaster Laboratories Pvt. Ltd. (India). The wastewater sample from the Direct Yellow dyeing

process was taken from the Troso home-based weaving industry located in Troso Village, Pecangaan District, Jepara Regency, Indonesia. Commercial chitin from shrimp shells was purchased from Multiguna C.V. (Indonesia). All reagents are of analytical grade.

Fungal Self-Immobilization in Chitin Matrix

Fungal cells' self-immobilization was performed using two methods. The first method is solid-state immobilization in chitin from shrimp shells. Chitin and a fungal starter culture were mixed in a 2:1 ratio. Then, chitin was sprayed with sterilized PDB medium until sufficiently moist to provide nutrition for the fungal cells. Subsequently, the fungal starter culture was sprinkled onto wet chitin and mixed well. The mixture of wet chitin and starter culture was weighed (0.25 g, 0.50 g, and 0.75 g) and wrapped in a muslin cloth to form bio-balls in three different sizes. The natural immobilization process was carried out in a dark place in a room-temperature incubator (30 ± 2°C) for 72 h. The second method is a submerged immobilization technique. Fungal inoculum was prepared by mixing 10% (w/v) of the starter culture into PDB medium. Chitin was weighed (0.25 g, 0.50 g, and 0.75 g) and wrapped in three different sizes of bio-balls. Then, the bio-balls were submerged in fungal inoculum and incubated at room temperature (30 ± 2°C) for 72 h. The morphological structure of mycelial growth in a chitin matrix and cell-loading capacity (CLC) were measured using scanning electron microscopy (SEM) and a weighing method (Fibriana et al., 2025).

Optimization of Bio-ball Adsorption Conditions in Direct Yellow-Contaminated Wastewater Treatment

The textile wastewater treatment was optimized using the one-factor-at-a-time method, varying factors that influence the adsorption process. One bio-ball was added to 150 mL of wastewater with adjusted pHs (5.0, 7.0, 9.0, and 11.0) and incubated at room temperature (30 ± 2°C) for 24 h in a still condition. The best pH, which gave the highest adsorption capacity, was selected and further used for the next factor. Subsequently, the treatment was optimized by varying the bio-ball quantity (1, 2, and 3), which were added to 150 mL of wastewater at pH 7.0. Samples were then incubated at room temperature (30 ± 2°C) for 24 h under still conditions. The best bio-ball quantity with the highest adsorption capacity was selected for the final wastewater treatment process.

Measurement of Adsorption Capacity

The calibration curve for the direct yellow-contaminated wastewater was determined by measuring the wastewater absorbance at 480nm using a UV-Vis spectrophotometer (UV2800 Double Beam UV-Vis Spectrophotometer). The linear regression equation was obtained in accordance with SNI 6989.80:2011. The equation $y = ax + b$, where x represents the relative concentration of direct yellow (mg/L) in the solution after dilution, and y represents the average absorbance value obtained from three repetitions of UV-Vis spectrophotometer measurements for each calibration point. Here, a represents the slope of the calibration curve and b represents the intercept. The calibration curve shows a linear relationship between concentration and absorbance, and the equation was used to calculate the concentrations of unknown samples from their absorbance readings. Thus, the final concentration of *Troso* woven fabric dye-contaminated after the adsorption process was determined by substituting the average

absorbance value into the calibration equation and solving for *x* as follows:

$$x = \frac{y-b}{a} \quad \text{(Eq. 1)}$$

where:

x = final concentration of dye-contaminated wastewater after the adsorption process (mg/L)

y = average absorbance of the sample from the UV-Vis spectrophotometer measurement

The adsorption capacity (*qe*) of the immobilized *R. oligosporus* in chitin matrix was calculated as follows:

$$qe = \frac{(C0-Ce)}{m} \times V \quad \text{(Eq. 2)}$$

where:

qe = adsorption capacity (mg/g)

C_0 = initial solution concentration (mg/L)

C_e= final solution concentration (mg/L)

m = adsorbent mass (g)

V = volume of the solution (mL)

Color Analysis of Wastewater

The color of the wastewater before and after treatment using immobilized *R. oligosporus* in chitin matrix was analyzed using the Color Detector application. The wastewater was filtered and centrifuged at 3000 rpm for 10 min to remove the debris. Then, wastewater clarity was measured to determine color removal (%) from the direct yellow concentration (mg/L) before and after treatment. The calculation is as follows,

$$\% \text{ Color removal} = \frac{Co-Ce}{Co} \times 100\% \quad \text{(Eq. 3)}$$

where:

C_0 = initial dye concentration before treatment (mg/L)

C_e = final dye concentration after treatment (mg/L)

Statistical Analysis

This study used a quantitative approach, with ANOVA and post-hoc test (Duncan) to analyze data and identify measurable relationships among variables (Waruwu et al., 2025).

RESULTS AND DISCUSSION

Fungal Self-Immobilization in Chitin Matrix

The determination of the optimal immobilization method aimed to establish the optimal conditions for binding *R. oligosporus* mycelium biomass to the shrimp-shell chitin matrix. In this study, two immobilization methods were used: solid-state and submerged techniques. The results of the cell-loading capacity (CLC) of the chitin matrix in these two methods are shown in Table 1.

Table 1.
Cell-loading capacity (CLC) of chitin matrix in the self-immobilization techniques of R. oligosporus

Chitin matrix size (g)	Cell-loading capacity (CLC) in $g_{biomass}/g_{matrix}$	
	Solid-state immobilization technique	**Submerged immobilization technique**
0.25	0.68 ± 0.18a	2.41 ± 1.41a
0.50	0.80 ± 0.18b	1.46 ± 1.07a
0.75	**1.19 ± 0.29c**	1.47 ± 0.42a

Data was obtained from the average of n = 2

The CLC of the chitin matrix in the solid-state immobilization technique shows that CLC values increase with increasing chitin weight, with the highest value observed at 0.75 g (1.19 ± 0.29 $g_{biomass}/g_{matrix}$). This result indicates that an increase in chitin weight supports the immobilization capacity of fungal mycelium. The one-way ANOVA also shows a p-value of 0.009 (< 0.05), suggesting that treatment variation significantly affects CLC values. The CLC results from the submerged technique show that variations in chitin weight affect the immobilization capacity of *R. oligosporus* biomass. Chitin at 0.25 g produced the highest average CLC value (2.41 ± 1.41 $g_{biomass}/g_{matrix}$). Based on statistical analysis (ANOVA), a significance value of 0.217 ($p > 0.05$) indicates that the difference in CLC values between chitin weight variations is not statistically significant.

The observed differences may be attributed to the characteristics of each immobilization system. In submerged conditions, smaller chitin matrices facilitate better nutrient diffusion and a more uniform biomass distribution, resulting in higher apparent CLC values (Liu et al., 2022; Pandey, 2003). In contrast, in solid-state conditions, increasing chitin mass provides a larger surface area that supports fungal hyphal growth and colonization on the matrix, leading to increased biomass accumulation and more consistent CLC trends (Barrios-González, 2012; Bukhari, 2022).

The solid-state immobilization technique was selected as the best method due to its ability to form more stable bonds between fungal mycelium and the chitin matrix. This condition is thought to support more uniform mycelium growth and attachment, thereby increasing the CLC value. Although the highest CLC was obtained in the submerged system, the solid-state immobilization method was selected as the optimal approach due to its more consistent CLC trend. Furthermore, the solid-state immobilization technique was selected as the optimal method, as it provides better structural stability of the bio-ball, more uniform mycelial attachment, and greater practical potential for wastewater treatment systems (Liu et al., 2022; Ogawa et al., 2024). The addition of chitin mass increases the matrix volume and surface area for immobilization, thereby increasing the surface area available for binding by *R. oligosporus* (Andriani & Irdawati, 2025). The advantage of this method lies in the faster growth time of *R. oligosporus*. The amount of PDB in the solid-state immobilization technique was lower than in the submerged technique, and the process was simpler than the immersion method. Fungal spores begin to appear within the first 24 h after inoculating the matrix with low water content, which promotes the growth of *R. oligosporus* and inhibits decay (Bukhari, 2022).

Characterization of Fungal Mycelium Profile in Chitin Matrix

Microscopic observation was performed to visually assess the immobilization performance using a solid-state technique, as shown in Figures 1 and 2.

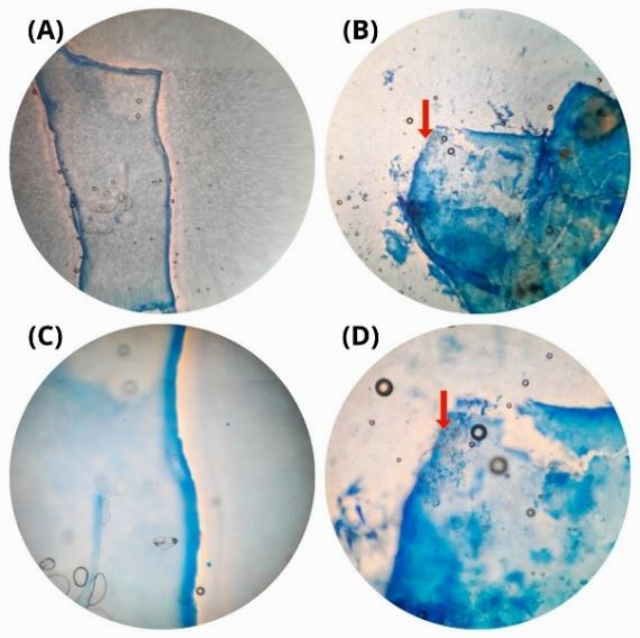

Figure 1.
Fungal mycelium profile in chitin matrix (A) and (B) magnification at 100×; (C) and (D) 400×, (A) and (C) are chitin matrix only without fungal cells, whereas (B) and (D) are chitin matrix with fungal cells. The red arrows show the mycelium growth within the matrix.

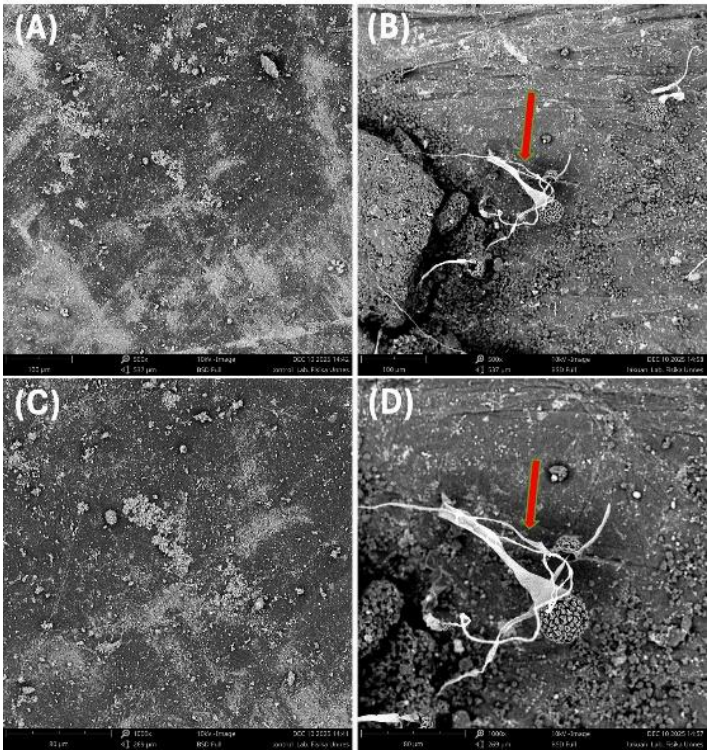

Figure 2.
Fungal mycelium profile in chitin matrix (A) and (B) magnification at 500×; (C) and (D) 1000×, (A) and (C) are chitin matrix only without fungal cells, whereas (B) and (D) are chitin matrix with fungal cells. The red arrows show the mycelium growth within the matrix.

Based on Figure 1, there are differences in the surface conditions of the chitin matrix only and immobilized fungal mycelia in the chitin matrix. The surface condition of immobilized chitin showed *R. oligosporus* mycelia growing and adhering to the surface of the shrimp shell chitin. In contrast, the surface of non-immobilized shrimp-shell chitin was flat. Optical microscope observations yielded less detailed results, and a more thorough analysis using a scanning electron microscope (SEM) was required. SEM analysis was performed to examine the microenvironment of *R. oligosporus* immobilized on chitin and to characterize the biosorbent for use in adsorption. SEM analysis was performed on the chitin matrix alone and on immobilized *R. oligosporus* in the chitin matrix, as shown in Figure 2. The SEM analysis of the chitin matrix (Figure 2A, 2C) reveals a surface with an uneven, rough structure. At both 500× and 1000× magnifications, no hyphae-like filaments are visible on the chitin surface. Meanwhile, SEM analysis of immobilized fungal mycelia in the chitin matrix (Figure 2B, 2D) at both magnifications reveals an uneven, rough chitin surface. At 1000× magnification, the chitin surface shows the presence of long filament structures resembling hyphae and sporangia attached to the chitin matrix surface.

Optimization of Bio-ball Adsorption Conditions in Direct Yellow-Contaminated Wastewater Treatment

The calibration curve for Troso woven fabric dye-contaminated wastewater (direct yellow) was measured at 480 nm, and the results are shown in Figure 3.

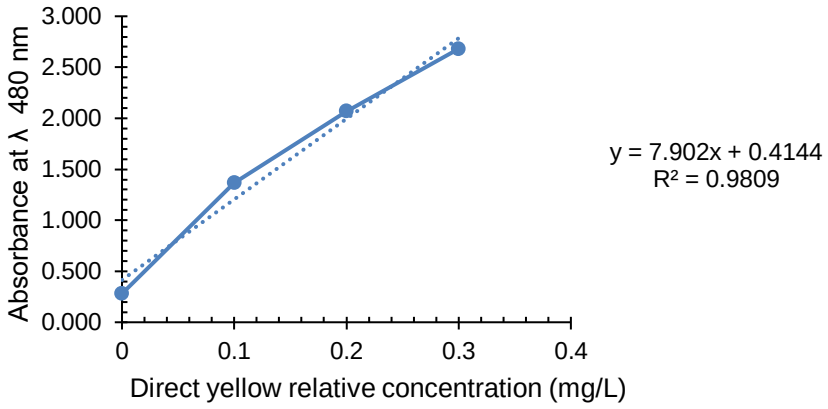

Figure 3.
Calibration curve of direct yellow at a wavelength of 480 nm

The linear regression equation $y = 7.902x + 0.4144$ was obtained with an R^2 value of 0.9899. According to SNI 6989.80:2011, a calibration curve is considered linear if the regression correlation coefficient (r) is ≥ 0.995. The result indicates that the relationship between the concentration of direct yellow and absorbance is linear, meeting the linearity criteria required by the standard. This equation was used to calculate direct yellow concentration (mg/L) from wastewater samples. Then, the adsorption capacity (*qe*) was also calculated.

The immobilized fungus in a chitin matrix was then used to optimize wastewater treatment using the one-factor-at-a-time method, in which each parameter was varied while the others were held constant.

Therefore, the impact of the changed parameter can be determined (Latip et al., 2023). The first optimization was carried out by varying pH (5.0, 7.0, 9.0, and 11.0). The effects of pH variation on wastewater samples and the bio-ball's adsorption capacity are shown in Table 2. Based on testing the effect of pH on adsorption capacity, the optimal pH was 7.0, providing the highest adsorption capacity (66.208 mg/g) and color removal (34.97%). The decrease in concentration at pH 7.0 is consistent with similar studies on anionic dyes using fungal biomass, which show that the optimum pH is neutral or slightly acidic. At a pH close to neutral, the degree of protonation of amino and carboxylate groups on the surface of the mycelium is in a condition that allows for a combination of electrostatic and non-electrostatic interactions, such as hydrogen bonding, thereby increasing the stability of the adsorbate-adsorbent complex (Salvi & Chattopadhyay, 2017). At pH 5.0 and 11.0, the biosorbent's surface charge changes, affecting adsorption capacity. At pH 5.0, excess H^+ ions will protonate the chitin functional group $–NH_2$ (amine) in acidic conditions, forming $–NH_3^+$ (Khairi et al., 2025), thereby saturating the biosorbent more quickly and decreasing adsorption capacity. Meanwhile, at pH 11.0, OH^- ions reduce the biosorbent's affinity by changing the surface charge of the biosorbent to a negative charge, which has the potential to reduce the adsorption capacity toward direct yellow dye (adsorbate), which is negatively charged as an anionic dye (Putri, 2024).

Table 2.
Effect of pH variation on wastewater treatment process and the adsorption capacity of the immobilized R. oligosporus with 1 bio-ball for 24 h

pH	Absorbance (480nm)	Initial dye concentration (C_0) (mg/L)	Final dye concentration (C_e) (mg/L)	Adsorption Capacity (*qe*) (mg/g)	% Color removal
5.0	4.000 ± 0.000	1.42 ± 0.00	3.95 ± 0.00	-350.992 ± 0.000a	-185.38a
7.0	0.871 ± 0.1077	1.42 ± 0.00	0.92	**66.208 ± 14.358c**	**34.97 ± 7.58c**
9.0	1.082 ± 0.0627	1.42 ± 0.00	1.09	43.408 ± 8.365b	20.13 ± 4.42b
11.0	4.000 ± 0.000	1.42 ± 0.00	3.95 ± 0.00	-350.992 ± 0.000a	-185.38a

Based on the observation result, the wastewater samples appeared more concentrated. They showed a color change to brown at pH 5.0 due to the acidic pH triggering protonation of the chromophore group in the dye molecule, thereby altering the conjugated bond system and making the dye structure less stable, resulting in a brown color (Arummidah, 2023). The adsorption capacity in was also negative; thus, no color removal occurred. The ANOVA indicated that the differences in adsorption capacity among treatments (pH 5.0 and 11.0 with 7.0, 9.0) were statistically significant ($F = 2360.173$, $p = 0.000$) (Table 2). After 24 h of adsorption, the wastewater color in each treatment became lighter. This is because most of the dye molecules have been bound to the surface of the chitin matrix through several attractive forces, namely the charge attraction between the $–NH_3^+$ group on chitin and the negatively charged sulfonate ($-SO_3^-$) groups on the dye molecules, plus the presence of hydrogen bonds between polar groups and the attractive forces between aromatic rings that cause the hydrophobic parts to come closer together (Mahatmanti et al., 2022). This combination of interactions causes more dye molecules to adhere to the

chitin matrix, reducing the amount of dye remaining in solution and making it appear much paler. The variation in the number of biosorbents used in this study was 1, 2, and 3 bio-balls. The adsorption capacity data and % color removal results from the variation of bio-ball number are shown in Table 3.

Table 3.
Effect of the number of bio-ball variations on wastewater treatment process and the adsorption capacity of the immobilized R. oligosporus at pH 7.0 for 24 h

Bio-ball/s	Absorbance (480nm)	Initial dye concentration (C_0) (mg/L)	Final dye concentration (C_e) (mg/L)	Adsorption Capacity (*qe*) (mg/g)	% Color removal
1	0.497 ± 0.029	1.42 ± 0.00	0.55 ± 0.03	116.074 ± 3.915a	61.33 ± 2.07a
2	0.531 ± 0.119	1.42 ± 0.00	0.58 ± 0.12	111.541 ± 15.908a	58.88 ± 8.41a
3	0.448 ± 0.079	1.42 ± 0.00	0.50 ± 0.08	122.608 ± 10.595a	64.73 ± 5.59a

Based on the results, the application of 3 bio-balls showed the highest adsorption capacity, with a *qe* value of 122.608 mg/g. However, the variation in the number of chitin balls did not show a consistent trend in adsorption performance. Although an increase in the number of bio-balls may enhance the effective surface area and the availability of active adsorption sites (Wijayanti et al., 2019), this effect was not statistically significant under the studied conditions. Furthermore, a one-way ANOVA and post-hoc test (Duncan test) indicated that differences in adsorption capacity among treatments (1, 2, and 3 bio-balls) were not statistically significant ($F = 0.730$, $p = 0.520$). Prior to the analysis, Levene's test confirmed the homogeneity of variances ($p = 0.064$), indicating that the assumption for ANOVA was satisfied. These findings suggest that increasing the number of bio-balls does not significantly affect adsorption performance. The observed variation may be attributed to differences in biomass distribution, contact efficiency, and possible mass-transfer limitations during adsorption, as well as to the relatively higher variability observed in certain treatments.

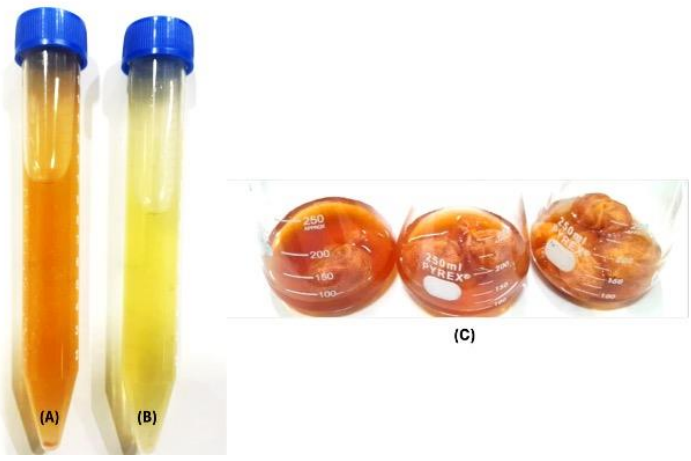

Figure 4.
Wastewater appearance after treatment with pH 7.0 and 3 bio-balls for 24 h at room temperature (A) before treatment, (B) after treatment, and (C) wastewater biosorption.

Figure 4 shows the effect of pH 7.0 and 3 bio-balls on the color removal of the wastewater. of chitin biosorbent on the reduction of dye color at pH 7 before and after a 24-h contact process. Figure 4A shows that the wastewater appears more cloudy, indicating that the dissolved dye concentration remains high and that fine particles remain suspended throughout the sample. The color was detected as golden orange-brown. After 24 h of contact (Figure 4B), the solution appears lighter and more transparent (clear olive yellow) because the dye molecules have been absorbed by the surface of the chitin spheres, as indicated by the change in color of the biosorbent from white to orange (Figure 4C), as well as the reduction in dye remaining in the aqueous phase. In addition, the particles initially suspended in the wastewater appear to settle to the bottom of the Erlenmeyer flask, which can be explained by flocculation and particle entrapment by the chitin matrix, followed by gravitational settling, thereby reducing turbidity and making the water appear more transparent.

This phenomenon is in line with the adsorption and flocculation mechanisms in chitin/chitosan biosorbents, where the active groups on the chitin surface bind dye molecules and fine suspended particles, forming larger and heavier aggregates that easily settle due to the influence of gravity, resulting in a decrease in turbidity and color intensity of the solution. Studies on the adsorption of dyes by chitin and fungal mycelium show that both materials exhibit significant adsorption capacity. In a study by Jóźwiak et al. (2023), pure chitin had an adsorption capacity for azo dyes such as Reactive Black 5 and Reactive Yellow 84 of 121.15 mg/g and 138.55 mg/g, while fungal mycelium as biomass also showed good adsorption capacity for the azo dye Remazol Black (434 mg/g) (Kodal & Aksu, 2017). The combination of both in an immobilization system is expected to increase the effective surface area and the availability of functional sites (amide, –OH, and fungal polysaccharides), potentially resulting in improved adsorption performance compared to single adsorbents.

CONCLUSION

This study shows that immobilizing *R. oligosporus* on a shrimp-shell chitin matrix successfully increased the adsorption capacity of dye-contaminated from *Troso* woven fabrics. The best technique for immobilizing the fungal species is the solid-state direct contact method (cell-loading capacity or CLC at 1.19 $g_{biomass}/g_{matrix}$). Characterization by scanning electron microscopy (SEM) showed that mycelium was successfully immobilized on the chitin surface, forming a hyphal structure that increased adsorption capacity. Based on the optimization results, the highest adsorption capacity (122.608 mg/g) and color removal (64.73%) were recorded at pH 7.0, employing 3 bio-balls for 24 h at room temperature. These results indicate that the combination of chitin and *R. oligosporus* has excellent potential as a biosorbent for the efficient treatment of textile wastewater.

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